COMMISSION OF THE EUROPEAN COMMUNITIES

EURATOM

PROGRAMME

RADIATION PROTECTION

PROGRESS REPORT

1982

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EURATOM

Beretning
Program
STRÅLINGSBESKYTTELSE

Tätigkeitsbericht
Programm
STRAHLENSCHUTZ

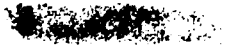
Progress Report
Programme
RADIATION PROTECTION

Rapport d'Activité
Programme
RADIOPROTECTION

Rapporto d'attività
Programma
RADIOPROTEZIONE

Verslag van de werkzaamheden
Programma
STRALINGSBESCHERMING

1982



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*** Biology Group of the Commission of the European Communities DG XII, Biology, Radiation Protection and Medical Research, at the Ispra Establishment of the Joint Research Centre.

I

EINLEITUNG

INTRODUCTION

INTRODUCTION

DE

I. EINLEITUNG

Der Euratom-Vertrag beauftragt die Kommission der Europäischen Gemeinschaften mit der "Untersuchung der schädlichen Auswirkungen von Strahlungen auf Lebewesen". Fünf mehrjährige Forschungsprogramme auf dem Gebiet des Strahlenschutzes wurden seit 1960 durchgeführt. Sie haben einschliesslich des laufenden Programms für 1980-1984 zur objektiven Beurteilung der Auswirkungen und Gefahren ionisierender Strahlungen sowie zum Schutz des Menschen und seiner Umwelt beigetragen. Wenn auch Fälle übermässiger Strahlung äusserst selten sind, so müssen die Forschungen auf dem Gebiet des Strahlenschutzes doch fortgesetzt werden, um die Kenntnisse über den Schutz der Bevölkerung und der Arbeitskräfte gegen alle möglichen Gefahren ionisierender Strahlungen zu erweitern. Damit wird eine risikofreiere Kernenergiegewinnung sichergestellt und ein Beitrag zur Lebensqualität geleistet.Das Programm der Gemeinschaften nimmt im Rahmen der europäischen Forschungstätigkeiten auf dem Gebiet des Strahlenschutzes eine strategische Stellung ein, da es durch Koordinierung aller Anstrengungen auf diesem Gebiet auf einen äusserst wirkungsvollen Einsatz der begrenzten Ressourcen abzielt, was in unserer durch Einschränkungen gekennzeichneten Zeit eine Notwendigkeit ist.

Der Tätigkeitsbericht für 1982 des Strahlenschutzprogramms der Kommission gibt einen Überblick über die Forschungsarbeiten, die im Rahmen von rund 320 Einzelprojekten - auf sechs Bereiche verteilt - durchgeführt wurden: Strahlendosimetrie und ihre Interpretation; Verhalten und Kontrolle der Radionuklide in der Umwelt; somatische Sofortwirkungen, somatische Spätwirkungen und genetische Wirkungen ionisierender Strahlung sowie Abschätzung der Strahlenrisiken.
In diesem Bericht wird auf über 900 Veröffentlichungen hingewiesen, woraus die Bedeutung und der Umfang des Gemeinschaftsprogramms hervorgeht.

Die Aktionen der Kommission beschränken sich jedoch nicht nur auf eine Beteiligung an den laufenden Forschungen sondern befassen sich ferner damit, bestehende Daten zu Themen von dringendem Interesse

auf dem Gebiet des Strahlenschutzes zu revidieren und - falls erforderlich - Arbeiten über neu auftretende Probleme einzuleiten.

Konferenzen und Seminare, die unter der Schirmherrschaft der Kommission abgehalten wurden, haben sich in dieser Hinsicht als sehr wirkungsvoll erwiesen. Unter den behandelten Themen befanden sich Neutronenkanzerogenese, Risiken der Tritiumexposition und Mikrodosimetrie (s.Kapitel IV). Sitzungsberichte werden regelmässig zur Verfügung gestellt, um den Austausch von Informationen zu erleichtern und zu erweitern (s.Kapitel V).

Dieser Bericht gibt einen Querschnitt der laufenden Forschungstätigkeiten in Europa wieder und vermittelt darüber hinaus einen Überblick über die Fortschritte im Strahlenschutz; er sollte sich - so hoffen wir - sowohl für Forscher als auch für beschlussfassende Organe auf dem Gebiet des Strahlenschutzes als eine nützliche Informationsquelle erweisen.

E. BENNETT

Direktor
Gesundheit und Sicherheit

F. VAN HOECK

Direktor
Biologie,Strahlenschutz und
Medizinische Forschung

I. INTRODUCTION

The Euratom Treaty requires the Commission of the European Communities to "study the harmful effects of radiation on living organisms". Five multiannual research programmes on radiation protection have been carried out since 1960 and these, including the current 1980-1984 programme, have contributed to the ongoing evaluation of the effects and hazards arising from ionizing radiation and to the protection of man and his environment. While it is true that cases of excessive irradiation are extremely rare, research on radiation protection must continue to improve knowledge on the protection of the public and the workers from all potential hazards arising from ionizing radiation, thus ensuring a safer production of nuclear energy and contributing to the quality of life. The Community programme occupies a strategic position in the overall research activities on radiation protection in Europe, since it aims at using the limited resources most effectively by coordinating all efforts in this area - a necessity in this time of austerity.

The 1982 progress report of the Commission's Radiation Protection Programme presents in a condensed form the research work carried out in about 320 individual projects covering six sectors: radiation dosimetry and its interpretation; behaviour and control of radionuclides in the environment; short-term somatic effects, late somatic effects and genetic effects of ionizing radiation; and evaluation of radiation risks. More than 900 publlilications are referred to in this report, providing an indication of the effectiveness and wide scope of the programme.

The activities of the Commission are not, however, restricted to participation in ongoing research but include the reviewal of existing data on subjects of immediate interest from the radiation protection standpoint and, where appropriate, the initiation of work on any new problems.

Conferences and seminars held under the auspices of the Commission have proved valuable in that connection. Among the subjects dealt with have been neutron carcinogenesis, risks of exposure to tritium, and microdosimetry (see Chapter IV). Proceedings are regularly made available in order to facilitate and extend the exchange of information (see Chapter V).

Besides giving a cross-section of ongoing research in Europe, this report provides an overview of the advances made in radiation protection and should, we hope, prove useful both to research workers and to decision-making bodies concerned with the radiation protection.

E. BENNETT

Director
Health and Safety

F. VAN HOECK

Director
Biology, Radiation Protection
and Medical Research

I. INTRODUCTION

Le traité Euratom a chargé la Commission des Communautés européennes de "l'étude des effets nocifs des radiations sur les êtres vivants". Depuis 1960, cinq programmes de recherche pluriannuels de radioprotection ont été menés à bien et, en incluant le programme 1980-1984 en cours, ils contribuent à une évaluation objective des effets et des dangers des rayonnements ionisants et à la protection de l'homme et de son environnement. S'il est vrai que les cas d'irradiation excessive sont extrêmement rares, les recherches en matière de radioprotection doivent néanmoins continuer à améliorer les connaissances sur la protection du public et des travailleurs contre tous les dangers éventuels des rayonnements ionisants afin de garantir ainsi une production d'énergie nucléaire plus sûre, et de contribuer à la qualité de la vie. Le programme communautaire occupe une place stratégique dans les activités globales de recherche sur la radioprotection en Europe, du fait qu'il vise à une utilisation la plus efficace possible des ressources limitées, par une coordination de tous les efforts dans ce domaine, ce qui est indispensable dans la période d'austérité actuelle.

Le rapport d'activité 1982 sur le Programme de Radioprotection de la Commission résume les travaux de recherche effectués dans le cadre de 320 projets environ, répartis selon six secteurs: dosimétrie des rayonnements et son interprétation; comportement et contrôle des radionucléides dans l'environnement; effets somatiques à court terme, effets somatiques à long terme et effets génétiques des rayonnements ionisants ainsi que l'évaluation des risques d'irradiation. Ce rapport fait référence à plus de 900 publications, ce qui témoigne de l'efficacité et de l'étendue du programme.

Toutefois, les activités de la Commission ne se limitent pas à une participation aux recherches actuelles mais comprennent l'examen des données existantes sur des thèmes présentant un intérêt immédiat pour la radioprotection et, le cas échéant, l'amorce de travaux sur les nouveaux problèmes qui se posent.

Les conférences et les séminaires tenus sous les auspices de la Commission se sont révélés efficaces à cet égard. Ces réunions ont porté entre autres sur les effets cancérigènes des neutrons, les risques d'une exposition au tritium et la microdosimétrie (voir chapitre IV). Des comptes rendus sont établis régulièrement afin de faciliter et élargir l'échange permanent de l'information (voir chapitre V).

Ce rapport, tout en présentant un profil des recherches en cours en Europe, donne un aperçu des progrès en matière de radioprotection et, nous l'espérons, sera utile aux chercheurs et aux organismes décideurs concernés par la radioprotection.

E. BENNETT
Directeur
Santé et Sécurité

F. VAN HOECK
Directeur
Biologie, Radioprotection
et Recherche Médicale

II

Mitglieder im Jahr 1982 des Beratenden Programmausschusses

"BIOLOGIE - GESUNDHEITSSCHUTZ"

Members in 1982 of the Advisory Committee on Programme Management

"BIOLOGY - HEALTH PROTECTION"

Membres en 1982 du Comité Consultatif en Matière de Gestion de Programme

"BIOLOGIE - PROTECTION SANITAIRE"

Mitglieder im Jahr 1982 des Beratenden Programmausschusses
"BIOLOGIE - GESUNDHEITSSCHUTZ"

Members in 1982 of the Advisory Committee on Programme Management
"BIOLOGY - HEALTH PROTECTION"

Membres en 1982 du Comité Consultatif en Matière de Gestion de Programme
"BIOLOGIE - PROTECTION SANITAIRE"

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III

FORSCHUNGSTÄTIGKEIT STRAHLENSCHUTZ

RESEARCH IN RADIATION PROTECTION

RECHERCHE EN RADIOPROTECTION

III A

STRAHLENDOSIMETRIE UND IHRE INTERPRETATION

RADIATION DOSIMETRY AND ITS INTERPRETATION

LA DOSIMETRIE DES RAYONNEMENTS ET SON INTERPRETATION

Weitere Forschungsarbeiten zu diesen Themen werden auch in folgenden Tätigkeitsberichten beschrieben :

Further research work on these subjects is also described in the following progress reports :

D'autres travaux sur ces thèmes de recherche sont également décrits dans les rapports suivants :

III D.	Gössner, W./Kellerer, A.M./ Spiess, H.	GSF Neuherberg/Univ. Würzburg/Univ. München	BIO 461 D
III D.	Palmer, G.H./Ramsden, D.	UKAEA Winfrith	BIO 380 UK
III D.	Simmons, J.A.	PCL London	BIO 381 UK
III D.	EULEP	Bordeaux	BIO 390 D
III E.	Kiefer, J.	Univ. Giessen	BIO 392 D
III E.	Leenhouts, A.P.	ITAL Wageningen	BIO 409 NL
III E.	Peirson, D.H.	UKAEA Harwell	BIO 460 UK
III E.	Smith, H.	NRPB Chilton	BIO 413 UK
III E.	Pohlit, W.	GSF Frankfurt	BIO 394 D
III F.	Jacobi, W./Drexler, G.	GSF Neuherberg	BIO 458 D
III F.	Jacobi, W./ Paretzke, H.G.	GSF Neuherberg	BIO 422 D
III F.	Reissland, J.A.	NRPB Chilton	BIO 500 UK
III F.	Reissland, J.A.	NRPB Chilton	BIO 501 UK

Progress Report
1982

Contractor:

REP-Institutes of the
Organization for Health
Research, TNO
P.O.Box 5815
NL-2280 HV Rijswijk

Contract no.: BIO-A-300-81-NL

Head(s) of research team(s):

Prof. G.W. Barendsen
Radiobiological Institute
TNO
P.O.Box 5815
NL-2280 HV Rijswijk

General subject of the contract:

Evaluation of the biological effectiveness of various types of radiation for different types of damages in cultured mammalian cells.

List of projects:

1. Measurement of the biological effectiveness of fast neutrons of different energies for cell reproductive death and for chromosome aberrations in different types of cultured mammalian cells.
2. Measurement of the biological effectiveness of different radiations for malignant transformation of cultured cells.

Title of project nr. 1:

Measurement of the biological effectiveness of fast neutrons of different energies for cell reproductive death and for chromosome aberrations in different types of cultured mammalian cells.

Head of scientific staff:
G.W. Barendsen and J. Zoetelief

The dose-effect relationships obtained for induction of cell reproductive death and of chromosome aberrations (dicentrics and centric rings) by different qualities of radiation and for different cell lines have been analysed for non-cycling cells from plateau phase cultures exposed to single doses of radiation. Experiments with fractionated doses have been performed but the results are not yet complete enough to be analysed in detail. The studies on chromosomal aberrations were restricted to dicentrics and centric rings, which are mainly produced in the G_0 and G_1 phases of the cell cycle. This endpoint is of importance, since the cell proliferation at risk in humans is not rapidly proliferating, i.e. most cells will be in the G_0 or G_1 phase of the cell cycle.

The RBE values for neutrons obtained for both biological endpoints are presented in the table for the different cell lines employed. The results for levels of effect corresponding to different doses of 300 kV X rays are given. Corrections for the contributions of the photon components of the neutron beams are not included in this table. It can be seen from the table that RBE decreases with increasing dose of X rays. No significant differences are observed between the RBE values for the different biological endpoints. The largest RBE values (average for cell death and chromosome aberrations) are found for 0.5 MeV neutrons, which show values of about 8, 12 and 12 at the low dose levels for R-1,M, RUC-2 and V-79 cells, respectively. These RBE values do not exceed significantly the $\bar{Q}$ value of about 10. For 4.2 MeV neutrons, the mean RBE values at low dose levels are about 3, 6 and 7 for R-1,M, RUC-2 and V-79 cells, respectively. However, for the experiments with this type of neutrons, the relative photon contribution to the total dose was rather

high (26 per cent). Correction for the photon contribution will increase the RBE values to about 4, 8 and 9 for 4.2 MeV neutrons alone; the relevant $\bar{Q}$ value is about 8. The mean RBE values at low doses for 15 MeV neutrons are about 2.5, 6 and 5 for R-1,M, RUC-2 and V-79 cells, respectively, and these values can be compared with $\bar{Q}$ values of about 7.

It can be concluded that the mean RBE values for R-1,M cells are lower than those for the relatively resistant RUC-2 and V-79 cells, which are similar. The RBE values for these latter lines extrapolated to levels corresponding to low doses of X rays do not exceed the quoted $\bar{Q}$ values. However, due to the large uncertainties in the RBE values, especially at the low dose levels, a variation by a factor of 2 to 3 cannot be excluded. Since the linear dose term is most important for the evaluation of the effectiveness of small doses relevant for radiation protection and for the derivation of RBE values, a more accurate determination is required than is possible from single dose data. Therefore studies will be continued with fractionated dose experiments for photons since the largest uncertainty in the derived RBE values is due to the photon results.

Table

RBE OF NEUTRONS AS A FUNCTION OF DOSE OF 300 kV X RAYS FOR INDUCTION OF CELL INACTIVATION AND CHROMOSOME DAMAGE*

dose of X rays (Gy)	15 MeV neutrons		4.2 MeV neutrons		0.5 MeV neutrons	
	cell death	chrom.aberr.	cell death	chrom.aberr.	cell death	chrom.aberr.
			R-1, M cells			
0.001	3.1 { 4.7 / 2.3 }	1.9 { 2.5 / 1.2 }	3.9 { 6.1 / 2.8 }	1.9 { 2.5 / 1.2 }	8.6 { 13 / 6.4 }	7.0 { 9.2 / 4.4 }
1	2.7 { 4.3 / 2.0 }	1.8 { 2.4 / 1.2 }	3.4 { 5.5 / 2.5 }	1.8 { 2.4 / 1.2 }	7.6 { 12 / 5.8 }	6.6 { 9.1 / 4.4 }
2	2.4 { 3.9 / 1.9 }	1.7 { 2.4 / 1.2 }	3.0 { 5.0 / 2.3 }	1.7 { 2.4 / 1.2 }	6.7 { 11 / 5.2 }	6.3 { 9.0 / 4.4 }
5	1.8 { 3.1 / 1.5 }	1.5 { 2.4 / 1.2 }	2.3 { 4.0 / 1.8 }	1.5 { 2.4 / 1.2 }	5.0 { 8.6 / 4.1 }	5.4 { 8.8 / 4.4 }
			RUC-2 cells			
0.001	5.4 { 47 / 3.1 }	7.5 { 16 / 4.6 }	5.0 { 50 / 2.7 }	6.9 { 15 / 4.2 }	13 { 110 / 4.4 }	11 { 36 / 7.1 }
1	4.5 { 13 / 3.7 }	5.7 { 11 / 3.8 }	4.2 { 10 / 4.0 }	5.2 { 9.7 / 3.4 }	11 { 32 / 8.8 }	9.0 { 17 / 5.8 }
2	3.8 { 8.3 / 3.1 }	4.6 { 7.7 / 3.2 }	3.6 { 6.4 / 3.4 }	4.2 { 7.1 / 2.9 }	9.4 { 21 / 7.5 }	7.3 { 13 / 4.9 }
5	2.7 { 4.1 / 2.2 }	2.9 { 4.3 / 2.2 }	2.5 { 3.1 / 2.3 }	2.7 { 4.0 / 2.0 }	6.5 { 10 / 5.1 }	4.6 { 7.0 / 3.4 }
			V-79 cells			
0.001	4.2 { 11 / 2.5 }	5.5 { 14 / 3.7 }	4.9 { 13 / 3.2 }	8.5 { 25 / 4.8 }	9.3 { 25 / 6.3 }	15 { 38 / 9.6 }
1	3.8 { 8.6 / 2.1 }	3.9 { 8.3 / 2.9 }	4.5 { 10 / 2.5 }	6.1 { 15 / 3.7 }	8.4 { 20 / 4.6 }	10 { 23 / 7.5 }
2	3.5 { 6.8 / 2.1 }	3.1 { 5.9 / 2.3 }	4.1 { 8.0 / 2.4 }	4.7 { 10 / 3.0 }	7.7 { 16 / 4.5 }	8.1 { 16 / 6.1 }
5	2.7 { 4.2 / 1.9 }	1.8 { 3.1 / 1.5 }	3.2 { 4.9 / 2.2 }	2.8 { 5.5 / 2.0 }	6.1 { 9.5 / 4.0 }	4.8 { 8.3 / 3.9 }

Title of project nr. 2:

Measurements of the biological effectiveness of different radiations for malignant transformation of cultured cells.

Head of project and scientific staff:
G.W. Barendsen and J. Zoetelief.

Studies have been carried out on morphological transformation of cells in culture by X-rays, 15 MeV neutrons and 0.5 MeV neutrons. As discussed in earlier reports it is considered necessary to obtain data for different cell lines, because the absolute effectiveness as well as the Relative Biological Effectiveness can vary among cell lines. Significant variations in RBE among cell types have been amply demonstrated for the induction of cell reproductive death and of chromosome aberrations, and similar differences are likely to be found for cell transformation.

With C3H 10T½ cells various groups of investigators have studied cell transformation in culture by different radiations and in different conditions and therefore we have started experiments with this cell line. The systems of culture and assay differ among laboratories and as a consequence the actual transformation frequencies observed vary also. In our studies we have irradiated cells in plateau phase and after treatment cultures were trypsinized immediately. Subsequently 10^5 cells were seeded per dish in 10 dishes. After a culture period of 6 weeks, fixation and staining reveals transformed foci which are densely stained, piled up cells with a criss-cross pattern of growth.

Data have now been obtained for doses of up to 4 Gy of X-rays, 1.5 Gy of 15 MeV neutrons and 0.5 Gy of 0.5 MeV neutrons.

The results have been analysed by fitting curves which to a first approximation can be represented by:

$$F(D) = a_1 D + a_2 D^2 \qquad (1)$$

The data for X-rays yield values of $a_1 = 1.5 \times 10^{-5}$ Gy^{-1} and $a_2 = 1.0 \times 10^{-5}$ Gy^{-2}. For 15 MeV neutrons a value of $a_1 = 1.2 \times 10^{-4}$ Gy^{-1} and for 0.5 MeV neutrons a value of $a_1 = 3.0 \times 10^{-4}$ Gy^{-1} were obtained. The parameter a_1/a_2, which represents the dose at which the linear and quadratic terms contribute equally to the total frequency of transformation is only evaluable for X-rays and yields a value of 1.5 Gy.

The absolute values of the transformation frequencies are a factor of about 20 smaller than those obtained by other investigators who plated fewer cells per dish. In a few experiments we observed indeed an increase in the transformation frequency for lower cell numbers plated per dish. However, because the reproducibility was better with larger numbers of plated cells we have retained the original system.

With respect to the RBE values which can be deduced from our data, a relation is shown with the dose. At very small doses the RBE tends to a maximum value of about 20 for 0.5 MeV neutrons and a value of about 8 for 15 MeV neutrons. These values are somewhat larger than for cell killing, but further studies are required to establish significant differences.

With the newly developed WAG/Rij embryo cell line, experiments have been started on cell transformation by X-rays, but data for neutron irradiations require confirmation before an analysis can be made.

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

J. Zoetelief and G.W. Barendsen, 1982.
RBE of neutrons for induction of cell reproductive death and chromosome aberrations in three cell lines. In: Neutron Carcinogenesis, Eds. J.J. Broerse and G.B. Gerber, EUR 8084, Commission of the European Communities Luxembourg, 357.

G.W. Barendsen, 1982.
Quantitative relations between effective and sub-effective cellular lesions in radiation carcinogenesis. In: Neutron Carcinogenesis, Eds. J.J. Broerse and G.B. Gerber, EUR 8084, Commission of the European Communities Luxembourg, 407.

II. Short Communications, Theses, Internal Reports, Patents ...

J. Zoetelief, W.C. Kuijpers, A. Wittwer and G.W. Barendsen, 1982.
Comparison of dose-effect relationships for induction of cell reproductive death and chromosome aberrations by photons and neutrons of different energies. Int.J.Radiat.Biol., 42, 76.

Progress Report
1982

Contractor:

Association pour le Développement
de la Physique Atomique, ADPA
Route de Narbonne 118
F-31062 Toulouse-Cédex

Contract no.: BIO-A-295-81-F

Head(s) of research team(s):

Prof. D. Blanc
Centre de Physique Atomique
Université Paul Sabatier
Route de Narbonne 118
F-31062 Toulouse-Cédex

General subject of the contract:

Transport simulation of particles, and measurement of the ionization potential of some polar liquids approximating to biological media.

List of projects:

1. Transport simulation of particles : applications to dosimetry, microdosimetry, radioprotection and radiobiology.
2. Measurement of the ionization potential of some polar liquids approximating to biological media.

Title of project n°1

TRANSPORT SIMULATION OF ELECTRONS, PHOTONS AND HEAVY PARTICLES BY MONTE-CARLO METHODS.

Head of project and scientific staff :

J.P. PATAU, M. MALBERT, M. LHERMINE, A. PIQUEMAL, J.C. PERES.

Nous avons pratiquement terminé une étude sur le transport des électrons dans l'eau au dessous de 30 keV, dont le but était de déterminer l'influence des liaisons chimiques sur les distributions axiales et radiales de dépôts d'énergie.

Nous avons considéré dans un cas l'eau naturelle en phase liquide à l'état "moléculaire", dans l'autre le milieu ralentisseur est constitué par l'ensemble des atomes isolés de cette même molécule.

Outre les dépôts d'énergie cette étude compare les parcours moyens, la portée, les distances radiales atteintes par les faisceaux, les transferts d'énergie linéiques et les faisceaux rétrodiffusés.

Ce travail nous permettra d'évaluer les incertitudes sur les quantités physiques obtenues en simulant le transport des électrons de basses énergies dans des matériaux dont on ignore les sections efficaces moléculaires.

Nous avons mis au point un modèle de simulation de l'interaction inélastique hadron-nucléon entre 1 et 20 GeV. Cette simulation comprend deux phases :

1) la création de particules très excitées lors de la collision,
2) la désexcitation de ces particules, en cascade.

Nous avons utilisé le modèle thermodynamique statistique de HAGEDORN et un traitement cinématique relativiste. L'identité quantique des particules secondaires stables est fixée à l'aide du modèle statistique de FERMI.

Le code de simulation permet :

- de reproduire les corrélations cinématiques des particules excitées ou stables émises depuis la même particule excitée,

- de conserver l'histoire entière d'une désexcitation et de l'étudier en fonction du temps dans le contexte du noyau.

La simulation reproduit correctement les multiplicités et les corrélations cinématiques ; dans le cas des collisions "non diffractives", les quantités de mouvement longitudinales et transversales des particules secondaires sont également en bon accord avec les résultats expérimentaux.

Un modèle de simulation de l'interaction nucléaire inélastique des protons de 50 à 350 MeV tenant compte de l'existence de sous-structures - deutons, tritons, ions ^{3}He et alpha présentes dans les noyaux-a été élaboré. Cette première simulation, fondée sur le "modèle du spectateur" conduit à des résultats encore médiocres, que nous comptons améliorer par la simulation du phénomène de "pick-up".

List of publications in 1982

I - Publications.

"Contribution à la simulation de l'interaction inélastique hadron-nucléon entre 1 GeV et 20 GeV" ; A. PIQUEMAL, M. MALBERT, M. LHERMINE et J.P. PATAU ; 8 th Symposium on Microdosimetry, JULICH (R.F.A.), 27 Sept.-1° Oct. 1982.

"Etude dosimétrique de l'Iridium 192 par la méthode de Monte-Carlo" ; J.P. PATAU et C. CAZES, XXI° congrès de la Société des Physiciens des hôpitaux d'Expression Française, BORDEAUX, juin 1982.

II - Thèses.

DUPRE Corinne : "Contribution à l'étude de la réponse d'un scintillateur liquide organique irradié par des photons par la méthode de Monte-Carlo" ; Thèse de spécialité en physique radiologique, TOULOUSE, n° 2704, 25 octobre 1982.

PIQUEMAL Alain : "Contribution à la simulation par la méthode de Monte-Carlo de l'interaction inélastique hadro-nucléon entre 1 GeV et 20 GeV" ; Thèse de spécialité en physique radiologique, TOULOUSE, n° 2617, 19 mars 1982.

PERES Jean Claude : "Contribution à la simulation de l'interaction inélastique de protons de 50 à 350 MeV avec les noyaux par la méthode de Monte-Carlo" Thèse de spécialité en physique radiologique, TOULOUSE, n° 2711, 29 Novembre 1982.

Title of project n°2

MEASUREMENT OF THE IONIZATION POTENTIAL OF SOME POLAR LIQUIDS APPROXIMATING TO BIOLOGICAL MEDIA.

Head of project and scientific staff :

J. CASANOVAS, R. GROB, J.P. GUELFUCCI and R. LAOU SIO HOI.

Taking into account that our final objective is, as far as possible, to measure the ionization potential value of liquid water, we have in 1982 essentially attempted to improve the sensitiveness of the experimental set-up we have built for the determination of this value by an electrical way.

We have been able to decrease the amplitude of the signal to be measured down to 10^{-11} A and at the same time to increase the level of the background in which this signal has to be detected up to 10^{-3} A.

These values seem to us to be adapted for measurements on moderately polar liquids but they have still to be improved for water. This would be done at the beginning of 1983.

On the other hand the experimental set-up we have built for N_2O scavenging experiments has been tested. We have irradiated water containing about 10^{-4} M of N_2O with V.U.V. photons of several energies. The nitrogen originating from N_2O dissociation after electron capture has been detected with an Hewlett-Packard gas chromatograph type 5880 A.

At present these experiments did not still allow us to give a precise value for the photoionization energy threshold value of liquid water. However, we have observed that the minimum value for the lowering of the ionization potential of water in going from gas state of liquid state is about 3 eV. These experiments would be prosecuted in 1983.

Progress Report
1982

Contractor: **Contract no.:** BIO-A-296-81-F

Association pour le Développement
de la Physique Atomique, ADPA
Route de Narbonne 118
F-31062 Toulouse Cédex

Head(s) of research team(s):

Prof. D. Blanc
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Université Paul Sabatier
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Prof. J.L. Teyssier
Lab.des Radiations Ionis.
Université de Limoges
Rue A.Thomas 123
F-87060 Limoges Cédex

General subject of the contract:

Dosimetry of low doses by the electret and tracks effect. Application to the individual dosimetry of fast neutrons.

List of projects:

1. Dosimetry of low doses by the electret effect and tracks effect in polymers. Development of a read out system adapted to radiation protection.

Titled of project nr

Dosimetry of low doses by the electret and tracks effect. Application to the individual dosimetry of fast neutron.

Head of project and scientific staff :

Dr D. BLANC
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1 - Etude de la charge et de la décharge d'un électret

Nous avons essentiellement utilisé deux types d'électret : le polyéthylène basse densité (d ≃ 0,92) et le polypropylène (d ≃ 0,91) que nous avons chargé selon deux méthodes : la première correspond à la charge du matériau (polarisé sur sa face arrière positivement par exemple dans le cas d'une charge négative) en ions créés à l'aide d'un pistolet piézoélectrique ; la seconde est basée sur l'utilisation d'un microscope électronique dont l'ouverture du faisceau est commandée à l'aide d'un obturateur à iris et à commande mécanique réalisée avec un soufflet calorstat.

Les mesures des densités de charge des électrets sont effectuées par la méthode dite de compensation (annulation du potentiel électrique externe) jointe à l'utilisation d'une électrode vibrante.

Les charges maximales sont obtenues avec du polypropylène (P.P.) métallisé sur une face (arrière et polarisée à + 20 V) et soumis à un flux ionique produit par le pistolet piézolectrique:

une charge quasi permanente correspondant à un potentiel externe de - 200 V a été obtenue.

Actuellement, nous effectuons une étude systématique de la méthode de charge à partir du faisceau du microscope électronique, qui présente l'avantage d'indiquer la nature des charges (électrons) et leur énergie.

Les premiers résultats nous ont montré là encore que le P.P. métallisé sur une face présentait une charge plus élevée et plus stable que le P.P. non métallisé ou que le polyéthylène (métallisé ou non). La détermination des paramètres (énergie des électrons et temps d'exposition) correspondant à des caractéristiques de charge optimum sont actuellement en cours (1 s pour 40 keV vraisemblablement).

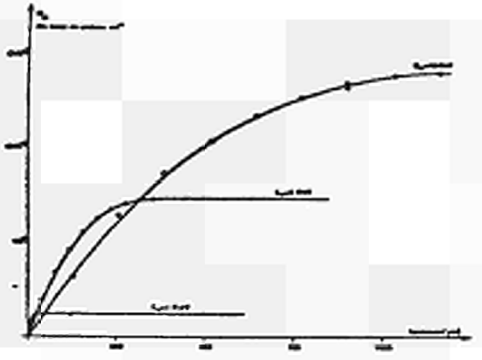
2 - Calcul de la sensibilité théorique aux neutrons rapides du dosimètre

Les distributions spectrales des protons émis par un radiateur de lyéthylène (rapport 1981) conduisent par intégration au nombre total N_H de protons émis. N_H a été étudié en fonction de l'angle d'émission, de l'épaisseur du radiateur, de l'énergie des neutrons. Il apparaît que :

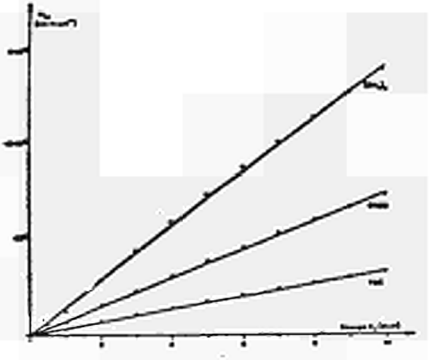
a) - les protons sont émis dans un angle inférieur à 60°
b) - un phénomène d'équilibre entre diffusion et absorption des protons au sein du radiateur existe au-delà d'une certaine profondeur (figure 1)
c) - N_H varie linéairement avec l'énergie des neutrons entre 1 et 10 MeV (figure 2).

Le nombre de traces de protons que peut enregistrer un détecteur solide visuel de traces placé derrière le radiateur dépend de ses performances : angle limite d'enregistrement, domaine d'énergie de sensibilité. L'intégration des spectres qui tient compte de ces limitations conduit à un nombre Φ_H de traces par cm^2. Nous en avons déduit un coefficient de rendement $\lambda = \Phi_H/\Phi_n$ si Φ_n est la fluence neutronique indicente. Si $U(E_n)$ est l'équivalent de dose déposée par fluence unité (ICRP rapport 21) la sensibilité aux neutrons est donnée par $S(E_n) = \lambda(E_n)/U(E_n)$.

Nos calculs nous ont montré que pour un dosimètre $(CH_2)_n$/CR 39, dans les conditions d'équilibre protonique, cette sensibilité évolue de $7\ 10^5$ traces cm^{-2} Sv^{-1} à $7\ 10^6$ traces cm^{-2} Sv^{-1} entre 1 et 10 MeV. Pour un dosimètre $(CH_2)_n$/CR 39 dans la même gamme d'énergie elle évolue entre $7\ 10^5$ et $3\ 10^5$ traces cm^{-2} Sv^{-1}. Le problème d'une réponse indépendante de l'énergie est à étudier.



- Figure 1 -



- Figure 2 -

List of publications in 1982

I - Publications in Scientific Journals, Monographs, Proceedings.

II - Short Communications, Theses, Internal Reports, Patents....

Rapport de stage D.E.A. - J.L. DUROUX
"Mesures électriques sur des polymères chargés". Septembre 1982.

Rapport interne - A. MOLITON, L. MAKOVICKA, B. GUILLE
"Les électrets" - Novembre 1982.

Progress Report
1982

Contractor:

REP-Institutes of the
Organization for Health
Research, TNO
P.O.Box 5815
NL-2280 HV Rijswijk

Contract no.: BIO-A-302-81-NL

Head(s) of research team(s):

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Radiobiological Institute
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NL-2280 HV Rijswijk

General subject of the contract:

Neutron dosimetry instrumentation for radiation protection and radiobiology.

List of projects:

1. Neutron dosimetry instrumentation for radiation protection and radiobiology.

Title of project nr. 1:

Neutron dosimetry instrumentation for radiation protection and radiobiology.

Head of project and scientific staff:
J.J. Broerse and J. Zoetelief.

The operation of tissue-equivalent (TE) ionization chambers under high gas pressures will increase the sensitivity and can provide information on the radiation quality. The reading of a chamber at a fixed value of the collecting potential increases with increasing pressure, but this increase is limited by ion recombination. With a thimble-type TE ionization chamber with a gas volume of about 1 cm3 measurements have been performed at pressures of up to 80 bar (8 MPa) of methane based muscle equivalent (TE) gas for ^{137}Cs gamma rays, 0,9 and 14,5 MeV neutrons (Zoetelief et al., 1981). The results previously obtained for TE gas at pressures of up to about 70 bar showed that the sensitivity of the ionization chamber operated at 600 V can be increased with reference to atmospheric pressure (1 bar) by factors of about 32, 15 and 6,3 for ^{137}Cs gamma rays, 14.5 and 0.9 MeV neutrons, respectively.

With the aim of obtaining a system with higher senstivity, the response of the high pressure TE ionization chamber has been studied as a function of pressure with gases of different composition for ^{137}Cs gamma rays. At a collecting potential of 600 V, the ionization chamber reading relative to the reading at 1 bar as a function of gas pressure is shown in the figure for various gases. As a function of gas pressure an increase in the relative reading is observed for all gases in the investigated pressure region. For pressures of up to about 10 bar (1 MPa) the increase in the sensitivity is approximately proportional to the pressure for all gases used.

For argon, initial recombination is small over the pressure region investigated. A supralinear relative reading as a function of gas pressure is observed for this gas. This might be expected on the basis of the Van der Waals' equation of state predicting that the number of argon atoms in the constant volume rises faster than the pressure. It is assumed that the ionization produced by secondary electrons (originating from the chamber

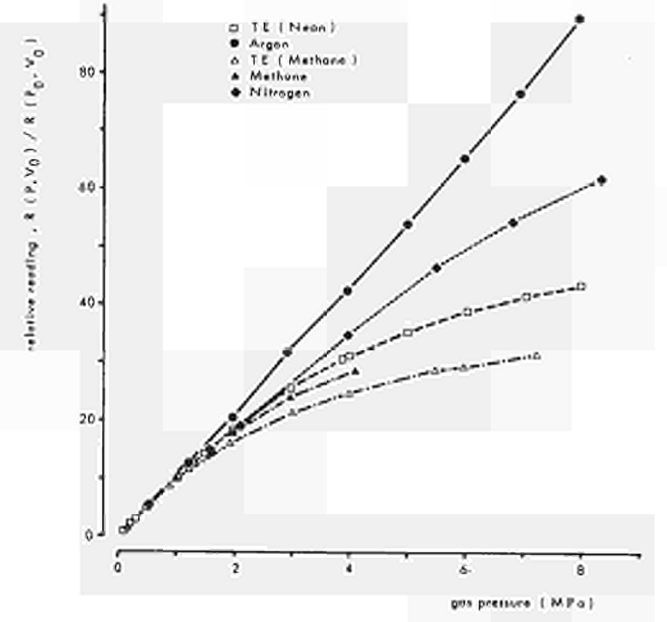
wall) in the gas is proportional to the number of atoms. For the other gases at higher pressures, deviations in the readings relative to those at 1 bar as a function of pressure occur due to initial recombination.

Argon is the most appropriate gas among the investigated ones for obtaining a sensitive dosimetry system for photons. For ^{137}Cs gamma rays, the ionization produced in the cavity is due to charged particles created in the wall. For lower energy neutrons, the contribution from charged particles created in the gas becomes predominant. For the latter situation argon is probably less suitable due to its small cross-section for neutron interactions.

Reference

Zoetelief, J., Engels, A.C., Bouts, C.J., Bennen, L.A. and Broerse, J.J. Response of tissue-equivalent ionization chambers as a function of gas pressure. In: Proc. Fourth Symp. on neutron dosimetry, EUR 7448, Commission of the European Communities, p. 315, 1981.

Figure: For various gases, pressure dependence of the reading of the TE ionization chamber relative to that at 0.1 MPa (1 bar) at a collecting potential of 600 V irradiated with ^{137}Cs gamma rays.



Progress Report
1982

Contractor:

United Kingdom Atomic
Energy Authority, UKAEA
Charles II Street 11
GB-London SW1Y 4QP

Contract no.: BIO-A-434-81-UK

Head(s) of research team(s):

Dr. A.C. Chamberlain
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AERE
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GB-Oxon OX11 ORA

Dr. J.W. LEAKE
Instr.& Appl.Physics Div.
AERE
Harwell, Didcot
GB-Oxon OX11 ORA

General subject of the contract:

Development of improved X-ray counters for assessment of plutonium in lungs.

List of projects:

1. Development of improved X-ray counters for assessment of plutonium in lungs.

Title of project nr 1.

Development of Improved X-ray Counters for Assessment of Plutonium in Lungs.

Head of project and scientific staff:

Dr A C Chamberlain
Dr J W Leake
Dr D Newton

The wiring of the prototype counter work was completed in the first half of the year; the frame contains 140 anode, 570 cathode and 165 drift wires. The counter envelope, with a temporary stainless steel window facility, was also ready by mid-year and the frame was put into the envelope and the whole was vacuum tested. The cathode strips (used to detect and hence reject electron events originating in the end MWA frame) are made of printed circuit board and the drift wire sub-assembly uses PTFE insulation: thus a long period of vacuum pumping was undertaken to reduce the effects of out-gassing from these materials. The counter was then filled with one atmosphere Argon-CO2 (98/2) and tests were carried out using a 22KeV source. This gas was chosen at this stage because it is cheaper than Xenon. The measurement with Ar/CO2 showed that the gain and resolution across the wires, ie from wire to wire, is not as uniform as had been hoped. The resolution varied between 10 and 13% between wires, while the gain varied by about plus or minus 1%. Measurements parallel to the wire direction gave resolution and gain variations of +/- 1% and +/- 2% respectively. Over a period of 20 days the resolution deteriorated from 10 to 11.9% while the gain changed by almost a factor of 2. The next stage was to pump out the counter, ie without exposing the inside to air, and then to refill it with a Xenon-CO2 (97/3) mixture. Measurements have been made using rise time and energy discrimination. These have proved very satisfactory but some shortcomings in the commercial electronic RTD system (rise time discrimination) have been observed. Preliminary results are shown in the table below:

Conditions	Count rate >0.5keV	Count rate in region 11-22KeV	Energy Channel 11-22KeV + RTD	With 5 Sided Guard
Lab natural bgd	21.32	3.1	0.38	0.12
^{60}Co at fixed distance to counter	2720	427	27.9	5.2
16 KeV Zr K X-rays	-	100% normalised	74.4%	69.4%

All count rates are counts/sec.

Progress Report

1982

BIO-A-307-81-UK

Contractor:

National Physical Laboratory
NPL
Teddington
GB-Middlesex TW11 OLW

Contract no.:

Head(s) of research team(s):

Dr. P. Christmas
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Teddington
GB-Middlesex TW11 OLW

General subject of the contract:

A study of the dosimetric applications of thermally stimulated exo-electron emission in beryllium oxide.

List of projects:

1. Thermally stimulated exo-electron emission (TSEE) dosimetry in beta-ray, X-ray and mixed neutron/gamma-ray fields.

Title of project nr BIO-A-307-81-UK.

The investigation of BeO exo-electron dosemeters.

Head of project and scientific staff: Dr M J Rossiter, Mr J L Makepeace.

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1. Equipment and procedure. A detailed description was given at the European Dosimetry Group meeting, Homburg, West Germany, in September 1981. The procedures have been modified only in the following respects. Firstly, the radiation facility housing cobalt-60 sources used for the routine calibration of BeO discs was dismantled prior to its relocation in another laboratory and was unavailable for the rest of the year. An alternative equipment using a Cs-137 source was then used for routine calibration, and the experimental value for the response of TSEE BeO in Cs-137 radiation relative to cobalt-60 is near 0.8, a low value for which we can offer no simple explanation. Secondly, whereas the BeO discs were each held individually in graphite cups for the majority of the irradiations, for the later measurements described here using tissue equivalent phantoms, it was found possible to remove the discs from the cups for the irradiations, to minimise discontinuities in the phantom as far as possible.

2. Fast neutron response of BeO dosemeters and the measurement of the gamma component of a collimated neutron field.

Details of the measurements of the fast neutron response of BeO dosemeters in graphite and teflon holders were given at the Homburg meeting.

The results were given as k_u values (near 2.5% in graphite, 4.2% in teflon irradiators) with an uncertainty of about $\pm$ 10% at the 95% confidence level. The graphite cup produced no significant anisotropy when teflon holders were used.

Using BeO discs in graphite holders and the above k_u values, measurements were made of the gamma component of the NPL collimated 14.7 MeV neutron beam at 65cm from the collimator. Gamma component values in the range 3% to 4% were measured but the uncertainty on these figures may be as high as $\pm$ 15% due to uncertainty in the k_u value and additional uncertainties arising from the photon energy dependence of the BeO response, and the presence of lower energy neutrons in the collimated beam. Furthermore, as geiger tube

and TLD measurements indicated gamma contributions near 6% to 7% it was clear that further experiments were required to establish the validity of the TSEE method. To date this has not been possible due to the utilisation of the collimated beam facility for other work.

3. Response to beta-radiations.

The response of BeO discs expressed in terms of absorbed dose to tissue relative to the response to cobalt-60 fell from a figure near 0.9 at 0.9 MeV mean beta energy (Sr/Y-90) to near 0.7 at 0.08 MeV (Pm-147) indicating that the dosemeters cannot be considered as 'infinitely thin'.

4. Photon-energy dependence (medium energy X-rays) and determination of depth doses.

This work has been conducted in collaboration with Mr M Fitzgerald, St Bartholomew's Hospital, London. The experiments described here may be viewed as a preliminary investigation of the use of TSEE BeO dosimetry in diagnostic X-ray work.

a) photon energy dependence. Between 25 and 110 keV the response of BeO dosemeters was constant at 60% ± 10% of their response to Co-60 radiation, in terms of absorbed dose in tissue.

b) BeO dosemeters (without graphite cups - see Section 1) have been used to determine depth dose curves for 90 and 120 kV X-ray beams in tissue equivalent phantoms with FSD 137 cm and field diameter 45 cm. Reasonable agreement was obtained with Grenz chamber measurements. The greater sensitivity of the BeO dosemeters allows their use at lower exposure levels than required by the Grenz chamber.

c) potential for bone-interface dosimetry. The ratio of response of BeO dosemeters under 7 mm bone-equivalent material relative to that under 7 mm muscle equivalent material was found to be (i) for 120 kV X-rays, 1.68, (ii) for 90 kV X-rays, 1.06. These numbers are uncorrected for attenuation in the bone-equivalent material, but in each case the ratios are much larger than those obtained with the Grenz ray chamber.

Progress Report
1982

Contractor:

National Physical Laboratory
NPL
Teddington
GB-Middlesex TW11 OLW

Contract no.: BIO-A-506-82-UK

Head(s) of research team(s):

Dr. P. CHRISTMAS
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and Acoustics, NPL
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GB-Middlesex TW11 OLW

General subject of the contract:

Investigation of low energy neutron sources in the energy range 5-100 keV using particle accelerators and their application to radiation protection dosimetry.

List of projects:

1. Investigation of low energy neutron sources in the energy range 5-100 keV using particle accelerators and their application to radiation protection dosimetry.

Title of project no. 1:
Investigation of low energy neutron sources in the energy range 5–100 keV using particle accelerators and their application to radiation protection dosimetry.

Head of project and scientific staff:

Dr J B HUNT

Introduction:

It has been demonstrated that in many working environments associated with the nuclear power programme, a significant part of the dose-equivalent to which personnel may be operationally exposed, is due to neutrons with energies below 100 keV. Many important operational neutron survey instruments are sensitive within this energy range, but with present techniques it is difficult to calibrate these devices due to the lack of suitable monoenergetic neutron sources.

It is the purpose of this project to investigate in detail the neutron-producing properties of (p,n) reactions with medium-weight nuclei, and in particular, the little-used $^{45}Sc(p,n)^{45}Ti$ reaction. The project is being carried out in co-operation with the Physikalish-Technische Bundesanstalt (P.T.B), making use of the facilities available at both laboratories. It is intended to intercompare the neutron fluence measuring systems at both laboratories and to calibrate typical neutron survey instruments and transfer devices over this neutron energy range.

Measurements of the $^{45}Sc(p,n)$ Excitation Curve:

Preliminary measurements have been made using the NPL long counters of the $^{45}Sc(p,n)$ neutron yield curves, as a function of the incident proton beam energy, at 0^{o} and at 60^{o} to the beam direction. These measurements were made using thin scandium metal targets, $\simeq 5$ $\mu g/cm^2$ thick, which is equivalent to approximately 0.3 keV energy loss for incident proton energies near the reaction threshold of 2.908 MeV.

An initial analysis of the data shows that there are a number of prominent resonances in the excitation curve that could be extremely useful for instrument calibration. However, these measurements have already

demonstrated that in order to make full use of these peaks it is essential to use targets with an equivalent thickness less than the energy width of the peak, and this means that an extremely stable accelerator is necessary. The measured excitation curve will be compared with time-of-flight neutron spectra measured at P.T.B.

A typical neutron survey instrument, the Harwell 0949/3, an updated version of the Harwell 0075, has been calibrated at about 8 keV and at about 27 keV and these measurements are at present being evaluated. This device will then be used as a transfer instrument in order to intercompare the fluence measuring systems at both laboratories.

Progress Report
1982

Contractor:
Commissariat à l'Energie
Atomique
CEN de Grenoble
B.P.n° 85 X
F-38041 Grenoble Cédex

Contract no.: BIO-A-293-81-F

Head(s) of research team(s):
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Service de Protection
CEA - CEN de Grenoble
B.P.n° 85 X
F-38041 Grenoble Cédex

General subject of the contract:

Design and construction of a dosimetry system for the determination of the quality factor and the dose equivalent in neutron-gamma mixed fields.

List of projects:

1. Design and construction of a dosimetry system for the determination of the quality factor and the dose equivalent in neutron-gamma mixed fields.

TITRE DU PROJET :

Etude et réalisation d'un système dosimétrique permettant la détermination du facteur de qualité et de l'équivalent de dose dans un champ mixte neutron-gamma.

Chef du projet et collaborateurs scientifiques

Mme HERBAUT
M.LEROUX
M.VIVIA

1 - INTRODUCTION

A partir des spectres microdosimétriques mesurés à l'aide d'un compteur proportionnel de ROSSI type FWT "Let 1/2" (/1/), il est possible d'obtenir la dose et le facteur de qualité dans un champ mixte neutron-gamma (/2/, /3/,/4/, /5/).

Le compteur type "Let 2"d'un diamètre géométrique intérieur 4 fois plus important,pourrait donc être utilisé comme capteur du futur appareil portable. Les caractéristiques de la maquette réalisée à partir d'une électronique à microprocesseur ont été définies et l'étude des performances de cette dernière devrait commencer au début de l'année 1983.

Parallèlement, avec la chaine spectrométrique existante, nous avons comparé deux capteurs Let 2 n° 152 et n° 1062.

2 - RESULTATS

Les caractéristiques principales de l'appareil portable sont les suivantes :
. Les signaux fournis par le préamplificateur de charge sont appliqués sur deux voies identiques,l'un d'un gain X1 pour les dépôts d'énergie importants, l'autre d'un gain X100 pour les faibles tailles d'évènement.

. Trois zones spectrales sont définies correspondant aux trois natures de particules secondaires suivantes : électrons, protons, ions lourds.

. La fréquence maximum admissible, quelle que soit la zone du spectre, est de 15 kHz : ceci correspond à une borne supérieure de l'ordre de 15 $rad.h^{-1}$.

. L'appareil possède deux modes de fonctionnement : étalonnage et mesure. Au bout d'un temps réglable les résultats sont imprimés en cGy ($cGy.h^{-1}$) et cSv ($cSv.h^{-1}$).

A l'aide de la chaine spectrométrique existante, deux détecteurs Let 2 ont été étudiés pour un diamètre simulé de 2 µm correspondant à une pression de remplissage de 26 mmHg, l'un (n°152) possède un gain constant sur une période de 1 mois (Fig 1) tandis que nous constatons pour l'autre une baisse voisine de 40 % de cette quantité pour une durée de l'ordre de 15 jours (Fig.2). Nous avons observé de plus pour ce détecteur l'influence relativement importante de la température (/6/).

La prise en compte de la composante photonique d'un champ mixte nécessite pour le capteur un bruit de fond aussi faible que possible. En effet cet inconvénient a des répercussions non négligeables sur la détermination de la contribution relative gamma d'un rayonnement neutron-gamma (Fig 3). Ces impulsions parasites, dues probablement à des microclaquages, sont d'autant plus importantes que la haute tension appliquée est plus élevée ou le diamètre simulé d_s plus petit : pour d_s = 3 µm et une haute tension de 840 V, elles correspondent respectivement à :

$3.6\ 10^{-3}$ rad.h^{-1} pour le détecteur n°152

$2.2\ 10^{-4}$ rad.h^{-1} pour le détecteur n°1062

Nous nous sommes attachés,à l'aide de ces détecteurs, à déterminer sous irradiation photonique pure au ^{60}Co dans des faisceaux caractérisés en exposition, les valeurs des paramètres midrodosimétriques $\bar{y}_F$, $\bar{y}_D$ pour plusieurs diamètres simulés (tableau 1) : nous pouvons constater qu'elles sont comparables à celles obtenues à l'aide du détecteur type "Let 1/2" (/7/).Ces mesures permettent de plus de calculer le diamètre géométrique de la cavité détectrice : une valeur moyenne de 5.70 cm a été obtenue en bon accord avec le constructeur.

3 - CONCLUSION

La détermination de la contribution photonique d'un champ mixte n-gamma et la stabilité en fonction du temps du gain du dosimètre sur des périodes longues nécessitent la réalisation de capteurs de grande fiabilité.

4 - REFERENCES BIBLIOGRAPHIQUES

/1/ FWT : Far West Technology :Inc Goleta (Californie)
/2/ LEROUX J.B,HERBAUT Y : EUR 7448 (293-304) 1981
/3/ LEROUX J.B,HERBAUT Y : EUR 7147 (1201-1209) 1980
/4/ BOOZ J,POLI A : EUR 7448 (237-261) 1981
/5/ HARTMAN,G,MENZEL H.G,SCHUMACHER H : EUR 7448 (235 - 236), 1981
/6/ LEROUX J.B, HERBAUT Y :8° Symposium on Microdosimetry (JULICH - SEPTEMBRE 1982) à paraître
/7/ LEROUX J.B - HERBAUT Y : Note d'étude SPR/GSTEC/LD N° 82-03 OCTOBRE 1982

5 - LISTE DES FIGURES ET TABLEAUX

Figure 1 : A = f(t) pour les détecteurs Let 2 N° 152 et n° 1054

Figure 2 : A = f(t) pour le détecteur Let 2 N° 1062. Influence de la température

Figure 3 : Comparaison des distributions ^{y}d (y) sous irradiation neutronique avec les compteurs Let 2 n° 152, n°1062 et Let 1/2

Tableau 1 : $\bar{y}_F$, $\bar{y}_D$ pour le détecteur type "Let 2" irradié au ^{60}Co

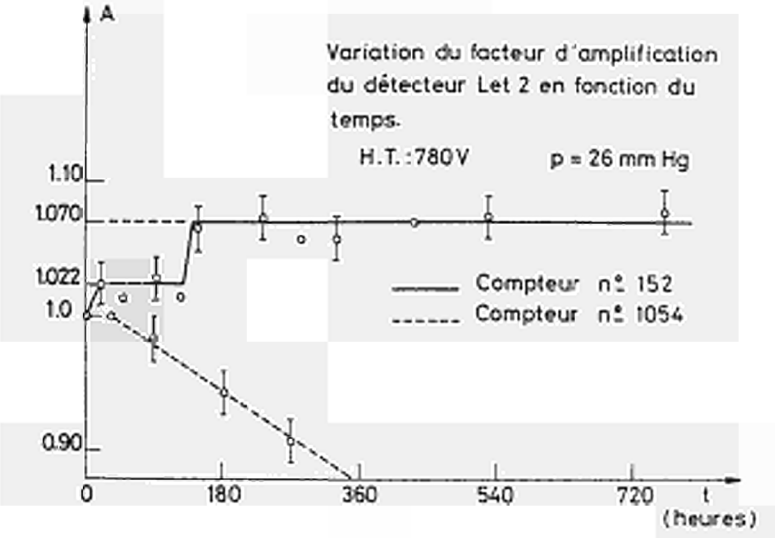
A
Variation du facteur d'amplification du détecteur Let 2 en fonction du temps.
H.T. : 780 V
p = 26 mm Hg
1.10
1.070
1.022
1.0
0.90
Compteur n° 152
Compteur n° 1054
0
180
360
540
720
t
(heures)

Fig. 1

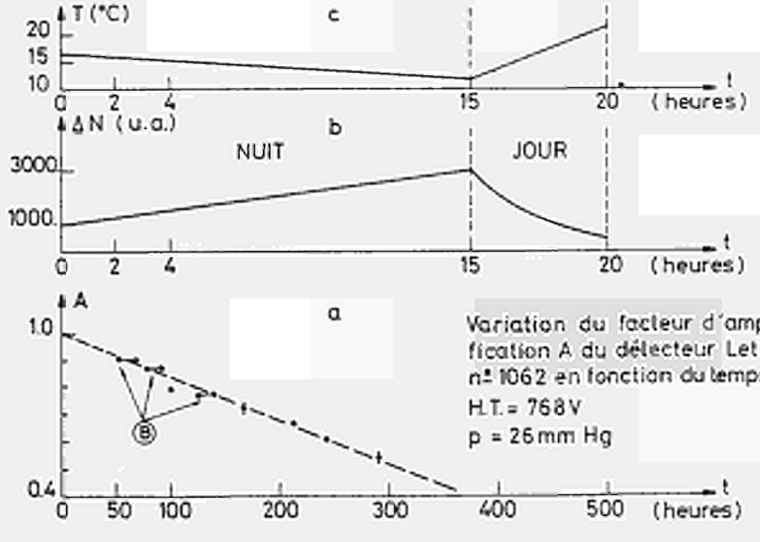
T (°C)
c
20
15
10
0
2
4
15
20
(heures)
ΔN (u.a.)
b
NUIT
JOUR
3000
1000
A
a
1.0
0.4
Variation du facteur d'amplification A du détecteur Let 2 n° 1062 en fonction du temps
H.T. = 768 V
p = 26 mm Hg
50
100
200
300
400
500

Fig. 2

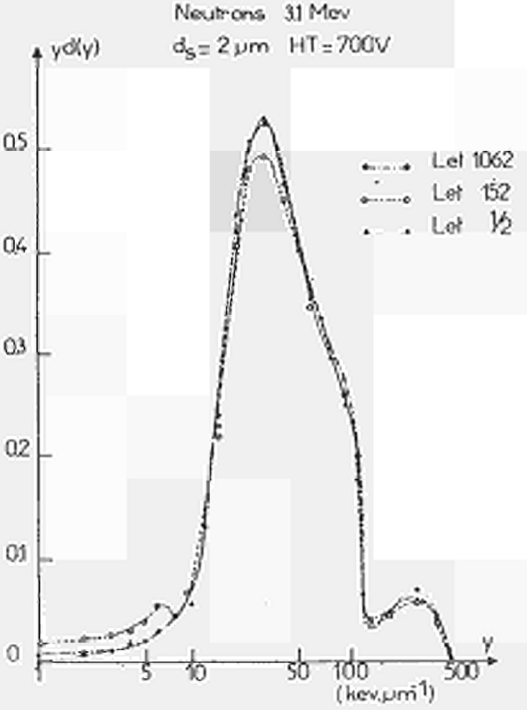

Fig. 3

ds (µm)	$\overline{y_F}$ (kev.µm^{-1})	$\overline{y_D}$ (kev.µm^{-1})	$\frac{\sigma^2}{\overline{y_F}^2}$ *
2	0.26	1.45	4.6
3	0.285	1.36	3.8
5	0.27	1.18	3.4
7	0.285	1.065	2.7

* $\frac{\sigma^2}{\overline{y_F}^2} = \frac{\overline{y_D}}{\overline{y_F}} - 1$

Paramètres microdosimétriques pour le détecteur type "Let2" irradié au CO60

Tableau I

LISTE DES PUBLICATIONS EN 1982

I -

LEROUX J.B, HERBAUT Y : Détermination du facteur de qualité dans les champs mixtes à 14.7 MeV et 3,1 MeV à l'aide du compteur Let 1/2.Premiers résultats obtenus avec le détecteur Let 2.

8° Symposium on Microdosimetry (Julich Septembre 1982). A paraître.

II -

LEROUX J.B, HERBAUT Y : Etudes microdosimétriques de rayonnements photoniques et neutroniques : Note d'Etude SPR/GSTEC/LD N° 82-03 OCTOBRE 1982.

Progress Report
1982

Contractor:
Kernforschungsanlage
Jülich GmbH., KFA
Postfach 1913
D-5170 Jülich 1

Contract no.: BIO-A-288-81-D

Head(s) of research team(s):
Prof.Dr. L.E. Feinendegen
Institut für Medizin
KFA Jülich GmbH.
Postfach 1913
D-5170 Jülich 1

General subject of the contract:

Biological effectiveness of incorporated radioactive nuclides and fast neutrons.

List of projects:

1. Biological effectiveness of incorporated radioactive nuclides other than bone seekers.
2. Assessment of the quality factor of neutrons and gamma-rays.
3. In vivo studies of biochemical reaction rates from low dose irradiation.

Title of project no 1:
Biological Effectiveness of Incorporated Radioactive Nuclides other than bone seekers.

Heads of project and scientific staff: J. Booz and G. Tisljar, B. Bauer, J. Humm, E. Pomplun, F.H.A. Schneeweiß, Th. Smit, D. Wermelskirchen

This year's work has concentrated on the microdosimetric and biological consequences of the Auger effect.

Using a Monte Carlo technique, photoelectric interactions in the phosphorus of the DNA have been simulated and the energy deposition due to Auger emission as well as the resulting multiple charge calculated. It appears that for sensitive volumes $\geq$ 3nm a biologically significant energy deposition occurs at all photon energies (see figure 1). However, the photoeffect in the phosphorus of DNA plays only a minor role in comparison to the interactions with other atoms (in particular oxygen) at energies above 537 eV. On the other hand, interactions with DNA bound phosphorus are important with respect to local energy deposition and to the mean dose in DNA for photon energies between the L-absorption-edge of P (131 eV) and 537 eV.

Further calculations have been performed on molecularly bound iodine studying the problem of neutralization of the multiple charge induced by Auger cascades. Several energy-transfer processes were distinguished and in a firstattempt a quantitative evaluation of their contributions to the total energy deposition estimated. These investigations led to a new term in the expression for the mass-energy-transfer-coefficient and to a new relation between kerma and dose.

Studies of the effects of low numbers of ^{3}H-decays accumulated in DNA of cultured human T1-cells were continued with ^{3}H-IUdR. Effects were compared with those of low doses of γ-rays (5-25 Gy). Induction of DNA strand breaks under aerobic and hypoxic conditions and measured by the DNA unwinding method served as the biological endpoint. SSB and DSB were determined individually by evaluation of their repair kinetics. For the oxygen enhancement ratio of total breaks, induced by ^{137}Cs γ-rays, a value of 3.1 ± 1.1 was found. It appears low which presumably is due to the low doses administered. SSB induced in the absence of oxygen could not be detected. This may be due to fast repair of SSB induced in nitrogen atmosphere.

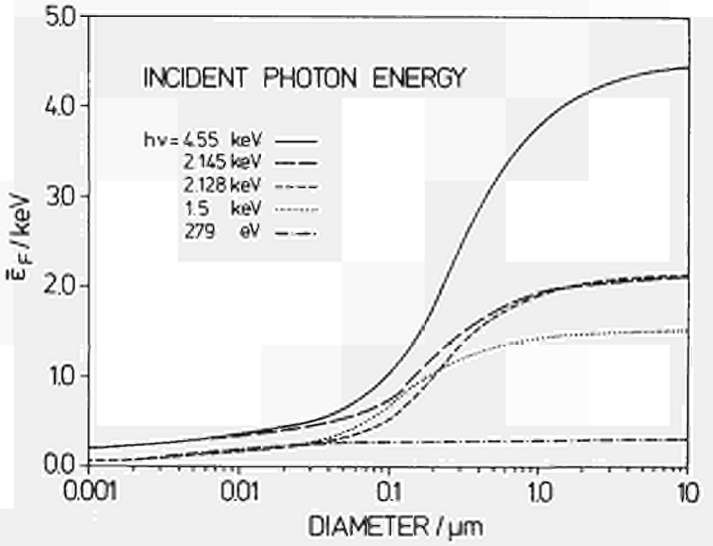

Fig. 1: Interaction of photons with phosphorus in DNA. Mean energy imparted, $\bar{\varepsilon}_F$, as a function of the size of the sensitive volume for different photon energies. For volume sizes $>$ 5µm, $\bar{\varepsilon}_F$ reaches approximately the incident photon energy i.e., nearly all emitted electrons will be absorbed. For volume sizes $\leq$ 10 nm the energy imparted mainly results from charge effect of the Auger cascade in phosphorus.

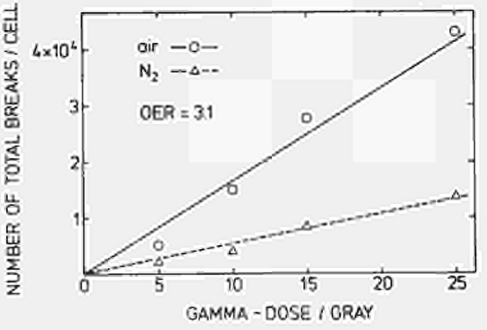

Fig. 2: OER of total breaks of DNA of cultured human T1-cells induced by low doses of ^{137}Cs γ-rays. A ratio of 3.1 was obtained from the slopes of the regression lines.

Title of project no 2:
Assessment of the Quality Factor of Neutrons and Gamma Rays

Head of project and scientific staff: J. Booz, L.E. Feinendegen, H. Neuhauß, E. Pomplun, Th. Smit

The theoretical and experimental investigations on the development of a radiation-quality and absorbed-dose monitor based on a microdosimetric counter have been continued.

Distributions d(y) from different types of mixed neutron-gamma radiation fields have been measured, collected and processed. Various methods of evaluating $\bar{Q}$ on the basis of microdosimetric counters have been tested. Finally the simple and sufficiently accurate Three Sections Method has been adopted for the monitor in development. Further, 3 different methods of accounting for overkill at y-values $>$ 175 keV/µm have been tested: a) no overkill, b) biological overkill, and c) overkill corresponding to a constant Q-value of 20. The latter method was adopted and with this correction the dose mean of d(y) turned out to be nearly independent of the simulated diameter between 0.5 and 10 µm. To obtain sufficient sensitivity, the counter was decided to have a size of 20 - 30 cm simulating 10 µm diameter. Since the helix as used in spherical counters presented principal problems resulting in limited resolution, cylindrical geometry was chosen with the height equal to the diameter.

From the electronic point of view, the availability of a logarithmic preamplifier is a must for the development of a portable monitor. The development of such a preamplifier has been initiated in collaboration with the Zentralinstitut für Elektronik, KFA Jülich. The first prototype has been built which is linear in its response function for the first decade and logarithmic for the others. The results are encouraging.

A test counter has been constructed with the first stage of the logarithmic preamplifier built in into the counter housing. Counter and preamplifier are being tested with different types of radiations.

Titel of project no 3: In Vivo Studies of Biochemical Reaction Rates from Low Dose Irradiation

Head of project and scientific staff: W. Porschen, H. Mühlensiepen, J. Marx, C. Lindberg, L.E. Feinendegen

It is the objective of this project to investigate the phenomenon of suppression of IUdR incorporation in cells after irradiation of mice with low and very low doses (0.5 to 10 rad) with the aim of developing an ultra-sensitive biological dosimeter.

In former years the effect itself has been carefully investigated and an assay system has been developed which allows for an observation of the effect at very low doses: At different times after irradiation, bone marrow cells are obtained and incubated in-vitro with tritiated thymidine for 10 minutes. Then the cells are centrifuged, washed and measured for the activity bound to cellular DNA. A pH-value of 7.4 and bicarbonate are required for observing the radiation induced depression of tracer uptake. The results showed that the depression of thymidine-uptake into the bone marrow cells after irradiation increased to a maximum at 4 hours and then disappeared to reach control within about 15 hours. A second assay system has then been developed in which blood serum of irradiated mice is added to the culture medium of unirradiated cells. These cells show the same depression of tracer uptake as described above.

Several test measurements brought us to the hypothesis that the inhibition of uptake was due to an increase of thymidine in the serum. In the last year this hypothesis has been proven right: Total body exposure of mice to gamma- or fast neutron-irradiation of 1 to 10 rad and even below 1 rad also produced the temporary appearance of thymidine in the circulating blood serum to a maximum at 4-5 hours and a return to normal values (4×10^{-5} molar) within about 15 hours. The time courses of the elevation of thymidine in the blood serum and of the reduction of tracer uptake into cellular DNA are concordant (see Fig. 1). Also the dependence on absorbed dose are similar for both effects. Further, depression of thymidine uptake was shown to be linked to an inhibition of thymidine kinase. With the exception of some degree of effect for deoxyuridine, no effect was observed for other nucleic acid precursors, nor for glucose or fatty acids.

Therefore it is concluded that the elevation of thymidine concentration in blood serum after irradiation at very low dose levels is due to a diminished rate of removal of thymidine from the reutilization pathway.

The target volume for the observed effect was calculated to be the entire cell. Thus single or very few radiation absorption events may trigger an intracellular response leading to a temporary inhibition of thymidine uptake in the hit cell.

The data indicate that the sensitive response of cells to single radiation absorption events may be a physiological answer of hit cells to background radiation and may play a role in the expression of effect from chronic low dose-rate exposure. Employment of this ultra-sensitive assay system was initiated investigating blood serum from healthy volonteers with the aim of utilizing this phenomenon for biological dosimetry.

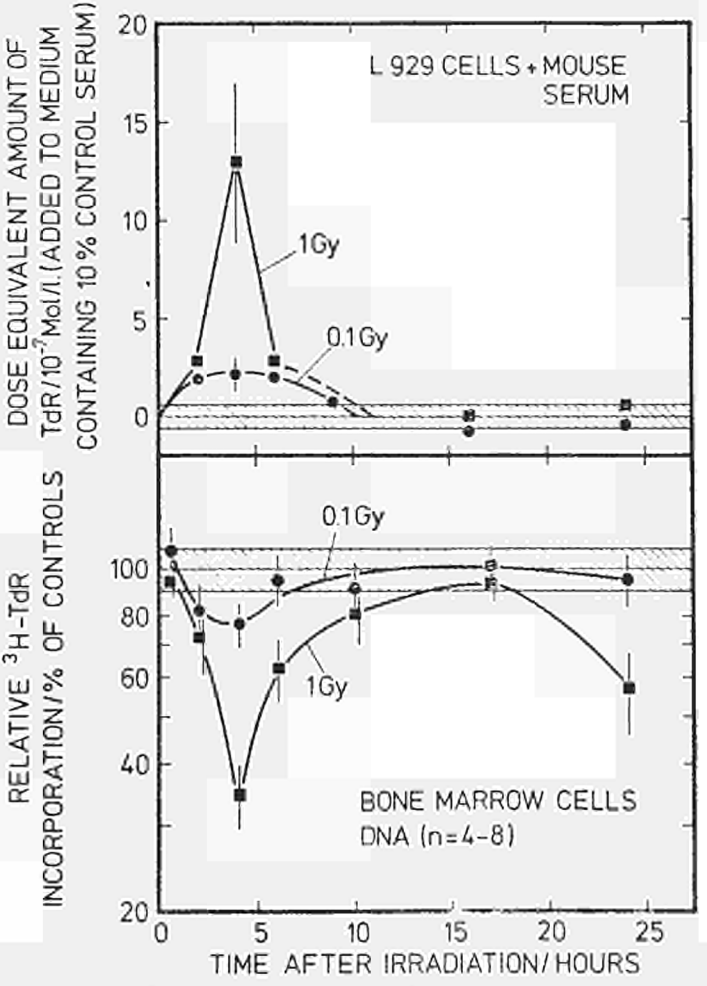

Fig. 1: Comparison of two radiation induced effects due to temporary disturbances of the thymidine reutilization pathway:
a) Elevation of thymidine in the circulating blood serum expressed in terms of 10^{-7} mol/l (top)
b) Incorporation of ^{3}H-TdR in bone marrow cells relative to controls (bottom)

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

Bauer, B.W. and Booz, J.:
Primary biological effects of ionizing radiation and their consequences: a theoretical approach.
Proc. 8th Symp. Microdosimetry, in press

Booz, J., Humm, J., Charlton, D.E., Pomplun, E., and Feinendegen, L.E.:
Microdosimetry of the Auger effect: the biological significance of Auger-electron cascades of phosphorus after low-energy photon interaction with DNA.
Proc. 8th Symp. Microdosimetry, in press

Charlton, D.E., Booz, J. and Pomplun, E.:
X-ray induction of the Auger effect: quantification of the different energy transfer mechanisms and dosimetric implications.
Proc. 8th Symp. Microdosimetry, in press

Feinendegen, L.E., Mühlensiepen, H., Porschen, W., Booz, J.:
Int. J. Radiat. Biol. 41 (1982) 139

Humm, J., Pomplun, E., Booz, J., Charlton, D.E.:
Energy and number distributions of electrons and photons emitted after photoelectric interactions of X-rays with phosphorus in DNA.
Proc. 8th Symp. Microdosimetry, in press

Tisljar-Lentulis, G., Henneberg, P., Feinendegen, L.E.:
The oxygen enhancement ratio for single- and double-strand breaks induced by tritium incorporated in DNA of cultured human T1-cells. Impact of the transmutation effect.
Radiation Research, in press

II. Short Communications, Theses, Internal Reports, Patents...

Booz, J., Braby, L., Coyne, J., Kliauga, P., Lindborg, L., Menzel, H.G. and Parmentier, N.:
Microdosimetry, Report of the Committee on Microdosimetry to the ICRU.
KFA Jülich, January 1982

Progress Report
1982

Contractor:
Gesellschaft für Strahlen- und Umweltforschung mbH.
GSF
Ingolstädter Landstr.1
D-8042 Neuherberg

Contract no.: BIO-A-287-81-D

Head(s) of research team(s):

Prof.Dr. W.Jacobi
Institut für Strahlenschutz
GSF
Ingolstädter Landstr.1
D-8042 Neuherberg

Dr. G. Burger
Institut für Strahlenschutz
GSF
Ingolstädter Landstr.1
D-8042 Neuherberg

General subject of the contract:

Determination of effective organ doses.

List of projects:

1. Determination of effective organ doses.

Title of project nr 1.
Determination of effective organ doses.

Head of project and scientific staff:

Dr.Burger	Dr.Kollerbauer
Dr.Drexler	Dr.Leuthold
Dr.Combecher	Dipl.Phys. Morhart
Dr.Schraube	Dipl.Phys. Wittmann

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1. Physical aspects of radiation effectiveness

There is sufficient indication now, that the target sizes of interest for some cellular endpoints are in the nanometer region, which can be best investigated by using low energy electrons. We have therefore further analysed single tracks of 1oo,2oo,5oo and 1ooo eV electrons in water. For each energy 1oo independent tracks were generated by means of the existing and continuously improved Monte Carlo codes. The spatial pattern of interaction events was fed piecewise into an arrayprocessor at a given local resolution represented by the pixeldistance in twodimensional planes of the array. An analysis of the pattern topology is then possible by means of mathematical morphology, as used in quantitative image analysis. It can be easily understood thatfor example a "blow" - operation applied upon each single volume element occupied by an event will deliver immediately Lea's associate volume, including the overlapping regions.The frequency distributions of associate volumes as function of size of the sampling element reveal the existence of event clusters and their amalgamation. The overlapping components of the ass.vol. deliver directly number distributions of events or even the corresponding energy distributions as being sampled randomly by the chosen target bodies.The method opens a new field of track structure analysis for the improvement of models of quantitative radiobiology and radiation risk analysis. /1/

The experimental investigations were restricted to the measurement of energy spectra and angular distributions of secondary electrons around collimated beams of 1.7 MeV/amu He-3 ions and 180 keV/amu deuterons in water vapour. Both ion beams have the same LET, but different electron starting spectra and hence radial energy deposition profiles. The results

were intercompared with numerical data from Monte Carlo calculations. To be able to perform such calculations for the mentioned ion beams the cross section library had to be extended. Measurements and calculations showed good agreement in a wide energy range. The intercomparison revealed however also some experimental problems measuring high electron energies, which have to be overcome in the future./2,3,4/

2.Organ and phantom models improvement and calculations of effective doses.

The electron transport was not treated in 1982. Instead of this increased emphasis was layed upon the final development of the sex specific phantoms ADAM and EVA. The design is based upon average organ masses, as given by ICRP 23, and the geometrical layout following the MIRD concept. From ADAM EVA was derived by first shrinking all organ and body sizes linearly down to a total body mass ratio of o.83. Than the ovaries, uterus and the breasts were added in a suitable manner. Finally a chin was intoduced to both phantoms to obtain a realistic thyroid geometry for external radiation, and eye lenses and skin were defined. The phantoms are described in/5/, and used extensively for Monte Carlo calculations of organ doses and the effective dose equivalent in case of external gamma and X-ray radiation./6/.

For the investigation of neutron organ doses the Monte Carlo calculations of the anthropoid phantom by means of the SAM-CE code could not be continued.Instead of this the versatile CHORD programme was improved.The new version is now applicable to the total phantoms at all exposure geometries, including the interesting isotropic situation.To get reasonable results for small organs, as testes, ovaries, thyroids and eyelenses an adjoint computation mode was developed. Finally revised depth doses as calculated in cylindrical phantoms by means of SAM-CE were introduced . It could be shown, that the depth doses used up to now in radiation protection from the Oak Ridge group (NCRP 38) have to be slightly revised. The new CHORD version was used to derive organ and effective dose equivalents assuming several exposure geometries for a series of compiled neutronspectra as occurring in realistic reactor fields and behind shields of several other neutron sources. It could be demonstrated, that conventional REM-counters under normal calibration and measurement conditions considerably overestimate the effective dose equivalent./7/

Publications:

/1/ Leuthold, G.,K.Rodenacker and G.Burger
Charged Particle Track Analysis by Pattern Recognition
8th Symposium on Microdosimetry, Jülich, 27.9.-1.10.82

/2/ Combecher,D.,G.Leuthold,J.Kollerbauerand G.Burger
Measurement of electron spectra in the track of various ions in water vapour.
8th Symposium on Microdosimetry, Jülich,27.9.-1.10.82

/6/ Kramer,R.and G.Drexler
On the Calculation of the Effective Dose Equivalent
Rad.Protection Dosimetry, Vol.3, No.1/2, pp.13-24,1982

Internal reports etc.:

/3/ Leuthold,G.
Messung mehrfach differentieller Elektronenverteilungen in einem Elektronenstrahl in Stickstoff. Thesis TU München, GSF-S 862.

/4/ Kollerbaur, J.
Sekundärelektronenspektren in der Spur schneller Ionen in Wasserdampf.Thesis TU München,GSF-S 859.

/5/ Kramer,R.,M.Zankl, G.Williams and G.Drexler
The Calculation of Dose from External Photon Exposures Using Reference Human Phantoms and Monte Carlo Methods.
Part I: The Male (ADAM) and Female(EVA) Adult Phantoms.
GSF-Report S-883, 1982

/7/ (Publication): Burger,G.and G.Wittmann
Organ Doses and Risks from Neutron Exposure
Neutron Carcinogenesis (J.J.Broerse,G.B.Gerber eds.)
Proceedings of a Seminar, EUR 8084, pp.255-274,1982

Progress Report
1982

Contractor:

Physikalisch-Technische
Bundesanstalt, PTB
Bundesallee 100
D-3300 Braunschweig

Contract no.: BIO-A-284-80-D

Head(s) of research team(s):

Prof.Dr. R. Jahr
Neutronendosimetrie
PTB
Bundesallee 100
D-3300 Braunschweig

Prof.Dr. H. Reich
Phot.& Elektronendosimetrie
PTB
Bundesallee 100
D-3300 Braunschweig

General subject of the contract:

Investigation of different dose equivalent quantities in phantoms externally irradiated with photons, electrons and neutrons.

List of projects:

1. Experimental determination of dose equivalent quantities in phantoms for photon and electron radiation.
2. Determination of different dose equivalent quantities for external exposure with neutrons.

Title of Project nr. 1

Determination of dose equivalent quantities in phantoms for photon and electron radiation.

Head of project and scientific staff:

Prof. Dr. H. Reich
Photonen- und Elektronendosimetrie
Physikalisch-Technische Bundesanstalt
Bundesallee 100, D-3300 Braunschweig

The present discussion concerning the definitions of the dose equivalent quantities defined in a 30 cm diameter tissue equivalent sphere which are finally to be used must be based on a knowledge of the characteristics of various feasible quantities. The problem is how these properties can be matched possibly by existing instrumentation or by (small) modifications thereoff. Investigations with the emphasis on angular dependence of selected quantities were carried out. It proved, that the angular response of commercial dosimeters is quite similar to that of the depth dose equivalent at a specified point in the sphere. That means that these instruments with non-isotropic response can be used for the determination of this quantity without the risk of its underestimation provided they are positioned so as to "face" the radiation source. The angular behaviour of other quantities proposed so far is not always in accordance with that of existing instrumentation. [1]

Similar considerations as to the angular dependence apply to the energy dependence of response with respect to sphere quantities. The only possibility to measure directly the true energy dependence of response of an instrument is to use monoenergetic radiation. As this is often not available or alternatively its dose rate may be too low, a method has been developed allowing the use of a spectrum of finite width for the determination of this intrinsic characteristic of the instrument. Up to now the result of such a measurement was generally

considered as being the true energy dependence of response, which, of course, it is not. Therefore a method was devised which is essentially a computer based decompositioning of the contributions from instrument properties and of those properties associated with the shape of the spectrum employed. This method was applied to a few selected instruments with the result that structures in the energy dependence became more pronounced. As the energy dependence of response of unmodified detectors is often similar to that of conversion factors such instruments are equally well suited to meet the requirements given with respect to sphere quantities and with respect to receptor free quantities. [2]

As the conversion factors from receptor free quantities like e. g. exposure to sphere quantities may depend strongly on photon energy, information on the spectral distribution of the radiation will often be required. Therefore an improved method to derive approximate X-ray spectral distributions from attenuation data has been developed which can be applied, if either time or facilities are not available for obtaining highly resolved spectra. It is well known that the tube voltage and the first and possibly second half value layer in a certain material characterize the spectrum only to a limited extent. In this situation a method is required which yields more precise information on the spectrum and which at the same time is simple enough to be applicable in a large number of laboratories. In an attempt to provide such a method measurements of attenuation curves in a range up to about the seventh half value layer combined with a computer evaluation have been examined with respect to their potentials. With such a procedure spectral information can be obtained which in contrast to quoting half value layers is unambiguous and of sufficient accuracy. The investigations represent also a first step in furnishing experimental data on photon spectra inside a phantom, a type of information which is currently only available from Monte Carlo calculations. [3, 4]

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

[1] B. Großwendt and K. Hohlfeld: Angular Dependence of Specified Depth Dose Equivalent Quantities in the ICRU Sphere for Photon Radiation. Rad. Prot. Dosimetry, in press.

[3] H. M. Kramer and H. von Seggern: The Determination of X-ray Spectra from Attenuation Data: Part I: The Potentials of Various Methods. Nucl. Instr. Meth., to be published.

[4] H. M. Kramer: The Determination of X-ray Spectra from Attenuation Data: Part II: Experimental Results, to be published.

II. Short Communications, Theses, Internal Reports, Patents ...

[2] H.-J. Selbach: Untersuchungen zur Energieabhängigkeit des Ansprechvermögens von Strahlenschutzdosimetern bezüglich der Meßgröße Tiefenäquivalentdosis. Report of Laboratory 6.41 in the Physikalisch-Technische Bundesanstalt, Braunschweig, January 1983.

Title of project nr 2: Determination of different dose equivalent quantities for external exposure with neutrons

Head of project and scientific staff : R. Jahr;
R. Böttger, H.J. Brede, G. Dietze, S. Guldbakke,
R. Hollnagel, H. Klein, H. Schölermann, B. Siebert

Theoretical concept for determining dose equivalents using individual monitors.

In radiation protection surveillance, organ doses, whole body doses or other suitable dose equivalent quantities are usually determined by means of individual monitors fixed on the exposed individual's trunk. The monitor readings generally depend on the exposure conditions such as the directional distribution and the energy spectrum of the radiation field. They may vary as a function of time, or as a function of uncontrolled, arbitrary movements of the exposed individual within the radiation field. A concept has been developed which allows the evaluation of monitor readings independently of these exposure conditions in terms of the desired dose equivalent quantities. In two publications /1, 2/, the design aim for the construction of an individual monitoring system is formulated as a mathematical relation. A distinction is made between "fitted" dosemeters satisfying this relation, and "non-fitted" dosemeters which do not. The calibration of both types is discussed in detail. For non-fitted dosemeters, procedures to determine the error limits are given. The application to accident dosimetry is briefly discussed.

Dose calculations and measurements

In publication /3/ it is shown that the mean quality factor for neutrons in tissue can be calculated in a good approximation directly from initial energy spectra of the secondary charged particles. Tables are given which make it possible to compute quality factors for monoenergetic neutrons from 11 meV to 20 MeV in any tissue-simulating material composed of H, C, N and O. The calculations are based on water as slowing down medium. This approximation is shown to be correct within 5 % at all energies considered.

In collaboration with the Institut für Biophysik der Universität des Saarlandes /4/, a consistency check was performed between kerma values in tissue-equivalent plastics (A 150) determined by means of microdosimetric methods, by means of an A 150 ionization chamber and by means of neutron fluence measurements in connection with calculated kerma factors. The inconsistencies of up to about 17 % observed between these three methods are discussed /4/.

Neutron cross section data

Uncertainties of measured neutron cross section data limit the accuracy of computed fluence distributions in phantoms and derived quantities such as absorbed dose (kerma) or dose equivalent (quality factor). This is clearly illustrated by the fact that the kerma factors for C-12 calculated at 14 MeV by different authors differ by as much as a factor 1.65 (M.A. Behrooz, et al., Phys. Med. Biol. 26 (1981) 507). Therefore, publication /5/ dealing with differential cross sections of the C-12(n,α)Be-9 reaction for neutrons between 8 and 10 MeV, and publication /6/ dealing with the differential cross section of the C-12(n,n)C-12 and the C-12(n,n')C-12* reaction at 10 MeV contribute to a reduction of such inconsistencies.

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings

/1/ B.R.L. Siebert, R. Hollnagel and R. Jahr: A Theoretical Concept for Measuring Doses from External Radiation Sources in Radiation Protection (accepted by Phys. Med. Biol.)

/2/ B.R.L. Siebert, R. Hollnagel and R. Jahr: Bestimmung von Teil- oder Ganzkörperdosen mit Personendosimetersystemen. 16. Jahrestagung des Fachverbandes für Strahlenschutz, München, 1982 (to be published)

/3/ B.R.L. Siebert, R.S. Caswell and J.J. Coyne: Calculations of Quality Factors for Fast Neutrons in Materials composed of H, C, N and O. 8th Symp. on Microdosimetry, Jülich, 1982 (to be published)

/4/ G. Dietze, S. Guldbakke, H.G. Menzel, H. Schuhmacher, G. Bühler: Correlated Microdosimetric, Dosimetric, Spectroscopic and Fluence Measurements with Monoenergetic Neutrons between 14 and 19 MeV. ibid. (to be published)

/5/ G. Dietze, H.J. Brede, H. Klein and H. Schölermann: Differential Cross Sections of the C-12(n,α)Be-9 Reaction in the Energy Range from 8 - 10 MeV. Internat. Conf. on Nuclear Data for Science a. Technology, Antwerp, 1982 (to be published)

/6/ H. Klein, B.R.L. Siebert, R. Böttger, H.J. Brede and H. Schölermann: Sample Size Corrections of Neutron Scattering Data and the Analysis of Angular Distributions. ibid. (to be published)

Progress Report
1982

Contractor:
Universität Würzburg
Sanderring 2
D-8700 Würzburg

Contract no.: BIO-A-286-81-D

Head(s) of research team(s):
Prof.Dr. A.M. Kellerer
Inst.für Med.Strahlenkunde
Univ. Würzburg
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General subject of the contract:

Microdosimetric studies and risk assessment for late somatic effects.

List of projects:

1. Studies in microdosimetry for application to radiation protection.
2. Biophysical and epidemiological studies of neoplasms in the Japanese survivors of the atomic bomb explosions.

Vertragspartner der Kommission:	Freistaat Bayern,vertreten durch die Julius-Maximilians-Universität Würzburg, Institut für Medizinische Strahlenkunde
Nr.des Vertrags:	BIO-286-81 D
Leiter der Forschungsgruppe:	Prof.Dr.Albrecht M.Kellerer

Allgemeines Thema des Vertrags:

Microdosimetric Studies and Risk Assessment for Late Somatic Effects

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This research program is directed towards the elucidation of the biophysical basis of the biological effectiveness of different types of ionizing radiations and at the application of these considerations to epidemiological studies of radiation carcinogenesis.

The development of Project 1 has been such that its objectives have been somewhat broadened. The computations of microdosimetric functions and parameters have been continued, but the linkage of microdosimetric problems to similar problems encountered in other fields of dosimetry, and in particular its connections to geometric probability, have taken added importance. Another new aspect has been the development - although at this point not yet the technical implementation - of a new method that permits the determination of microdosimetric variables in high intensity pulsed fields as they occur, for example, in the vicinity of accelerators.

The further execution of Project 2 will largely depend on the reconstruction of the dosimetry for Hiroshima and Nagasaki. As it appears now, the reassessed personal dose estimates will probably not be available before 1984; in the mean time the emphasis of the project has therefore been on the development of the suitable theoretical tools that can be utilized when the new data from Japan are available. Particular attention has been on the work on isotonic regression, and on the development of other non-linear optimization routines also in terms of maximum likelihood. Tentative studies with the new dosimetry have also been performed, but the intent was mainly a methodological one, rather than the wish to obtain final data now.

Results of Project No.1

Head of Project and Scientific Staff: Prof.Dr.A.M.Kellerer, Dr.F.Zilker,
Dr.H.-W.Thomas, Dr.H.Roos,
Dr.T.Fekete, Dipl.Biol.H.Wulf

Project Title

Studies in Microdosimetry for Application to Radiation Protection

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An important objective of the project has remained the computation of microdosimetric parameters and distributions for various radiations. For heavy ions we have performed further computations of such functions on the basis of an approximation that disregards straggling. For very small diameters and for sparsely ionizing radiations we have written our own program for the Monte-Carlo simulation of particle tracks, and have then made this program coherent with a similar program written by Dr.Brenner (Los Alamos) and Dr.Zaider (Columbia University).

The problem of the intersection of geometric objects with random structures, such as charged particle tracks, has been investigated further. In earlier work it had been shown that the proximity functions of the particle tracks and of the geometrical sites can be utilized to compute the important weighted mean event sizes for energy imparted, specific energy, or lineal energy. More general considerations of geometric probability have now shown that this is a particular aspect of far more general theorems. Such theorems permit the computation of the unweighted and the weighted mean overlap of randomly placed geometric objects. The unweighted mean values depend only on the fundamental parameters of the geometric ojects, such as volume and surface. The weighted mean values depend on the proximity functions and they determine the variance of the overlap for unweighted randomness.

In a more general approach a fundamental relation has been found for the average Minkowski functionals (measures of a geometric body) of the union of random sets that form a Poisson process in n-dimension space. This theorem can be based on the kinematic Fundamental Formula of W.Blaschke. Although it is of somewhat abstract nature it has various implications, relevant to microdosimetry, dosimetry, or other fields such as image

analysis. Among the results one may mention a very broad generalization of the so-called Cauchy theorem for the mean intersection of two geometric objects, one can also refer to the result that has been obtained for the mean number of clumps formed when figures are randomly placed in 2-dimensional space. The latter result has applicability to track etching dosimetry and has been utilized in an automatic computer evaluation that we have developed for the quantification of track etching.

Another result has been the derivation of a method that permits the determination of microdosimetric parameters in fluctuating radiation fields of high intensity. Up to now it was not possible to perform microdosimetric measurements when the dose rates or instantaneous dose rates were too large for the resolution of individual charged particles. The only possibility was then the application of the variance method. The variance method, however, requires repeated measurements over intervals with absorbed doses that are exactly constant. In fluctuating fields, as they are produced by the micro- and macro-pulses of accelerators, the constraint can never be fulfilled. As has now been found, the difficulty is removed by the utilization of a pair of microdosimetric detectors in simultaneous measurements during a series of consecutive intervals. The weighted mean values of the event sizes are then obtained from the variance of the response of one of the detectors minus the covariance of the response of the two detectors. In principle the method is also applicable to the determination of higher moments of the single event distribution. The technical implementation of the method is likely to be beyond the scope of this project. However, initial studies are being initiated now.

Publications:

Kellerer, A.M., Rossi, H.H. On the Determination of Microdosimetric Parameters in Variable Radiation Fields - The Variance-Covariance Method - Submitted to Radiat.Res.

Kellerer, A.M. On the Number of Clumps Resulting from the Overlap of Randomly Placed Figures in a Plane. J.Appl.Prob. to appear March 1983

Kellerer, A.M. The Kinematic Fundamental Formula - A Theorem with Implications to Dosimetry and Microdosimetry - Proceedings of the 8th Symposium on Microdosimetry.

Results of Project No.2

Head of Project and Scientific Staff: Prof.Dr.A.M.Kellerer, Dr.U.Mäder,
Dr.E.Kriener, H.Friede

Project Title:

Biophysical and Epidemiological Studies of Neoplasms in the Japanese Survivors of the Atomic Bomb Explosions

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Substantial agreement has been reached now on the need to abandon the former T-65 dosimetry established at Oak Ridge for Hiroshima and Nagasaki, The essential change has been that, according to the reevaluation in different laboratories, the neutron component is much smaller in Hiroshima than was assumed up to now. On the other hand, the γ-ray doses at larger distances in Hiroshima are now taken to be substantially higher than the values from the T-65 dose estimation. Claims that the risk estimates must be increased because the neutron component - formerly held to be responsible for most of the small dose effect in Hiroshima - has disappeared, are therefore erroneous. The increased γ-component can explain the observed effects in the range of small doses in Hiroshima without the need to invoke risk factors that are much in excess of those earlier assumed. The detailed analysis of leukemias, of other neoplasms, and of the observed chromosome aberrations will, however, require the newly determined personal dose estimates that include shielding factors for the individual survivors. These data are not, as yet, available and the final numerical computations will therefore have to await the completion of the dose reassessment.

In the meantime the project has been directed towards the development of the mathematical tools required for the ultimate analysis, and towards those evaluations that are meaningful even without individual new dose estimates.

In the work on statistical techniques we have given special attention to the method of isotonic regression that provides maximum likelihood solutions of the prevalence as a function of absorbed dose without the need to classify the estimated doses into a coarse grid of dose intervals.

Instead individual dose estimates can be utilized in the computations. 1-dimentional isotonic regression can be based on an established algorithm. 2-dimensional isotonic regression is more difficult. However, we have implemented a little known method earlier proposed by F.Gebhardt. The algorithm is now functional but we have not yet achieved a version that works for arbitrarily high numbers of observations. Work on this problem will therefore be continued. The use of non-linear optimization routines for more general regression that is subject to more sophisticated constraints than monotony has also been continued and we have established satisfactory algorithms that require the utilization of large computers and the relatively complex program GRGA of Abadie. Considerable additional effort has gone during the last year into the attempt to produce analogous routines suitable for small computers that could also be implemented at the computer center of RERF, Hiroshima, with its more limited capabilities.

As indicated in the previous report the non-parametric analysis of a chromosome aberrations data permits a meaningful analysis even without the determination of individual shielding factors. It has been performed both with the old and the new dosimetry. In addition a thesis has been completed during the past year with a reevaluation of the parametric analysis for chromosome aberrations. It has improved the earlier analysis by identifying the bias that was produced in previous studies by the coarse classification of doses. Furthermore the relation between the parametric analysis and non-parametric estimates of the RBE has been examined under the assumption that the presently assumed small neutron doses may still be responsible for the observed difference between the yields for Hiroshima and Nagasaki. The conclusion has been that RBE values of neutrons somewhat in excess of 100 at small doses could account for the observed differences.

Publications:

Kellerer, A.M. Biophysikalische Grundlagen der Wirkungen kleiner Strahlendosen. Atomwirtschaft Vol.27, 99.103, 1982.

Kriener, E. Dosis-Wirkungs-Beziehungen und RBW von Neutronen errechnet aus persistierenden Chromosomenaberrationen bei Überlebenden der Kernwaffenexplosionen von Hiroshima and Nagasaki.
Dissertation, Würzburg, 1982.

Progress Report
1982

Contractor:
University of Aberdeen
Old Aberdeen
Regent Walk
GB-Aberdeen AB9 2ZD

Contract no.: BIO-A-310-81-UK

Head(s) of research team(s):

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Dr. K.V. Ettinger
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General subject of the contract:

Application of lyoluminescence to radiation dosimetry and radiation protection.

List of projects:

1. Lyoluminescence dosimetry of incorporated emitters.
2. Lyoluminescence accident dosimetry with desiccated human tissue and personal effects of the victim.

LYOLUMINESCENCE DOSIMETRY OF INCORPORATED EMITTERS -Project 1

Head of research team: Prof. J R Mallard

Deputy project leader : Dr K V Ettinger

Colaborators : Dr A R Forrester , Dr U J Miola, S Dixon BSc,
G Shentall MSc, D Temperton MSc

Research on the dosimetry of emitters incorporated into biochemicals in the solid phase continued during the year 1982. One category of experiments dealt with quasi-homogeneous materials, i.e. biochemicals containing some amount of a labelled identical compound. The emitters are incorporated into the matrix by co-crystallization. In order to determine the incorporated activity it is necessary to measure concentration of the radioactive atoms by means of a scintillation counter in the preparation. It was found,that for tritium label up to few percent of activity may escape into air from the solution undergoing crystallization,after all the activity in reagents and mother liquor is taken into account. This effect is possibly present with other emitters as well,but at much lower scale.

It has been found that the response of homogeneous biochemicals ,which were used as lyoluminescent phosphors ,to internally incorporated tritium label indicates a small but statistically significant defect,amounting to about 10% in sugars and aminosugars (glucose,mannose,glucosamine).This defect does not depend on the position of the label (i.e. is the same within experimental errors for D-(1-^{3}H)-glucose, D-(2-^{3}H)glucose and for D-(6-^{3}H)- glucose). The dose defect was also the same for samples kept in the freezer (-30^{o}C)*during the time of storage and for samples kept in a vacuum desiccator. There is no measurable influence of the specific activity of the sample on the response defect. All the measurements were performed with sufficient reproducibility to assure that the observed effect does not arise from the conditions of read out.

In experiments in which small amounts of biochemicals with high specific activity were mechanically dispersed in inactive matrix, the response defect is of the same order of magnitudy. This applies when the dispersal matrix is of the same composition and when the dispersal matrix is different i.e. glucose in mannose matrix. However, for mechanical mixing (by grinding and blending) the statistical errors in read out are larger than for homogeneous mixtures.

For ^{14}C labelled glucose the effect was statistically insignificant after corrections for irradiation temperature were applied to the results, which were originally interpreted as indicating a presence of response defect. For ^{14}C if the effect exists it is smaller than 3% and for

* There is a pronounced effect of temperature during irradiation,i.e.during the storage on the lyoluminescent response,equal to about 0.5 - 0.3 %/deg.^{o}C ,depending on the material and temperature range. This correction has been determined with Co-60 gamma rays and was accounted for in the calculation of the response defect of biochemicals stored in low temperatures. This temperature effect is apparently associated with the yield of free radicals and has been observed directly in experiments with an ESR read out of free radical concentration. It is explicable on the grounds of non-stationary thermodynamics.

^{35}S-methionine it is smaller than 5 %.
Amongst the compounds in which internal doses were measured were 5-Iodo--2'-deoxy(6-^{3}H)uridine and 5-(^{125}I)Iodo-2'-deoxyuridine. It is now possible to relate doses from tritium labelled deoxyuridine to that of ^{125}I labelled material. It is still impossible to estimate the magnitude of response defect,if any, in 5 -(^{125}I)Iodo-2'-deoxyuridine,owing to uncertainty in the energies carried by individual Auger electrons.

The possibility of stimulating the Auger cascade emission from iodinated compounds has been investigated theoretically,together with the Brookhaven group and it is expected that some preliminary experimental irradiation of samples will be carried out using the Brookhaven Synchrotron Light Source. Similarly,preparations are being made for irradiations of samples containing bromine,using the Daresbury Light Source. The outcome of these preparations depends upon the availability of machine time.

In order to provide calibration of radiation doses imparted by very low energy emitters an electron gun has been tried,with accelerating voltage adjustable in the range of 5 -25 kV. The use of this gun as an irradiator requires preparation of very thin samples and the initial experiments has shown that deposition,stripping and other handling of thin layer targets introduce uncertainties that make it impossible to use the gun for calibration or even standardization of samples. A design has been initiated of a low energy X-ray source with an internal irradiation chamber i.e.without the use of a window.The source,permanently connected to a vacuum pump,will incorporate a thin aluminium foil as a heat shield between the generating assembly and the sample holder. The foil,of about 2 μm thick,will be much thinner than any exit window,because it will not be exposed to pressure gradient.

Apart from few sugars and amino acids most of lyoluminescent materials require radiation doses in the range of 5 - 100 Gy in order to obtain a response sufficient to reduce the read out errors below about 10 % ,if the dissolution is take place in pure water. A substantial increase of intensity of response, often up to 100 times and more, was observed in the presence of luminol and lucigenin . This increase in response was, however, accompanied by a steep increase in the chemical background, particularly pronounced in brominated and iodinated compounds.

The results of the research on the lyoluminescence dosimetry of incorporated emitters provide interesting information on the interaction of ionizing radiation with biochemicals forming part of living tissues like carbohydrates and amino acids. The lyoluminescence effect is a measure of ability of incorporated radionuclides to produce free radicals and thus a measure of the direct radiation effect in radiobiology.

LYOLUMINESCENT ACCIDENT DOSIMETRY WITH DESICCATED HUMAN TISSUES AND PERSONAL EFFECTS OF THE VICTIM - Project 2

Head of research team : Prof. J R Mallard

Deputy project leader : Dr K V Ettinger

Colaborators: Dr A R Forrester, S Dixon BSc, M Watkins MSc

The presence of free radicals in desiccated tissues i.e. hair,finger- and toenails and in flakes of epithelium following irradiation can be easily demonstrated by means of electron spin spectroscopy. These radicals persist for hours and even days, depending upon the amount of water still present in the tissue. It is an interesting observation that so little is known about recombination of radicals in tissue-like media. Calculations based on a theoretical model,developed in our laboratory, predict that in a viscous medium free radicals attached to the molecules not bound into a polymer would recombine with a rate inversely proportional to the square of the viscosity. The Arrhenius law should apply to this process,with an exponent depending upon the configuration of the molecule. Disappearance of free radicals in desiccated tissues is ,at present, not understood. The diffusion mechanism permits oxygen to permeate the tissues and peroxidize the radicals originally formed by radiation. It is a well known fact that the peroxy radicals are difficult to observe with ESR,but it appears that a genuine recombination mechanism operates here, despite the fact that radicals in collagen are trapped within a polymeric network and are relatively immobile.

There are at present three techniques available to visualize presence of free radicals in the irradiated tissues and in organic compounds in general. The most direct technique is the measurement of the ESR absorption. Lyoluminescence requires that the sample should be soluble and the third method is based on the chemical detection of free radicals e.g. by means of colorimetry.

In the previous report we have mentioned measurement of ESR signals in human hair following irradiation. There are two components of the ESR signals, which decay with different half lives, but could not be separated spectroscopically. One component decayed with a half-life of about 10 min, the other was characterized by a half-life of about 2 hours,with the actual value changing from sample to sample in the range of 1.5 to 6 hrs. No correlation was observed with the colour of hair,even with the totally grey hair included in the variety.
The instrument used for the measurement was Bruker ER-200 interfaced to a microcomputer. The analysis of factors affecting the sensitivity of ESR method indicates that lowering the temperature during the measurement down to liquid helium temperatures will give better detectivity. The higher the field in the cavity,the more sensitive the spectrometer. The design of the cavity is also important. We have found that dual transmission cavity system,followed by a parametric amplifier and balanced mixer is superior to the more conventional arrangement of reflection cavity, single mixer and no front end amplifier of any sort at all.

In conjunction with the Fourier Transform mode of operation the ESR method of detection of free radicals may be adequate for the measurement of accidental exposures to gamma radiation of an order of 1 - 5 Gy,using the effects of the victim made of plastic,leather,cotton,wool etc.This is our conclusion from measurements on a series of commercial acrylic plastics,including acrylic fibers, buttons and items of apparel.

The lyoluminescence technique of measuring the presence of free radicals in irradiated objects requires that the material is dissolved. Lyoluminescence in aqueous media is of limited use in the analysis of desiccated tissue samples, even if solubilizers based on sodium sulphide or tertiary amines are employed. The problem is that these solubilizers generate very strong luminescence,possibly as a result of involvement of oxygen radicals. This chemiluminescence interferes with the measurement of lyoluminescence, raising the minimum detectable dose to more than 8 - 12 Gy,which is beyond the practical applications of accident dosimetry. The use of radical scavengers ,described in the previous report, was partially successful.The work continues on the enzymatic digestion of irradiated samples as a technique for producing lyoluminescence without the disadvantages of strong chemiluminescent background.Other approaches to improve the range,viz.infra. It has been found that in order to accelerate the dissolution or digestion an efficient minification of material is required .Most of plastics being soft can be crushed almost into powder by freezing to liquid nitrogen temperatures,at which they become hard and brittle. The same applies to fingernails. Textiles can be reduced to fibres by a process of combing with a very fine metal comb, a modern analogue of making oakum. We have found this techniques very useful, yielding material suitable for lyoluminescence measurements.

Dissolution of diminuted acrylic resins (including perspex) in intensely agitated organic solvents yields a LL signal,which can be enhanced by the presence of sensitizers suitable for organic media,like rubrene. We were able to obtain an increase in the LL signal by a factor of about 10. The solvent in which LL is unsuccessful is dioxane,probably because of difficulty of purifying the solvent from traces of peroxides.

There is hovewer a caveat when using acrylic resins as lyoluminescence phosphors in accident dosimetry: some of the techniques used for polymerization involve use of initiators seeding the monomer with free radicals. We are at this moment looking for a reliable method of distinguishing the radicals produced in plastics by ionizing radiation from those introduced during the manufacture. Fortunately,not all plastics are produced in that way.

In order to increase the light yield of lyoluminescence we have investigated properties of sensitizers based on energy transfer to trivalent terbium and europium ions in solution. Such sensitizers are needed in accident dosimetry because of low light yield of materials used as accidental dosimeters. It has been found by us that free terbium ions are useful in aqueous media and that chelated terbium ions are very efficient enhancers of lyoluminescence in organic solvents. Doses below 5 Gy were detected in acrylic resins using chelated rare earth compounds as sensitizers based on energy transfer from relatively inefficient lyoluminescence emitters to highly efficient rare earths ions.

In dealing with LL of irradiated hair it was possible to obtain light from dissolution of hair cortex in 17% KOH after initial treatment with sodium sulphide to loose the scales. This work continues with other reagents.

List of publications in 1982

1. U J Miola, K V Ettinger and J R Mallard - Solid state lyoluminescence dosimetry for neutron therapy. Proc.World Congress on Medical Physics, Hamburg,Sept.1982. Ed.by W.Bleifeld et al. p.27.47
2. U J Miola, K V Ettinger and J R Mallard - New developments in clinical lyoluminescence dosimetry. Idem ,p.29.36
3. U J Miola, K V Ettinger ,S Srirath and J R Mallard - Lyoluminescence dosimetry for therapeutic dose range. Proc.Third World Congress on Nuclear Medicine and Biology,Paris,1982. Ed. C.Raynaud p.2926-2929 .
4. R G Fairchild@ A B Brill@and K V Ettinger - Radiation Enhancement with Iodinated Deoxyuridine, Invest.Radiol. 17,407-416,1982
5. K V Ettinger, A R Forrester and C J Hunter - Spin trapping and lyoluminescence, Can.J.Chem. 60.1549 - 1559,1982
6. K V Ettinger and K J Puite† - Lyoluminescence Dosimetry pt.I.Priciples. Int.J.Appl.Radiat.Isotopes 33.1115-1138.1982 .
7. K J Puite† and K V Ettinger - Lyoluminescence Dosimetry pt.II.State-of-the-art . Int.J.Appl.Radiat.Isotopes. 33.1139-1158.1982
8. C.Hunter, D.H.Temperton,K.V.Ettinger and A.R.Forrester - Lyoluminescence spectra of typical dosimeter materials. Int.J.Appl.Radiat.Isotopes, 33.1291-1298.1982

in addition two theses were accepted by the University of Aberdeen:

Ueber Jose Miola, Ph.D.thesis,Department of Bio-Medical Physics and Bio-Engineering (1982)

Glyn Shentall, M.Sc. thesis,Department of Bio-Medical Physics and Bio-Engineering. (1982)

A contributed paper presented during the Conference on Radioprotectors and Radiosensitizers (NBS,Washington,1982) has been issued as a laboratory report and is available upon request:
K V Ettinger, R B Hussain,S Dixon, D Temperton and W L McLaughlin* -
- Quenching of lyoluminescence by some radioprotectors and by superoxide dismutase.
*-Center for Radiation Research, NBS,Washington DC,20234 USA.

@ - Medical Research Center,Brookhaven National Laboratory,Upton, N.Y. 11973 USA

† - Foundation ITAL, 6700AA Wageningen, The Netherlands .

Progress Report
1982

Contractor:

The National Radiological
Protection Board, NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

Contract no.: BIO-A-308-81-UK

Head(s) of research team(s):

Mr. T.O. MARSHALL
Instrumentation and Equipment
NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

General subject of the contract:

The development of a rationalised approach to the dosimetry of external beta and photon radiation for radiological protection purposes.

List of projects:

1. Study of the principles to be embodied in detectors for the practical estimation of new quantities in radiation protection for area monitoring and personal monitoring.
2. Development of beta and gamma calibration standards for the direct determination of new radiation protection quantities.

Contractor:	National Radiological Protection Board Chilton, Didcot, Oxon OX11 ORQ
Contract No:	BIO-A-308-81-UK
Head of Research Team:	Mr T O Marshall
General Subject:	The development of a rationalised approach to the dosimetry of external beta and photon radiation for radiological protection purposes

Title of Project No 1:	Study of the principles to be embodied in detectors for the practical estimation of new quantities in radiation protection for area monitoring and personal monitoring
Project Leader and Scientists:	T M Francis, T O Marshall, D A J Morris, and P H Burgess

The objective of this project is to study the ways in which the response of existing survey instruments can be modified to have the correct response in terms of new dosimetric quantities (eg, dose equivalent rate at a depth of 1 g cm^{-2} in a tissue equivalent sphere of 30 cm diameter which is one of the possibilities being considered for introduction into applied radiological protection) in order to establish design criteria for future survey instruments.

During the period of this report work has concentrated on modifications to ion chambers. The chamber chosen on which to apply the modifications was that normally incorporated in the Eberline RO2 ion chamber monitor.

Two sets of modifications to the chamber, as a means of achieving a response which varies with energy in a manner suitable for the measurement of the dose equivalent H_d at a depth of 1 g cm^{-2} along the central axis of a tissue equivalent sphere of 30 cm diameter with the radiation directed along the central axis, were tried.

In the first case the collector electrode of the chamber, originally of synthetic resin bonded paper was replaced with one of aluminium of the same diameter. The thickness of the electrode was optimised to 2.12 mm. In addition the chamber was surrounded by a 1 cm thick perspex cap which covered all but the rear wall to which is attached the support plate.

The variation in response of the modified chamber with photon energy was observed to match the variation of dose equivalent H_d with energy to within 0.94 to 1.14 relative to the response to Cs-137 gamma rays, over the energy range tested, ie, 33 keV to 1.25 MeV.

The polar response of the chamber was also investigated over the same energy range. The variation of response over 4π solid angle, relative to the response at normal incidence to the front window, was within 0.8 to 1.0 for all energies of 48 keV and above. The minimum value for 33 keV photons was 0.72.

A second approach was made to design a chamber with a good performance for the measurement of H_d which with a simple adjustment could be used, in addition, for the measurement of skin dose. It was found that the chamber had the desired response. If a 1 mm thick aluminium collar was fitted externally around the cylindrical walls of the chamber, the diameter of the collector was reduced by 50%, the inside back wall was covered with aluminium of thickness 0.12 mm and a 19 mm deep collar of aluminium of thickness 0.12 mm placed inside the chamber with its mid line in the same plane as the collector electrode. The front window of thin aluminised melinex was covered with an easily removed aluminium disk of thickness 1 mm.

In this case the variation of response with energy for the measurement of H_d was found to be within 0.92 to 1.04, relative to the response to Cs-137 gamma rays, over the photon energy range 33 keV to 1.25 MeV. The polar response over the front 2π solid angle was found to be within 0.94 and 1.02 relative to the response for normal incidence over the energy range 33 keV to 1.25 MeV. Over the rear 2π solid angle the polar response was inferior with minimum values, relative to the response at normal incidence to the front face, of 0.62 at 33 keV, 0.72 at 48 keV and 0.77 at 65 keV. For all energies greater than 65 keV the response at all angles is always within 0.8 to 1.0 relative to the response at normal incidence to the front face.

Work is continuing in an attempt to improve the polar response over the backward 2π solid angle. The suitability of the chamber is also being investigated for the measurement of skin dose, ie, the dose equivalent at a depth of 0.07 mm in the ICRU sphere for both photon and beta radiations with the 1 mm aluminium disk removed from the front window.

Title of Project No 2: Development of beta and gamma calibration standards for the direct determination of the new radiation protection quantities

Project Leader and Scientists: T M Francis, T O Marshall, D A J Morris, and P H Burgess

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The objective of this project is to develop calibration standards for both photon and beta radiations which will allow direct calibration in terms of the proposed dose equivalent quantities and which can be directly traced to the existing metrological standards.

Based on satisfactory results obtained for the measurement of the dose distribution over a circular area of radius 10 cm in a slab phantom of sides 30 cm and thickness 11 cm (CEC Radiation Protection Progress Report 1981), it was decided that a cubic phantom should be investigated further as a substitute for the ICRU spherical phantom on which to base the standards. A cube of 30 cm sides was designed and constructed using tissue equivalent material MS20 (the material used for the construction of 30 cm diameter spherical phantom used in previous studies (CEC Radiation Protection Progress Report (1980)). The phantom consists of an assembly of closely machined slabs of various thicknesses with a facility for incorporating TL dosemeters at desired depths. The design also allows the measurement of the dose distribution over a circular area of radius 10 cm at each depth. Each of the slabs which constitute the phantom has been checked for density and x-ray attentuation characteristics to ensure uniform dosimetric properties throughout the phantom.

Lithium borate pellets (4.6 mm diameter x 0.8 mm thick) have been used for the measurement of absorbed dose within the phantom. The depths of measurements ranged from 0.7 to 6 cm from the front face of the phantom. At each depth the dose distribution was measured over a circular area of radius 10 cm from the central axis. The photon energies used for these measurements were 30.9 keV, 74.1 keV and 661.6 keV; these radiation qualities conformed to the reference radiation qualities specified by the ISO (1979). The results of the measurements indicate that the dose distribution over a circular area of radius 8 cm does not differ by more than 2% from the dose at the centre of the area and that a parallel plate ionisation chamber with an area at least equal to the above could be accommodated within the phantom without introducing significant

inaccuracies in the measurements. The depth dose distributions measured (mean dose over the area of the measurements) through the central axis of the cubic phantom were compared with those measured previously within the ICRU sphere (CEC Radiation Protection Progress Report (1980)). The distributions within the cubic phantom did not differ by more than $\pm$ 2% from those measured in the spherical phantom for photon energies of 74.1 keV and 661.6 keV. However, in the case of 30.9 keV, the distributions differed by approximately 7%. Whilst this is not considered highly significant and would not rule out use of a cubic phantom as the basis of the standard, further investigations are being carried out to check this result.

Based on these observations, preliminary work concerning the development of extrapolation chambers for use within the photon and beta-ray standards has begun. As far as possible these chambers will be constructed using MS20 but inevitably small amounts of other materials such as aluminium will also have to be used and the effects of this on the dose distributions has been studied. Measurements at an energy of 74.1 keV and at a depth of 700 mg cm^{-2} with and without a 2 cm thick aluminium ring of internal diameter 6 cm and external diameter 7 cm surrounding the lithium borate dosemeters indicate that the introduction of small quantities of aluminium into the phantom does not alter the dose distribution within the volume of interest significantly.

A prototype electrode assembly for use with an extrapolation ionisation chamber for the beta-ray standard has been designed and constructed. Special features have been included to overcome difficulties due to the relatively poor electrical insulation properties of MS20. Preliminary measurements using this electrode in an existing extrapolation chamber gave satisfactory results. Work has begun on the design of an in-phantom extrapolation chamber incorporating electrodes of a similar construction but with various collecting areas.

References

CEC Progress Report - Radiation Protection (1980)
CEC Progress Report - Radiation Protection (1981)
ISO 4037 'x and γ reference radiations for calibrating dosemeters and dose rate meters and for determining their response as a function of photon energy' (1979).

Progress Report
1982

Contractor:
University of Dundee
Nethergate
GB-Dundee DD1 4HN

Contract no.: BIO-A-463-81-UK

Head(s) of research team(s):

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Dr. D.E. Watt
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General subject of the contract:

Dosimetry and microdosimetry of intermediate energy neutrons.

List of projects:

1. Dosimetry and microdosimetry of intermediate energy neutrons.

Title of project nr 1.

Head of project and scientific staff : Dr. D.E.Watt, I.A.M.Al-Affan.

There are no direct methods of measuring the quality of neutrons in the intermediate energy range below 50 keV. The most satisfactory way of specifying quality is in terms of microdose spectra. These can be calculated and measured at energies above 50 keV[1,2,3]. In the present programme the intention is to calculate details of the microdosimetry at lower energies where energy transfer by elastic scattering becomes important as this enhances the energy deposition by the charged particle recoils. In parallel, a practical microdosimeter which is capable of selectively detecting low energy events associated with intermediate energy neutrons whilst excluding those events due to fast neutrons and other radiations is being developed.

Caswell's[4] basic formulae for calculation of energy deposition events due to insiders, starters, stoppers and crossers, using the continuous slowing down approximation have been modified to allow for the enhanced energy deposition in spherical volumes due to elastic scattering interactions which reduce the penetration depth of the charged particle recoils. Energy loss spectra have been obtained for neutron energies of 1, 10, 50, 100 keV in 0.2 µm and 1 µm tissue-equivalent spheres. From these frequency and dose distributions in lineal energy and in specific energy density have been calculated. Fig. 1 shows values of the frequency mean of y, $\bar{y}_F$ and the dose mean of y, $\bar{y}_D$ as a function of neutron energy. Also calculated are values of $\bar{\varepsilon}$, the energy average of event size as a function of the diameter of the sensitive site. The structure of the energy loss curves can be /

be interpreted in terms of the basic physics.

The effect of the modifications to the formulae is to increase the number of energy deposition events due to insiders and stoppers at the expense of the starters and crossers respectively. As may be expected, the degree of the effect increases with decreasing neutron energy, increasing sphere size, and the change is most significant for low energy deposition events. This latter is noticeable even for neutron energies of 1 MeV. A publication is in preparation.

Some technical difficulties are being experienced in the construction of a mesh inner wall in the co-axial cylindrical proportional counter in which the inner (microdosimeter) will be operated in anti-coincidence with the outer detector to record only insiders. This, combined with theoeretical and practical knowledge of the components of the microdose spectra for fast neutrons, should enable the full intermediate energy neutron contribution to be deduced.

References.

1. Caswell, R.S. and Coyne, J.J. EUR5452, 97-123, 1975.
2. Booz, J. and Coppola, M. EUR5122, 983-1000, 1974.
3. Rodgers, R.C. and Cross, W. EUR5122, 1027-1042, 1974.
4. Caswell, R.S. Rad. Res. 27, 92-107, 1966.

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

1. Cunningham, J.W., Al-Kazwini, A.T. and Watt, D.E. Penetration depths of low energy (< 100 keV) H^+, He^+ and N^+ ions in solid enzyme layers. 8th Symposium on Microdosimetry, 1982.

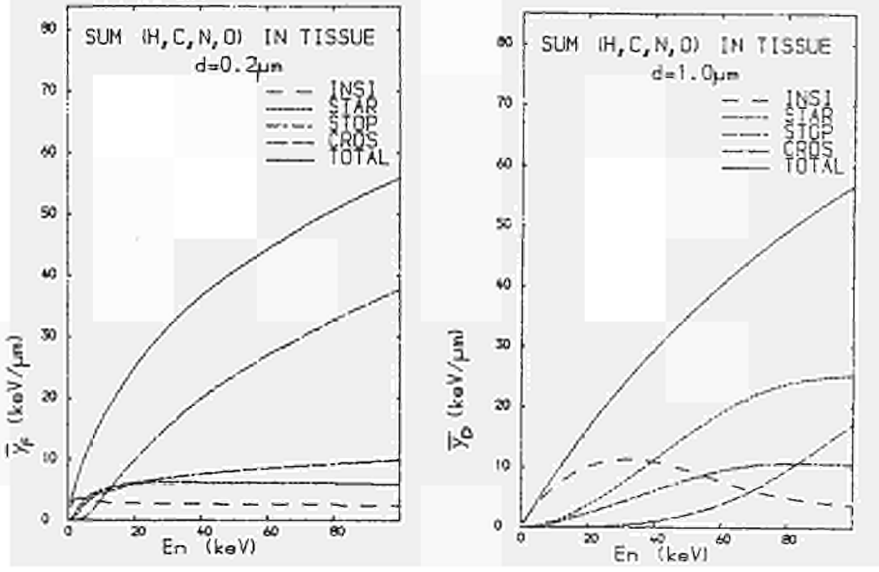

Fig. 1. Components of frequency and dose means of lineal energy transfer for intermediate energy neutrons in tissue spheres of diameter 0.2 μm and 1 μm.

Progress Report
1982

Contractor:
Com.Naz.per la Ricerca e
per lo Sviluppo dell'Energia Nucl.
et delle Energie Alternative, ENEA
Viale Regina Margherita 125
I-00198 Roma

Contract no.: BIO-A-298-81-I

Head(s) of research team(s):
Prof. P. Metalli
Div.Fisica e Scienze Biomediche
CSN della Casaccia, ENEA
C.P. 2400
I-00100 Roma

General subject of the contract:

Microdosimetric studies of radiological effects at low doses.

List of projects:

1. Microdosimetric studies of radiobiological effects at low doses.

Title of project nr 1

Microdosimetric studies of radiobiological effects at low doses

Head of project and scientific staff: Prof. M. Coppola
Dr. G. Bertoncello

Research on the biological effectiveness of radiation of various qualities was continued at low radiation doses for different biological end-points. This included the analysis of results of measurements already completed, the continuation of long range experiments already started before this year, and the performance and planning of new measurements.

Investigation of the effectiveness of fast neutrons to induce ovary tumors in female mice exposed to low neutron doses has been continued in collaboration with the Laboratory of Pathology. It is expected that all animals will come to death during the next year, and that data collection and analysis could start soon after.

In a cooperative endeavour with the Biology Group of Ispra cell survival of Nicotiana Pl. protoplasts irradiated in vitro with fast neutrons was further measured to complete the previous observations. In particular, additional irradiations of diploid cells were carried out with 16 MeV neutrons in fractionated mode, as an extension of the previous positive results, to study the influence of time between fractions as well as of size of fraction on the global effect. Survival curves were also repeatedly measured with 1 MeV neutrons.Finally a test of in vivo irradiation of small plants, both haploid and diploid, was initiated using 16 MeV neutrons, to detect somatic effect differences due to different repair capacity in the two species. The results are presently considered for evaluation.

An experiment designed to study the influence of the presence of heavier elements such as iodine on cell reproductive death of V-79 Chinese hamster cells irradiated with X- and ^{60}Co gamma-rays was completed and the results were published. This experiment has indicated that the increase of cell death is essentially due to an increased absorbed dose related to the higher photoelectric cross-

section of iodine, at least in the dose region of interest for diagnosis. Because of the potential implication of these results for the protection of patients undergoing X-ray examinations, and the possibility of comparison with actual clinical data, it has been planned to repeat a similar test on peripheral blood human lymphocytes.

Human lymphocytes have also been irradiated with 1 MeV neutrons at doses down to 2.5 mGy to ascertain the shape of the dose-effect curve for dicentric formation in the very low-dose region. Eurther irradiation series will be required to acquire the necessary statistical accuracy.

Micronuclei formation in the eye-lens proliferative epythelim of mice irradiated with X-rays and fast neutrons is under continuing investigation. The results of irradiations with 1 and 16 MeV neutrons for doses down to 10 mGy are now available and will soon be published. Further irradiations have recently been carried out at lower doses both in acute and in fractionated modes, and the histological samples have prepared for microscope observation. An attempt of increasing the reading efficiency and of improving the data analysis is presently underway by the introduction of a semi-automatic image analysis system, recently purchased.

Further preparation for the measurement of microdosimetric spectra in the biological channel of the TAPIRO fast reactor of Casaccia has been obtained with the construction of a capsule to contain the proportional counter that will be inserted into the channel down to the core region.

List of publications in 1982

1. G. Barile, G. Bertoncello, V. Capuano, M. Coppola, and M. Quintiliani
The influence of iothalamic acid on the biological effectiveness of photonic radiations. VIII Symposium on Microdosimetry, Jülich, Sept. 1982.

2. M. Coppola
Neutron effectiveness at low dose levels for other endpoints. In: Neutron Carcinogenesis, 343-356, EUR 8084en, 1982.

3. M. Coppola
Summary of discussions of Session V. In: Neutron Carcinogenesis, 437-444, EUR 8084en, 1982.

4. E. Magnien, M. Devreux, X. Dalschaert, M. Coppola
Unexpected dose-dependence of the radiosensitivity-ratio between haploid and diploid protoplasts. International Congress of Plant Tissue and Cell Culture, Tokyo, July 1982.

5. R. Cavalloro, M. Coppola, and G. Pozzi
Med-fly cell line: response to irradiations with fast neutrons and gamma-rays. Revue Canadienne de Biologie. In Press.

Progress Report
1982

Contractor:

Com.Naz.per la Ricerca e per
lo Sviluppo dell'Energia Nucl.
e delle Energie Alternative, ENEA
Viale Regina Margherita 125
I-00198 Roma

Contract no.: BIO-A-299-81-I

Head(s) of research team(s):

Prof. P. Metalli
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Dr. L. Tommasino
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CSN della Casaccia, ENEA
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General subject of the contract:

Personal dosimetry and area monitoring.

List of projects:

1. Personal neutron dosimetry by electrochemically etched CR-39 detectors.
2. Further developments of thin-film breakdown counters.
3. Electrochemically etched track detectors for the counting and spectrometry of alpha particles and their applications.

1. Personnel Neutron Dosimetry by Chemically and Electrochemically etched CR-39 Detectors.

L. Tommasino, L. Lembo, G. Busuoli, O. Civolani, G. Zapparoli.

In attempts to use CR-39 damage track detectors for personnel neutron dosimetry, doubts seem to arise whether to use the chemical etching, the electrochemical etching or a combination of both for the registration of damage tracks.

In 1982 systematic investigations on these different types of etching procedures have been continued with particular regard to the chemical etching and the electrochemical etching of prior-etched detectors.

In the case of the electrochemical etching (ECE), efforts have been made to simplify the ECE apparatus and the automation of track counting. By properly choosing the pre-etch time, the response of the electrochemically etched detectors has shown little energy dependence from 50 KeV to 20 MeV. Large track-sizes are obtained with about the same diameter throughout the entire energy range, which greatly facilitate the automation for track counting. Large areas of electrochemically etched spots can be easily scanned by a colony counter, even with small magnification. Investigations have just started in order to obtain convenient radiators, so that the response of electrochemically etched tracks induced by neutrons with energy below 10 KeV could be equal to that of fast neutrons. This will make it possible to obtain the measurements of neutron doses with any energy with a single track counting.

Parallel investigations have been carried out with chemically etched detectors. The response of these detectors versus the neutron energy has been obtained with irradiations carried out at CEA in France and sponsored by the european community. The preliminary results have confirmed the response of these dosimeters, which was obtained previously. The chemically etched detectors appear to have a treshold at about 200 KeV. For the detection of thermal and and intermediate neutron-energy, an albedo-type of dosimeter is used, where the neutron-induced alpha tracks from a $Li_2B_4O_7$ converter are registered by chemical etching. Irradiations to Pu-Li, Am-Be and ^{252}Cf sources have shown an increased sensitivity to low energy. However, a comprehensive analysis of this dosimeter remains to be made with beams of low-energy neutrons, hopefully with irradiations sponsored by the european community.

2. Further Developments on Thin-film Breakdown Counters.

L. Tommasino; F. Raponi.

Both the thin-film breakdown counter and the spark counter for damage tracks in insulators can detect up to a maximum number of events per cm^2. By increasing the detector area, the density of events which can be registered decreases since the evaporation of the thin Al electrode increases with the detector capacitance.

In 1982 we have developed a new capacitor system, where only a fraction of the energy stored in the capacitor contributes to each breakdown event.

A systematic investigation of this new capacitor has been started to improve the registration characteristics of the spark jumping counter. Similar considerations apply for the improvement of the detecting characteristics of the thin-film breakdown counter as well.

3. Electrochemically etched track detectors for the counting and spectrometry of alpha particles and their applications.

L. Tommasino, G. Sciocchetti, G. Zapparoli and F. Raponi

Extensive investigations on the electrochemical etching of both polycarbonate and CR39 detectors have provided important guidelines to fully exploit the advantages of this etching procedure.

If the time for track formation is much smaller than that of treeing propagation, the breakdown spots present uniform track sizes and limited overlapping. These characteristics are ideal for the track counting and its automation and when large signal-to-noise ratios are required. The track formation times can be easily reduced by chemically pre-etching the plastic detectors before the electrochemical etching. The high signal-to-noise ratio obtained in this way can be further increased by using a chemical reagent highly sensitive for track formation and electrochemically etching the detector with a different reagent capable of producing low background tracks.

With no pre-etching and relatively long track formation times, the breakdown spot-diameters become highly dependent on the particle type and energy. Alpha-energy discrimination can be easily obtained, which could be useful for the identification of both man-made and natural alpha-emitting radionuclides.

Finally efforts have been made in order to improve the spark counter for the detection of alpha tracks, which counter is still the most simple and sensitive technique for the track counting. Both the electrochemical etching and the spark counter make it possible the scanning of alpha tracks in such large detector areas that radon monitoring with particular new geometries can be designed.

In 1982,applications of these registration techniques in the field of radon dosimetry have been started with particular regard to the assessment of individual exposure to radon daughters. Efforts have been made to calibrate these systems both in the laboratory and in field operations. In particular a prototype radon chamber of 1.4 m^3 has been set-up. A different chamber with controlled environmental factors is under construction, while a large work chamber will be built in the future.

List of Publications in 1982.

1. J. Wong and L. Tommasino (1982) Energy Discrimination of Alpha Particles By Electrochemical Etching of Track Detectors. Nuclear Tracks 6 (1) 17

2. J. Wong and L. Tommasino (1982). The Frequency Response of the Electrochemical Etching. Nuclear Tracks. 6 (1)-24.

3. L. Tommasino, G. Zapparoli and F. Caiazzo. A new Method for Electrochemical Etching. I Results with DC voltage. In press

4. L. Tommasino, G. Zapparoli and F. Caiazzo. A new method for electrochemical etching. II Results with ac voltage. In press.

5. G. Espinosa Garcia, J.F. Gorzarri, L. Tommasino and F. Raponi. Electrochemical Etching Registration Efficiency and Track Formation Time. In press.

Progress Report
1982

Contractor:

Istituto Nazionale di Fisica
Nucleare, INFN
P.O.Box 56
I-00044 Frascati (Roma)

Contract no.: BIO-A-297-81-I

Head(s) of research team(s):

Prof. G. Moschini
Lab. Nazionale di Legnaro
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Via Romea 4
I-35020 Legnaro-Padova

General subject of the contract:

Dosimetry and hazards of neutrons at energies between 15 and 50 MeV.

List of projects:

1. Dosimetry and hazards of neutrons at energies between 15 and 50 MeV.

Title of project nr. BIO-A-297-81-I

DOSIMETRY AND HAZARDS OF NEUTRONS AT ENERGIES BETWEEN 15 AND 50 MEV

Head of project : G. MOSCHINI

Scientific staff: P. COLAUTTI, B.M. STIEVANO, G. TALPO, D.E. WATT, M.A. BEHROOZ

We emphasize that Istituto Nazionale di Fisica Nuclea re has approved financially the project only during the month of October 1982, and that Tandem accelerator has not been available as neutron source due to problems con nected with official authorizations.

For this reason, experimental set-up for neutron spec trometry and neutron dosimetry has been assembled at 7 MV Van der Graaff accelerator of Laboratories of Legna-ro.

In order to obtain neutron beams with well-known ener gy and flux, a device utilizing the associated particle technique(with time of flight capability) has been built and tested. Owing to the backing of standard T targets, calibration is allowed for neutron energies near 16 MeV.

For higher and lower energies, thin and self-suppor ting D and T targets will be used; to avoid their punc-ture during irradiation, a rotating target holder has been built.

By using neutron beams produced by 7 MV accelerator, on line n-γ discrimination and dose measurements with ionization chambers have been checked and optimized.

Besides, a neutron collimator made with tungsten blocks plus auxiliary shielding has been planned and ordered.

To obtain microdosimetric parameters, some routines for data analysis, graphics and relative display have been prepared.

It has been got ready a program which, by using the Cashwell's method, allows the computation of microdosimetric spectra for neutron energies lower than 1 MeV.

Finally, it has been improved a program which computes total kerma factors, partial kerma factors and microdosimetric spectra for neutrons in the 20 to 60 MeV energy range.

Owing to the shortage of cross section data, actually the program allows calculations up to 30 MeV.

Progress Report

1982

Contractor:
Universität des Saarlandes
Stadtwald
D-6600 Saarbrücken

Contract no.: BIO-A-289-81-D

Head(s) of research team(s):

Prof.Dr. H. Muth
Institut für Biophysik
der Univ. des Saarlandes
Boris Rajewsky Institut
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Prof.Dr. R.E. Grillmaier
Institut für Biophysik
der Univ. des Saarlandes
Boris Rajewsky Institut
D-6650 Homburg (Saar)

General subject of the contract:

In vitro and in vivo investigations of radiation induced chromosome damages and microdosimetric techniques for neutrons and high-LET radiations.

List of projects:

1. In vitro and in vivo investigations of radiation induced chromosome damages and of the underlying primary mechanisms with regard to applications in biological dosimetry.
2. Application of microdosimetric techniques to practical problems in dosimetry of neutrons and high-LET radiations and in radiation protection dosimetry.

Title of project nr. 1: In vitro and in vivo investigations of radiation induced chromosome damages and of the underlying primary mechanisms with regard to applications in biological dosimetry.

Head of project and scientific staff:
Prof. Dr. R.E. Grillmaier, Dipl.-Phys. H.K. Stanger,
Dipl.-Math. H. Klein, E. Fiedler

I. The investigation of blood samples obtained from radiotherapy patients started in 1981, has been continued. In order to obtain statistically reliable results, the examination of a total of 35 to 40 patients is planned. During 1982 the blood samples of twelve additional patients were studied. (Three patients were examined already in 1981.)

The samples were taken immediately before and after the first, second and in some cases third irradiation fractions. (Patient irradiations were performed using either high energy electrons or cobalt-60 gamma rays. With the exception of one patient suffering from a gynaecological tumor, the patients were treated for lung cancer.) Radiation damage and cell activity were examined in terms of chromosome aberration rate in lymphocytes, the PHA stimulated lymphocyte transformation and the rosette formation. The results of this examination are setout in Tables 2 and 3. Table 1 demonstrates the results of the lymphocyte transformation and rosette formation test after in-vitro irradiation of blood.

In order to obtain results also at the very low dose range investigations of the chromosome aberration and the micronuclei rate in lymphocytes of patients receiving X-ray irradiation during heart-catheterization are in progress.

II. We intend to determine the type and relative amount of radicals induced by ionizing radiation in the nuclei of living cells. (The radicals are measured by EPR-techniques.) The following procedure is applied: firstly the complex system as a whole as well as its single components are irradiated and measured separately. Thereafter a spectrum is constructed using the signals of the components (EPR-spectra), to

which each of the single constituents contribute proportional to its mass in the complex system. Comparing the original with the constructed system-spectrum, conclusions can be drawn regarding the type of radicals and their frequency.

To date we have measured frozen chromatin solutions ("complex system") extracted from calf thymus cells, commercially available DNA and histone in addition to the different histone constituents H1, H2A, H2B, H3 and H4 as well as the nucleic acids guanine and thymine. (The samples were irradiated at 77 K.) We found in all samples (at 80 K) predominantly OH-radicals which ceased to exist after annealing at 140 K. The total amount of radicals decreased according. After the OH radicals disappeared we identified $\dot{G}^{+}$, $\dot{T}^{-}$ and $\dot{T}$-H radicals both in chromatin and DNA, although in chromatin another not yet identified broad EPR-signal was observed, probably attributable to histone radicals. In histone samples we observed broad, as yet unidentified signals unlike those found in chromatin.

The "chromatin" spectrum were constructed by adding the histone and DNA spectra (at 140 K) based on a radical concentration ratio of 3:2 (according to the measured weight ratio of 3:2 for histone and DNA in chromatin). A comparison of this spectrum with the experimentally obtained spectrum (at 140 K) indicates a loss of histone radicals in the real chromatin spectrum, whereas the DNA radical concentration is increased. There is also a qualitative difference between the two spectra indicating that different radicals are involved. A reconstruction of the chromatin spectrum using the single spectra of H1, H2A, H2B, H3 and H4 instead of the histone sample was not able to reduce the discrepancies. It therefore cannot be excluded, that a spin transfer occurs from protein to DNA within the chromatin.

Peroxy radicals were observed in all samples after further annealing to temperatures slightly below melting point. Preliminary investigations of irradiated cell nuclei suspension, also extracted from calf thymus cells, revealed no peroxy radicals. For this reason investigations using only deoxygenated samples are at present in progress.

LYMPHOCYTE-TRANSFORMATION-TEST measured by the uptake of ^{3}H-thymidin					ROSETTE-FORMATION-TEST	
number of observations	dose (rad)	β-count-rate (cpm) before irradiation	β-count-rate (cpm) after irradiation	ratio of count rates after/before irrad. %	rosettes found per 200 cells before irradiation	rosettes found per 200 cells after irradiation
5	200	$\bar{x}$ = 58464 s = 13357	$\bar{x}$ = 62553 s = 14481	107	33	30
6	500	$\bar{x}$ = 48683 s = 11239	$\bar{x}$ = 22951 s = 3417	48	43	48
5	1000	$\bar{x}$ = 50415 s = 2809	$\bar{x}$ = 11919 s = 1886	24	26	37

Table 1

Donor	Whole body averaged dose per irrad. session (Gy)	Ratio of transformed cells After irrad./before irrad. 1.Irrad.	2.Irrad.	3.Irrad.	Rates of Rosette formation After irrad./before irrad. 1.Irrad.	2.Irrad.	3.Irrad.
OS	0.15	0.94	-	-	1.32	-	-
HU	0.302	0.92	-	-	1.05	-	-
ZI	0.228	1.00	0.70	-	0.95	0.90	-
GR	0.199	0.75	-	-	1.50	-	-
DH	0.203	1.09	0.74	-	1.34	0.51	-
HA	0.218	1.00	0.77	-	1.96	1.24	-
NI	0.23	0.95	1.33	-	1.65	0.87	-
AD	0.296	0.30	0.59	-	1.28	-	-
ST	0.212	0.98	0.84	1.34	1.42	0.98	0.52
SE	0.258	0.93	-	-	0.70	0.89	-
SÜ	0.07	0.08	-	-	1.51	-	-
PF	0.229	2.70	1.11	-	0.94	0.62	-

Table 2

Donor	Dose (Gy)*	Chromosomeaberration rate of radiotherapy patients 1. Irradiation Before Ac.	Dic.	After Ac.	Dic.	2. Irradiation Before Ac.	Dic.	After Ac.	Dic.	3. Irradiation Before Ac.	Dic.	After Ac.	Dic.
OS	0.15	2.7	0.4	5.0	0	6.0	0.9	-	-	-	-	-	-
HU	0.302	0.5	0.5	4.7	0.5	2.3	0	-	-	-	-	-	-
ZI	0.228	2.1	0	4.8	0.5	2.2	0	11.4	1.0	-	-	-	-
GR	0.199	0.5	0	4.3	0.4	3.1	0	-	-	-	-	-	-
DH	0.203	5.1	0.9	2.6	0	3.1	1.8	11.1	2.6	5.1	0.9	-	-
NI	0.23	4.7	0.4	11.6	0.5	12.6	1.4	14.3	2.2	-	-	-	-
SCH	0.266	1.9	0.5	3.1	0.5	1.3	0	5.0	1.8	-	-	-	-
MA	0.221	1.9	0	6.0	0.5	6.5	0.5	-	-	-	-	-	-
FE	0.352	4.9	0.4	10.1	2.9	7.9	1.0	8.9	1.8	9.1	1.8	-	-
SA	0.195	2.7	0.5	11.2	3.0	6.8	0	7.6	3.6	3.4	0.5	-	-
KR	0.06	2.8	0.5	1.3	1.3	10.9	2.3	5.4	0	7.5	2.3	5.7	1.4
KE	0.247	5.1	0	10.8	1.9	7.6	2.2	9.1	1.8	6.8	1.4	-	-

* Whole body averaged dose per irradation session

Table 3

List of publications in 1982

GRILLMAIER, R.E., A. PAPPAS, R. DIETZ
Alterations of the genetic material and activation of lymphocytes in cancer patients after radiotherapy.
5th European Immunology Meeting, Istanbul-Turkey, June 1-4,1982, Postersession.

STANGER, H.K., E. FIEDLER, R.E. GRILLMAIER, R. KESSLER
Mechanism of radiation-damage in chromatin: DNA associated proteins as radical scavengers?
Proceedings of the World Congress on Medical Physics and Biomedical Engineering 1982. Sept. 5-11,, 1982. Edited by W. Bleifeld, D. Harder, H.-K. Leetz and M. Schaldach. Contribution 29.40

FIEDLER, E., H.K. STANGER, R.E. GRILLMAIER
A comparative ESR-study of X-irradiated frozen solutions of DNA, histones and chromatin from calf thymus.
Proceedings of the World Congress on Medical Physics and Biomedical Engineering 1982. Sept. 5-11, 1982. Edited by W. Bleifeld, D. Harder, H.-K. Leetz and M. Schaldach. Contribution 29.41

FIEDLER, E., H.K. STANGER, R.E. GRILLMAIER
Conduction of radiation damage in chromatin: An ESR-study.
Proceedings of the Eighth Symposium on Microdosimetry.
27. September - 1. October 1982, Jülich, Fed. Rep. of Germany. In press

STANGER, H.K, E. FIEDLER, R.E. GRILLMAIER
Mechanisms of radiation damage in chromatin: Radical transfer between DNA and histone.
Proceedings of the Eighth Symposium on Microdosimetry.
27. September - 1. October 1982, Jülich, Fed. Rep. of Germany. In press

Title of project nr. 2: Application of microdosimetric techniques to practical problems in dosimetry of neutrons and high-LET radiations and in radiation protection dosimetry

Head of project and scientific staff:
Dr. H.G. MENZEL, Dr. H. SCHUHMACHER, Dipl.Phys. G. BÜHLER

The evaluation of the correlated microdosimetric, dosimetric, and fluence measurements with monoenergetic neutrons of 13.9, 15.0, 17.0, and 19.0 MeV has been completed. All measurements were performed at the Physikalisch-Technische-Bundesanstalt (PTB), Braunschweig; the determination and evaluation of fluence, fluence spectra and the ionization chamber dosimetry were carried out by members of PTB staff (Dr. G. Dietze, Dr. S. Guldbakke). 17 and 19 MeV neutron beams were contaminated by scattered and parasitic neutrons of lower energies. Microdosimetric and fluence measurements were also performed for non-tritiated but otherwise identical targets in order to determine the contribution of parasitic neutrons. The fluence measurements enabled the microdosimetric distributions to be normalized to neutron fluence. A comparison of fluence normalized distributions for four neutron energies is set out in Figure 1. This type of normalization also enables the kerma per unit neutron fluence to different secondary particles to be assessed.

The energy transferred to alpha-particles per incident neutron increases from 13.9 MeV to a maximum at 17 MeV neutron energy and is reduced again at 19 MeV. This resembles the energy dependance of the cross-section for the reaction $^{12}C(n,n')\,3\alpha$. The expected constant kerma factor for hydrogen in this energy range was confirmed within the experimental uncertainty.

The kerma and kerma factors for A150-plastic were derived from the microdosimetric measurements and compared to data obtained by both ionization chamber measurements and evaluations from published kerma factors. The results show that at all energies the proportional counter data are lower than the tabulated values. Significant evidence points to the fact that

the discrepancies are partly explained by a systematic error in the proportional counter measurements namely that the geometrical cross section of the counter is smaller than presumed. A preliminary correction for this error provides very good agreement between the microdosimetric determination of kerma factors and the tabulated values at 13.9 and 15 MeV. A difference at 19 MeV is, however, still apparent. On the other hand there is a very good agreement between ionization chamber and proportional counter results after the preliminary correction.

The results obtained by the correlated microdosimetric and spectroscopic measurements will be compared to calculations of ionization yield distributions. These calculations are being performed at the time of writing and will be completed together with Dr. Coyne (National Bureau of Standards, Washington) during his stay in Germany. The comparison of calculated and measured data will enable critical testing of physical data relevant to neutron and radiation protection dosimetry.

In order to improve the accuracy of proportional counter measurements and to be able to better assess the uncertainties involved, investigations of systematic uncertainties inherent to the measuring principle were made. a) Three calibration alpha-sources as normally mounted into proportional counters were investigated. It was found that the energy loss of alpha-particles in the gold layer covering the source was not negligible as had been expected assuming a layer thickness as given by the manufacturer. There were also differences in energy loss between identical nuclide sources. This result suggests that the real energy of alpha-particles entering the gas cavity should be used in the calibration procedures and that an uncertainty of 2.7-4.5% is introduced into the calibration procedure due to variations in the thickness of the gold layer. b) From the evaluation of absorbed dose it was presumed that the counting volume of the proportional counters is smaller than is to be expected from the nominal diameter. X-ray photographs of chambers and further dose measurements in well-known radiation fields have supported this presumption. At the present time further investigations to determine the real chamber volume are in progress.

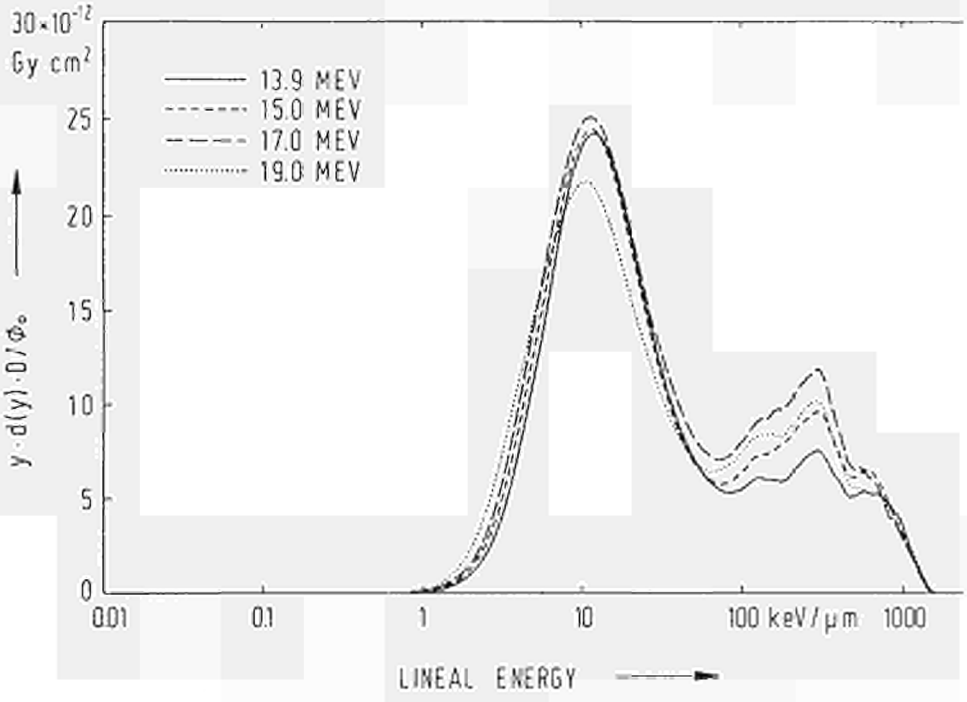

Fig. 1: Dose distributions per logarithmic increment of lineal energy, y, normalized to the neutron fluence at the detector position. Only neutrons of the respective nominal energies were considered for the normalization.

List of publications:

I. G. DIETZE, S. GULDBAKKE, H.G. MENZEL, H. SCHUHMACHER, G. BÜHLER:
Correlated Microdosimetric, Dosimetric, Spectroscopic, and Fluence Measurements With Monoenergetic Neutrons Between 14 and 19 MeV.
Proc. 8th Symposium On Microdosimetry, Jülich 1982, in press

H.G. MENZEL, G. BÜHLER, H. SCHUHMACHER:
Investigation of Basic Uncertainties In The Experimental Determination of Microdosimetric Data.
Proc. 8th Symposium On Microdosimetry, Jülich 1982, in press

F. ZYWIETZ, H.G. MENZEL, D. VAN BEUNINGEN, R. SCHMIDT:
Biological and Microdosimetric Intercomparison of 14 MeV d-t Neutrons and 6 MeV Cyclotron Neutrons.
Int. J. Radiat. Biol. 42, 223 (1982)

II. G. DIETZE, S. GULDBAKKE, G. BÜHLER, H.G. MENZEL, H. SCHUHMACHER:
Correlated Microdosimetric, Dosimetric, Spectroscopic, and Fluence Measurements With Monoenergetic Neutrons between 14 MeV and 19 MeV.
Progress Report Neutronen Dosimetrie
Physikalisch-Technische-Bundesanstalt, July 1982

Progress Report
1982

Contractor:
Universität des Saarlandes
Stadtwald
D-6000 Saarbrücken

Contract no.: BIO-A-482-82-D

Head(s) of research team(s):

Prof. Dr. H. Muth
Institut für Biophysik
Der Univ. des Saarlandes
Boris Rajewsky Institut
D-6650 Homburg (Saar)

Dr. H.G. Menzel
Institut für Biophysik
der Univ. des Saarlandes
Boris Rajewsky Institut
D-6650 Homburg (Saar)

General subject of the contract:

Applicability of tissue-equivalent proportional counters in radiation protection dosimetry.

List of projects:

1. Principal and practical aspects of the applicability of tissue-equivalent proportional counters in radiation protection dosimetry of complex radiation fields.

Title of project nr. 1: Principal and practical aspects of the applicability of tissue-equivalent proportional counters in radiation protection dosimetry of complex radiation fields.

Head of project and scientific staff:
Dr. H.G. Menzel, Dr. R. Dudler, Dr. H. Schuhmacher

The application of microdosimetric proportional counters in routine radiation protection dosimetry requires considerable modification and simplification of the standard technique of experimental microdosimetry. Some of the uncertainties which may be introduced into the determination of dose equivalent H and effective quality factor $\bar{Q}$ were investigated for different types and energies of radiations.

Within the neutron energy range of 0.2 to 19 MeV we were able to show that a direct specification of the quality factor in terms of lineal energy y provides as good an approximation as any other method used to calculate effective quality factors from microdosimetric spectra. As the specification in terms of y is particularely simple it appears to be advantageous for applications in routine dosimetry. Moreover, the measurable quantity lineal energy y is directly related to the dose equivalent, the quantity used for risk assessments.

The influence of the resolution of the pulse height analysis on the precision in the determination of $\bar{Q}$ and H was studied using computer simulations of measurements. For monoenergetic neutrons between 0.2 and 19 MeV, for pure gamma-rays and for mixed radiations it was established that a pulse height analysis using a fixed pattern of only 16 channels provides results which are not significantly different to values obtained by more sophisticated methods. In Figure 1, $\bar{Q}$ evaluated from spectra for monoenergetic neutrons with the two approximations ($\bar{Q}$ specified in terms of y and a resolution reduced to 16 channels) is given as a function of neutron energy and compared to the curve of values suggested by ICRP Reprot 21.

A first comparison was made for values of dose equivalent H obtained by

the simplified microdosimetric method and other methods. At high neutron energies a very good agreement was found between results based on fluence measurements and the data from microdosimetry. The same holds for ionization chamber measurements with gamma-rays. A difference of about 50% was ascertained when compared to a commercial "rem"-counter in a mixed neutron and gamma-ray radiation.

The statistical uncertainty in determining H and $\overline{Q}$ may be a limiting factor in the use of proportional counters because of the usually quite low dose rate encountered in radiation protection conditions. An assessment was carried out on the relative standard deviation of H as function of absorbed dose and detector size. For instance, for a counter 1/2" in diameter a relative standard deviation of 10% is obtained for an absorbed dose of 10^{-5} Gy. By chosing a suitable detector diameter statistical uncertainty should not present a problem.

Microdosimetric measurements in phantom positions could only be performed for 15 MeV neutrons. At a depth of 2 cm in a 30 cm^3 water phantom the value for $\overline{Q}$ is 6% lower and for H 3% higher than in the free air position.

All investigations mentioned will be extended to cover lower neutron energies. Microdosimetric measurements at low energies down to thermal neutrons are scheduled for 1983. The results obtained to date have provided important criteria for the design of a routine instrument. Parts of the design are being completed and work on other details is in progress. The studies have been undertaken in co-operation with the Zentrale Einrichtung Strahlenschutz und Dosimetrie at Deutsches Krebsforschungszentrum, Heidelberg (Dr. Hartmann).

List of publications

I. HARTMANN, G., MENZEL, H.G., SCHUHMACHER, H., KRAUSS, O.:
Some studies on principal and practical aspects of the applicbility of ROSSI-type counters in radiation protection.
Proc. 8th Symposium on Microdosimetry, Sept. 1982, Jülich (in press)

MENZEL, H.G., HARTMANN, G., KRAUSS, O.:
Anwendung eines Detektors vom Rossi-Typ im Strahlenschutz
Proc. 16th Annual Meeting of Fachverband für Strahlenschutz,
Oct. 1982, München (in press)

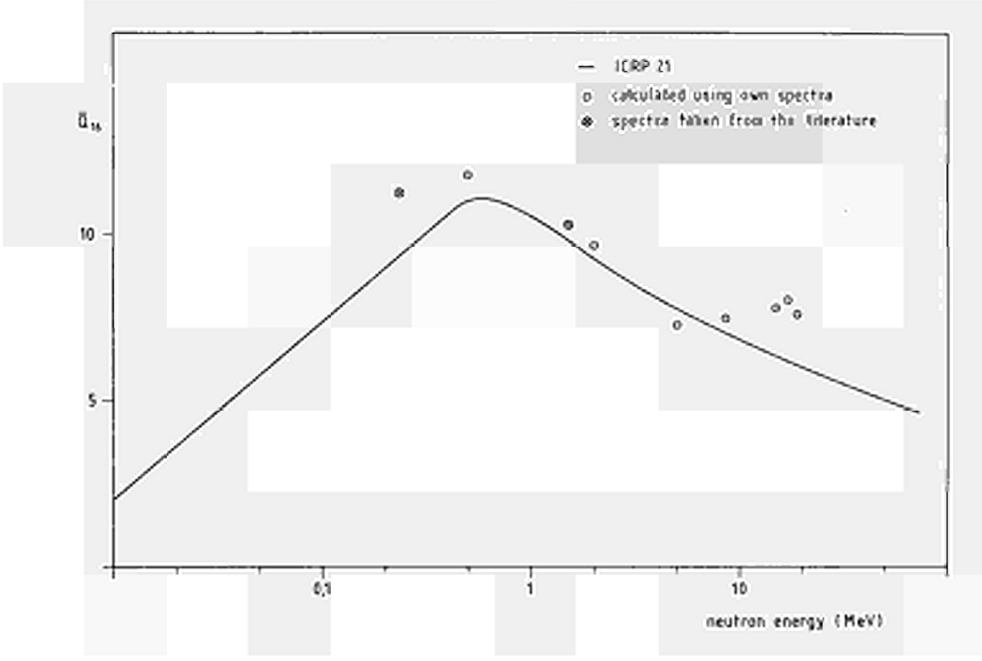

Figure 1: Effective quality factor $\bar{Q}$ as a function of neutron energy calculated from microdosimetric spectra for standard neutron beams using the two described approximations. The solid curve is from ICRP-Report 21.

Progress Report
1982

Contractor:
Commissariat à l'Energie
Atomique, CEA
CEN de Fontenay-aux-Roses
B.P. n°6
F-92260 Fontenay-aux-Roses

Contract no.: BIO-A-433-81-F

Head(s) of research team(s):
Dr. N. Parmentier
IPSN - DPR
CEA - CEN FAR
B.P. n°6
F-92260 Fontenay-aux-Roses

General subject of the contract:
Device for dose equivalent measurement in mixed fields.

List of projects:

1. Device for dose equivalent measurement in mixed fields.

Title of project nr 1

DEVICE FOR DOSE EQUIVALENT MEASUREMENT IN MIXED FIELDS

Head of project and scientific staff :

NGUYEN Van Dat

The first prototype of the dose equivalent meter has been tested during six months with the original gas filling (see figure 1).

The data processing unit consists of a single chip based on the Z 8 microprocessor. The 2 K resident utilitary programs allows to reduce the size of the external software located in a 4 K ROM.

The general organization for data processing unit, illustrated by figure 2, has been structured in order to obtain a fast data acquisition before storage in a 1 K RAM buffer.

The results of preliminary tests in monoenergetic neutron beams from 360 keV to 16.030 MeV are shown in figure 3 comparing with ICRP 21 recommended curve and with calculated values by STINCHCOMB and BORAK.

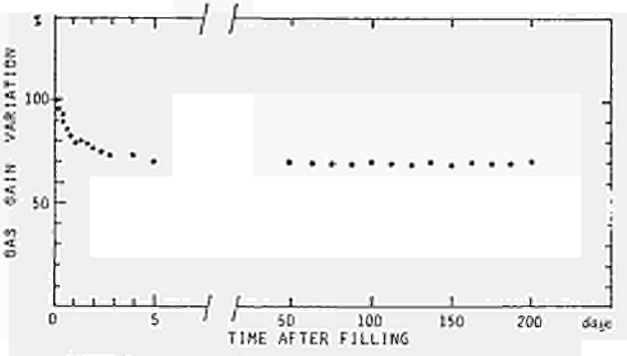

FIGURE 1 - GAS GAIN VARIATION VERSUS TIME AFTER FILLING

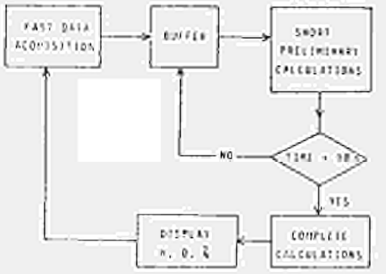

FIGURE 2 - GENERAL ORGANIZATION FOR DATA PROCESSING UNIT

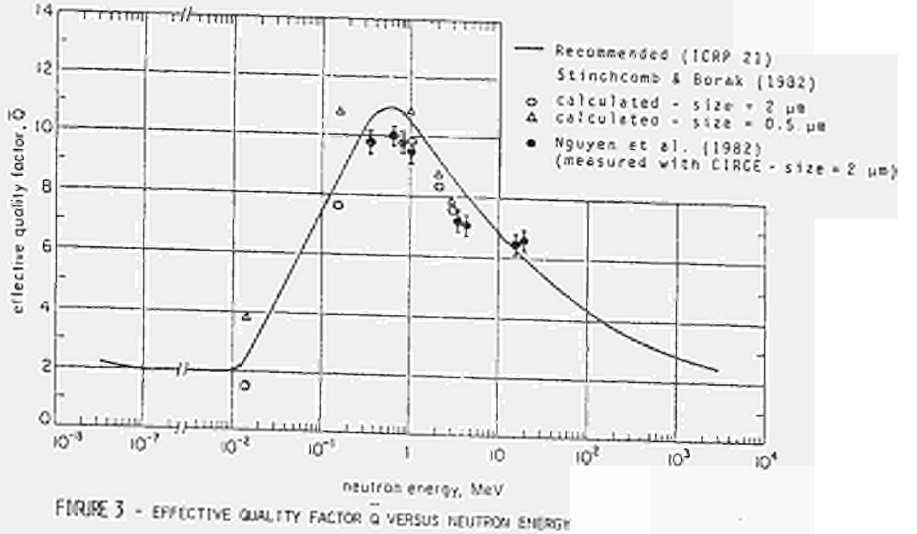

FIGURE 3 - EFFECTIVE QUALITY FACTOR $\bar{Q}$ VERSUS NEUTRON ENERGY

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

(néant)

II. Short Communications, Theses, Internal Reports, Patents. . .

NGUYEN V.D., LUCCIONI C., CHUITON R., CHAPUIS J.C., RICOURT A., MUNTEAN I. , PARMENTIER N.
State of progress in ambient dose equivalent meter device (CIRCE). Communication presented at the 8th Symposium on Microdosimetry at JULICH (R.F.A.) Sept. 1982.

Progress Report
1982

Contractor:

United Kingdom Atomic Energy
Authority, UKAEA
Charles II Street 11
GB-London SW1Y 4QP

Contract no.: BIO-A-305-81-UK

Head(s) of research team(s):

Dr. D.H. Peirson
Env.& Medical Sciences Div.
AERE
Harwell, Didcot
GB-Oxon OX11 ORA

General subject of the contract:

Radiation dosimetry and spectrometry.

List of projects:

1. Neutron spectrometry.
2. Development of a fast neutron personnel dosimeter using recoil ions.

Title of project nr 1 Neutron spectrometry

Head of project and scientific staff: H J Delafield
L H J Peaple
R Birch
K G Harrison

Introduction

Since neutron dosimetry is becoming increasingly important in the nuclear industry, as outlined in the last progress report, a neutron spectrometry system with high resolution is being developed for radiological protection measurements. It is based on a set of proportional counters filled with hydrogen at various pressures to cover the energy range from about 20 keV to 1 MeV. An organic scintillator (Harrison, 1981) will be used to extend the range to about 7 MeV.

The programme is divided into two parts;

(i) The establishment of a basic spectrometry system using spherical hydrogen proportional counters (Type SP2, diameter 4 cm). This system will be used to characterise reference radiation fields and to measure spectra in field situations where the fluence-rate is sufficient.

(ii) The development of a system using larger volume counters of higher sensitivity suitable for neutron spectra measurements in the field at about the maximum permissible flux level.

Basic spectrometry system (SP2 counters, diameter 4 cm)

The spectrometry system has been established using counters, which were manufactured and filled with hydrogen to pressures of 0.5, 1, 3 and 10 atm* by the Atomic Energy Establishment Winfrith (Dr A Goodings). The counters are operated with main amplifiers employing double delay line shaping with 10 μs lines.

An extensive set of measurements has been made to determine the experimental response functions for the 1, 3 and 10 atm counters. Effectively monoenergetic neutrons of energies 100 to 600 keV were generated at 0° by the ^{7}Li (p,n) ^{7}Be reaction, and from 600 keV to 1.9 MeV by the ^{3}H (p,n) ^{3}He reaction, using the IBIS Van der Graaff accelerator at Harwell. In addition accurate energy calibration measurements have been made for the 3 counters at selected neutron energies at the National Physical Laboratory.

The energy equation obtained with, for example the 3 atm counter was;

$E = (2.088 \pm 0.006)n - (9.1 \pm 1.3)$ keV where n is the channel number,

related to pulse height and ionisation. The energy corresponding to zero ionization is -9.1 keV, and the neutron energy equivalent to the ^{3}He (n,p)^{3}H reaction is 782 keV. The energy equations were obtained from the measured response functions which approximated to rectangular pulse height distributions. The channel number n_o corresponding to a neutron energy E_o

was determined accurately using a fitting technique which optimised n_o, the counter resolution and a straight line through a selected portion of the distribution.

The calibrated set of SP2 counters have been used to measure the spectrum in free air, at 20 cm from a 5 Ci Am-Li neutron source loaned by NPL. The spectrum was derived using the unmodified unfolding code SPEC 4 which employs calculated response functions adjusted by functions related to earlier experimental measurements. Comparison of these functions with the results of present measurements is proceeding.

High sensitivity spectrometry system

To achieve a higher detection sensitivity, counters of larger volume than the type SP2 (diameter 4 cm) are required. Exploratory measurements were therefore undertaken using a scaled up design of this spherical counter known as a type SP6 (diameter 15 cm). Initial trials with such a counter showed that it should be possible to operate at an adequate gas gain and filling pressure. However the pulse height distribution consisting of the calibration peak and tail given by the ^3He (n,p)^3H reaction showed some distortion. An attempt to overcome this by the addition of 5% methane to the hydrogen filling to improve the counter pulse shape was tried but was unsuccessful. Hence an investigation was begun into the characteristics of large volume cylindrical counters.

A cylindrical counter of about 5 cm diameter and 30 cm long was used for the investigations. Trial fillings of 0.8 atm methane, and 0.8 atm hydrogen plus 5% methane, both gave an energy resolution of about 3% based on the ^3He (n,p)^3H reaction resulting from a trace of helium gas in the counter.

The greatest disadvantage in adopting a cylindrical counter for field spectrometry is that unlike the spherical counter its response is no longer isotopic. Hence exploratory measurements have been made of the response function of the counter to monoenergetic neutrons as a function of the angle of incidence of the neutron beam. With the counter filled with 2.4 atm hydrogen, 5% methane and a trace of helium measurements were made with monoenergetic neutrons of energies 250, 500 and 700 keV (within the measurement range of the counter) and at 1.2 MeV (above measurement range). These results are currently being assessed. The response functions at 250 and 500 keV are not very dependent upon the angle of incidence, but at higher neutron energies a greater variation in the response function is observed.

References

Harrison, K. G. (1981), AERE-R 9780.

* 1 atm ≃ 0.101 MPa

Title of Project nr 2 Development of a Fast Neutron Personnel Dosimeter Using Recoil Ions

Head of project and scientific staff: K G Harrison
Miss R M Haigh

Work during 1982 has primarily concentrated on the collaborative testing of new samples of CR-39 plastic manufactured by Bristol University and Pershore Mouldings Ltd in progressively better-controlled clean conditions, which have included extensive filtering and outgassing of the monomer prior to casting. Whole sheets of plastic were tested for uniformity of response (to ^{252}Cf fission neutrons) and variability in background, when processed using electrochemical etching. Some of the sheets now being produced would be acceptable for operational use and work is now directed towards the manufacture of substantial batches of sheets to the acceptable standard.

We have also collaborated with Dr Portal's Group (Fontenay-aux-Roses) in an "Intercomparison of Ion Recoil Neutron Dosimeters" organised through a working group of CENDOS. Irradiations with ^{252}Cf fission neutrons and at monoenergetic energies between 0.29 MeV and 15.2 MeV were undertaken in France. We used the same CR-39 plastic and processing conditions as in our earlier study at AERE (1). The results were in good agreement (± 25%) with predictions made from our earlier work, except at one energy (3.2 MeV) where there was an unexplained discrepancy of a factor of four, which is still being investigated. We were also able to determine that the plastic did not age significantly over a period of one year between manufacture and exposure, and that fading (of latent tracks prior to processing) was not significant up to at least seven months. We could not detect any build-up of background due to radon over a period of eighteen months with the plastic stored in polyethylene bags. We also noted that linearity of response with dose existed with electrochemical etching up to at least 15 mSv.

A design study for a prototype operational CR-39 dosimeter and processing system has been started. There appear to be no major difficulties in designing a simple, safe and efficient multiple electrochemical etching apparatus.

Theoretical work to understand the response of the plastic to neutrons when processed by electrochemical etching will now be undertaken in 1983.

Reference

(1) K.G. Harrison and C.A. Perks. "Neutron Dosimetry Studies using CR-39 Plastic", Proc. 11th Intl. Conf. on Solid State Nuclear Track Detectors Bristol 1981 (Pergamon Press, Oxford) p.461-464.

Publications

CENDOS Report on the Intercomparison of Ion Recoil Neutron Dosimeters (Medioni, Lembo and Harrison) is in preparation.

Progress Report
1982

Contractor:
United Kingdom Atomic Energy
Authority, UKAEA
Charles II Street 11
GB-London SW1Y 4QP

Contract no.: BIO-A-306-81-UK

Head(s) of research team(s):
Dr. D.H. Peirson
Env.& Medical Sciences Div.
AERE
Harwell, Didcot
GB-Oxon OX11 ORA

General subject of the contract:

Studies in microdosimetry, cellular radiobiology and track structure.

List of projects:

1. Cellular radiobiology.
2. Track parameters of ionizing radiations.

Title of project nr 1 Cellular radiobiology

Head of project and scientific staff: P D Holt
G J Cambray
J A B Gibson
Miss B Hindmarsh
Mr C J Roberts

The aim of this work is to obtain experimental data on the radiation responses of cells (reproductive death, mutation, chromosome aberration and transformation) which may be used to construct and test theoretical models of radiation response (Contract No. BIO-E-460-81-UK). Such models are necessary in order to predict the risk of carcinogenesis or hereditary damage from exposures of populations to very low doses or at very low dose-rates.

A mutation assay involving the inactivation of the HGPRT locus has been developed and validated for BHK/21 Cl. 13 cells, and the results compared with the equivalent assay for V79/4 cells.

The two assay systems are remarkably similar with respect to response to γ-radiation, although V79 cells are aneuploid with 20 chromosomes and BHK diploid with 44 chromosomes. This work is being prepared for publication in Mutation Research. Some preliminary work on neutron-induced mutation in these systems has been undertaken.

It was hoped that an assay for radiation-induced cell transformation could be set up with the BHK cells, based on the ability of transformed cells to grow in soft agar. However, as in other laboratories which have attempted to use this system, it has not been possible to obtain sufficient reproducibility. A clone of C3H/10T½ cells has been obtained and some initial transformation experiments have been carried out. The results are promising.

A project has recently begun to investigate the involvement of cell surface proteins in the process of radiation-induced transformation, using cell fractionation, affinity chromatography, electrophoresis and electro-focussing. Two putative transformation-specific surface glycoproteins have so far been detected.

The studies on radiation-induced chromosome aberration in V79 cells have been followed up using the BHK cells, and a value has been found for the RBE of neutrons similar to that found for V79 cells. The correlation between cell killing and asymmetrical exchanges is very consistent. This work is at present being prepared for publication.

To simplify asymmetrical exchange detection a new assay is under development based on the micronucleus test of Heddle (Mutat. Res. 18, 187, 1973). This test relies on the fact that acentric fragments are released from the nucleus after the second division and form a micronucleus; this can be detected by staining methods and is indicative of an asymmetrical exchange event.

Publications

Roberts C.J. and Holt P.D. The production of chromosome aberrations in Chinese hamster fibroblasts by gamma and neutron radiation Int. J. Radiat. Biol. 41. 645-656.

Roberts C.J., Carver S.K. and Holt P.D. Partial characterisation of neutron induced HGPRT mutants of V79/4 Chinese hamster fibroblasts. To be submitted to Int. J. Radiat. Biol.

Roberts C.J. and Holt P.D. The effect of incubation conditions on the radiosensitivity of BHK 21 C13 Syrian hamster fibroblasts. To be submitted to Int. J. Radiat. Biol.

Holt P.D. and Roberts C. J. RBE values for chromosome aberrations and survival in BHK 21 C13 Syrian hamster fibroblasts exposed to high and low LET radiations. To be submitted to Int. J. Radiat. Biol.

Title of the project nr. 2

TRACK PARAMETERS OF IONIZING RADIATION

Head of Project and scientific staff : M Marshall
T Budd
G P Stonell

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1. Introduction

The cloud chamber is designed primarily to investigate the spatial distribution of ionizations in the tracks of charged particles. The aim is to produce data and parameters which are relevant to biological modelling (e.g. project 1) and microdosimetry (e.g. contract no. BIO-A-460-81-UK)

2. Analysis of Electron and Alpha-particle Tracks

Work in the improved T.E. gas mixture developed in 1980 has been completed for the present. Detailed results are presented in two papers to be published in Radiation Research.

3. Cloud Chamber Operation with Pure Water Vapour

Work to this end has progressed slowly. Various faults developed in the chamber and associated equipment so that a major overhaul was required. Nevertheless conditions were achieved which gave pictures of distinct clusters of droplets produced by aluminium x-rays with a low density of background droplets. Subsequently the background has been considerably greater but the cause has not yet been established. However running the chamber on pure water has such great advantages that work in this direction is continuing. Experimentally it overcomes the problems of ensuring that the chamber contains the correct mixture. Theoretically it enables direct comparisons with calculated distributions. We intend to obtain results for low-energy electrons (from x-rays), alpha particles below 5 Mev and protons below about 350 keV.

Considerable interest has been expressed in this study by others in the field. Discussions have taken place with Wilson (Battelle) and Brenner (Los Alamos) as well as with members of the European Dosimetry Group. The heavy-ion Monte-Carlo program of Wilson which uses the electron track program of Paretzke (GSF, Munich) has been installed at Harwell. It will be

used to generate proton and alpha-particle distributions for comparison with experimental cloud-chamber data.

4. Preparations for a Study of Proton Tracks

Work to attach the chamber to a 560 keV Van der Graaf accelerator is nearly complete. A scattering chamber has been made to reduce the proton flux to the required few protons per millisecond. A series of cloud chamber entrance windows are being calibrated for energy losses so that the proton energy on entry to the chamber is known.

Publications.

BUDD T. and MARSHALL M., Microdosimetric properties of electron tracks measured in a low-pressure cloud chamber. Radiat. Res.93,19-32(1983) (In press).

BUDD T., KWOK C.S., MARSHALL M. and LYTHE S., Microdosimetric properties of alpha-particle tracks measured in a low-pressure cloud chamber. Radiat. Res. (In press).

BUDD T., MARSHALL M. and KWOK C.S., Microdosimetric properties of particle tracks measured in a low-pressure cloud chamber, Proc. Eighth Symposium on Microdosimetry, Julich, September 1982 (In press).

Progress Report
1982

Contractor:
United Kingdom Atomic Energy
Authority, UKAEA
Charles II Street 11
GB-London SW1Y 4QP

Contract no.: BIO-A-459-81-UK

Head(s) of research team(s):
Dr. D.H. Peirson
Env.& Medical Sciences Div.
AERE
Harwell, Didcot
GB-Oxon OX11 ORA

General subject of the contract:
Radiation dosimetry and spectrometry.

List of projects:

1. Examination of the photo transfer process in TL materials.

Title of the project nr. 1

PHOTO-TRANSFER IN THERMOLUMINESCENT DOSIMETRY

Head of Project and scientific staff : M Marshall
J A Douglas
D M Baker

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1. Introduction.

This work is concerned with the use of U.V. photo-transfer in various aspects of thermoluminescent dosimetry. These include determining the optimum conditions for dose re-estimation in thermoluminescent LiF, using re-estimation as a means of measuring doses at high ambient temperatures and using U.V. radiation to reduce the background of LiF TLD's during sensitization. Glow curves are recorded for analysis with a view to obtaining a coherent picture of the effects.

2. Current state.

Little work on this project has been possible in 1982 because of other pressures. Methods of recording glow curves are being improved using a microcomputer coupled to the TLD reader. Techniques for cleaning the LiF TLD's are being investigated to try to reduce spurious readings and improve the precision of measurement.

Optical emission spectra as a function of readout temperature have been obtained for us by the University of Sussex for various TL materials which had been subjected to a range of treatments. In particular they have studied dosimeters from the same batch and given the same treatment as used in our experiments. Results show that the optical spectra do not change significantly from one glow peak to another in the same material. Although this simplifies the analysis it means that desireable glow peaks cannot be enhanced by a suitable choice of optical filter and photomultiplier tube in the TLD reader.

Further experimental studies and the analysis of glow curves will continue in the next year.

Progress Report
1982

Contractor:
Commissariat à l'Energie
Atomique, CEA
CEN de Fontenay-aux-Roses
B.P. n°6
F-92260 Fontenay-aux-Roses

Contract no.: BIO-A-292-81-F

Head(s) of research team(s):
Dr. G. Portal
Serv.Techn.d'Equipt.de Prot.
CEA - CEN FAR
B.P. n°6
F-92260 Fontenay-aux-Roses

General subject of the contract:
Dosimetry of neutrons around installations using or processing nuclear fuel.

List of projects:
1. Design and construction of individual neutron dosimeters.
2. Design and construction of a portable environmental neutron dosimeter.

Title of project nr 1 -

Etude et réalisation de dosimètres individuels neutrons.

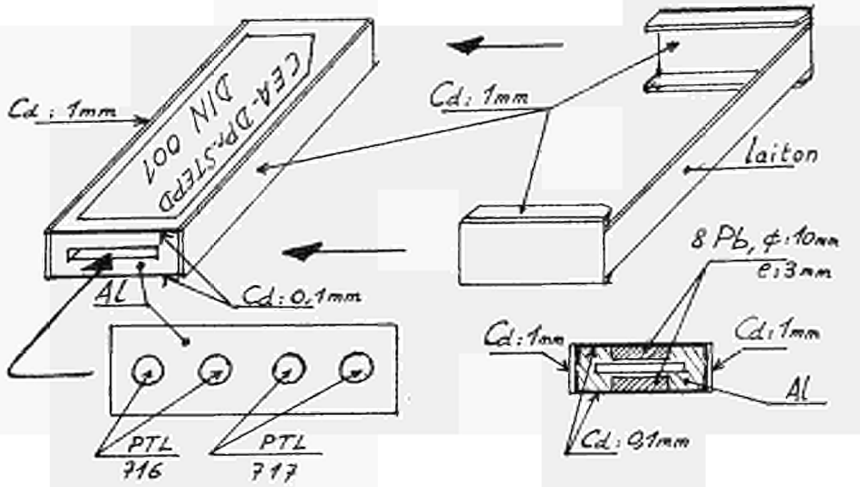
Chef du projet : M. BUXEROLLE

Participants : M. BUXEROLLE, R. MEDIONI

Deux voies sont prospectées pour l'étude des dosimètres individuels faisant intervenir l'effet d'albédo : les produits thermoluminescents (TLD) et les détecteurs solides de traces (DST).

Dosimétrie par TLD -

La fabrication du boîtier du dosimètre individuel a été réalisée en petite série selon le croquis ci-dessous :



Longueur : 55 mm - Largeur : 28 mm

Epaisseur : 10 mm - Masse : 56 g avec plomb
35 g sans plomb

Les huit disques de plomb placés de part et d'autre des pastilles thermoluminescentes ne sont utiles que dans les installations de technologie du plutonium.

Des essais systématiques du dosimètre ont été entrepris auprès de sources étalonnées, auprès de PWR ainsi que dans des ateliers de technologie du plutonium.

Le dosimètre a participé au "Joint American-European Personnel Monitoring Intercomparison 1982".

L'équivalent de dose est calculé par la relation: $H = F.(\overline{L_6} - \overline{L_7})$

$\overline{L_6}$ et $\overline{L_7}$ sont les moyennes des lectures (en röntgen équivalent ^{60}Co) sur les PTL 716 et 717; F est le facteur de conversion en rem/R.

Avec le filtre de cadmium de 0,1 mm d'épaisseur, F = 0,04 rem/R pour des énergies de neutron E_n, inférieures à 10 keV, et la réponse est plate. Le but recherché : utilisation du dosimètre comme complément des émulsions nucléaires, semble donc assez correctement atteint.

Par contre pour E_n>10 keV, la dépendance énergétique est forte et nécessite si le dosimètre est seul un étalonnage particulier pour chaque condition d'utilisation.

Pour des spectres très dégradés (^{252}Cf avec 15cm d'eau lourde; environnement d'un PWR) : F ≃ 0,5 rem/R.

Pour un spectre de fission avec des écrans : F ≃ 1 à 2,5 rem/R

Pour un spectre de ^{252}Cf sans écran : F ≃ 5 rem/R

Pour un spectre de source Am-Be : F ≃ 9 rem/R

Parallèlement, des études ont été faites sur la réponse des PTL 716 aux neutrons lents : facteur d'autoprotection, influence de l'orientation du PTL dans un courant de neutrons.(1),(2).

Dosimétrie par DST -

Le détecteur utilisé est du nitrate de cellulose CN-85 (épaisseur 10 µm) recouvert d'une couche de tétraborate de lithium.

En 1982 une étude de dosimétrie dans des laboratoires de Fontenay-aux-Roses traitant des matériaux transuraniens a conduit à l'utilisation de ce dosimètre pour les travailleurs de ces zones (3). Les résultats principaux sont les suivants : Le coefficient d'étalonnage (trace-équivalent de dose) en 6 points différents varie d'un facteur 1 à 3. Les équivalents de dose sont calculés à partir d'une spectrométrie par le système multisphère. Dans ces conditions la limite inférieure de détection atteint 70 mrem environ. L'utilisation d'un convertisseur au bore naturel améliore d'un facteur 4 la sensibilité de détection.

Le tableau ci-dessous résume les résultats obtenus :

Postes de travail	1	2	3	4	7	8
Réponse avec tétraborate de lithium (trace/cm2 pour 1 mrem)	55,3	42,2	40,3	31,4	30,2	89,2
Réponse avec bore naturel (trace/cm2 pour 1 mrem)	195	163	147	174	118	376

Title of project nr 2 -

Etude et réalisation de dosimètres d'ambiance à neutrons portatifs.

Chef du projet : M. MOURGUES

Participants : M. MOURGUES, R. MEDIONI

La détection des neutrons au niveau de l'ambiance nécessite de recourir, afin d'obtenir une certaine sensibilité, à des capteurs du type à "modérateur". Ce principe, lorsqu'il est appliqué à des rem-mètres de conception traditionnelle (courbe de réponse équivalente à la fonction d'équivalent de dose $\hat{H}$) conduit à des appareils lourds et volumineux. Un nouveau concept de dosimètre est né de l'analyse des champs de rayonnement neutronique dans les installations présentant ce risque, en particulier les centrales PWR. Un des projets issus de ce concept, le RIF, en a parfaitement démontré la validité à savoir : un gain de sensibilité mais surtout un gain de poids. Il est toutefois apparu qu'un capteur plus sensible permettrait de couvrir parfaitement la gamme de mesures pour ce type d'installation.

Réalisation du prototype -

En conservant la même structure "à plateaux" que pour le modèle précédent, le diamètre de la chambre à fission a été porté à 60 mm pour une même longueur de 120 mm. La charge massique en ^{235}U qui était de 2 $mg.cm^{-2}$ a été réduite à 1,37 $mg.cm^{-2}$ afin d'améliorer la pente de la fonction de discrimination. Pour une augmentation de la masse du dépôt fissile d'un facteur 3 (2492 mg contre 810 mg), la sensibilité est multipliée par 4, ce qui réduit d'autant le seuil de détection de l'appareil. La gamme de mesure est désormais comprise entre 1 $mrem.h^{-1}$ et 10 $rem.h^{-1}$ pour des spectres dégradés tels que ceux rencontrés dans l'enceinte de confinement des centrales PWR.

En ce qui concerne l'électronique, elle est dans son ensemble identique à celle du prototype précédent. Les modifications portent sur l'affichage qui est maintenant du type alphanumérique à double système d'unités (mrem et mSv).

Le poids de l'appareil n'est pratiquement pas modifié (2 kg).

Essais du prototype -

. Référence Am-Be et Cf 252 : la sensibilité obtenue est respectivement de 90 et 100 impulsions par mrem.

. Source Silène de Valduc : le réacteur solution Silène de Valduc constitue en France une référence pour les études de dosimétrie. Le point de mesure étant situé à 6 m du réacteur, la réponse du RIF a été trouvée égale à 3200 impulsions par mrem, équivalente à celle des rem-mètres du commerce.

. Enceinte de confinement des centrales PWR : la sensibilité de détection est désormais de 20 000 impulsions par mrem pour ce type de mesure, ce qui représente un gain d'un facteur 6 par rapport aux rem-mètres traditionnels.

Conclusion -

Destiné à la dosimétrie de routine au niveau de l'ambiance dans les installations industrielles à risque neutronique, ce nouvel appareil possède désormais le capteur qui le rend apte à couvrir la gamme de mesure comprise entre 1 $mrem.h^{-1}$ et 10 $rem.h^{-1}$ avec une précision satisfaisante.

List of publications in 1982

I - Publications in Scientific Journals.

(1) The response of LiF Thermoluminescent detectors to thermal neutrons. Frantisek SPURNY et Michel BUXEROLLE - à paraître dans Jaderná Energie-Tchécoslovaquie.

(2) Influence of light transmission on the response of LiF Thermoluminescent detectors to thermal neutrons. Michel BUXEROLLE et Frantisek SPURNY - à paraître dans Radiation Protection Dosimetry.

II - Short Communications, Internal Reports,

(3) Dosimétrie par détecteurs solide de traces dans un laboratoire de transuraniens de Fontenay-aux-Roses.
R. MEDIONI, F. BERMANN, J.M. BORDY. (en préparation)

Progress Report
1982

Contractor:
Commissariat à l'Energie
Atomique, CEA
CEN de Fontenay-aux-Roses
B.P.n° 6
F-92260 Fontenay-aux-Roses

Contract no.: BIO-A-483-82-F

Head(s) of research team(s):
Dr. G. PORTAL
Serv.Techn.d'Equip.de Prot.
CEA - CEN FAR
B.P.n° 6
F-92260 Fontenay-aux-Roses

General subject of the contract:
Development of a personal substitution technique in the event of accident gamma irradiation.

List of projects:

1. Dosimetry in the event of accidental gamma irradiation using clothes - Phenomenological study.
2. Dosimetry in the event of accidental gamma irradiation using clothes.

Title of project n° 1 DOSIMETRIE EN CAS D'IRRADIATION GAMMA ACCIDENTELLE A PARTIR DE VETEMENTS. ETUDE PHENOMENOLOGIQUE.

Head of project and scientific staff: P.KELLER, P.IACCONI
D.LAPRAZ .

Le travail qui a été réalisé cette année a eu essentiellement pour but l'étude phénoménologique et l'étude de faisabilité d'une dosimétrie accidentelle dans le cas de personnes ne portant pas de dosimètres individuels. Le produit constituant le détecteur de base qui a été choisi pour sa présence très fréquente dans l'habillement des personnes susceptibles d'être irradiées, est le coton sous la forme de tissu de coton commercial. Ce choix a été en outre guidé par le fait que le coton a une structure cristalline relativement bien connue et se prête à un certain nombre de techniques d'investigation simples dont l'émission exoélectronique.

Cette dernière a été choisie pour sa grande sensibilité en dosimétrie des rayonnements: à titre d'exemple la sensibilité de l'oxyde de Beryllium est de 4.E4 électrons/uGy (1).

La première partie du travail a été consacrée à une analyse bibliographique des propriétés cristallographiques et physiques du coton, poursuivie par l'analyse de ses propriétés après irradiation par des rayonnements ionisants: UV lointains, rayons X et gamma. Avant tissage le coton subit un certain nombre de traitements chimiques dont le plus important, puisque il modifie sa structure intrinsèque, est le mercerissage. Ce dernier consiste en un trempage en milieu basique (18-30% NaOH) suivi d'un rinçage à l'eau. Le coton mercerisé cristallise dans le système monoclinique avec pour formule condensée ($C_6H_{10}O_5$)n où n>3500. Le coton devient plus brillant, plus résistant et son affinité tinctoriale est améliorée. De nombreux autres traitements chimiques sont effectués tels que l'encollage ou le paraffinage, l'ennoblissement, le blanchissement, la teinture ou l'impression et enfin l'apprêt. La très grande diversité des traitements et produits utilisables, 1300 colorants directs, 400 colorants de cuve et 500 colorants réactifs permettent plus de 15000 combinaisons (2).

La température de décomposition du coton (chaine cellulosique) est d'environ 265°C, sa dégradation ne peut être observée par les méthodes physiques classiques (RMN, absorption UV, diffraction X,...) qu'à la suite de fortes irradiations (1.E4 Gy).

Les échantillons mesurés (plusieurs centaines) ont été obtenus à partir de 4 tissus de coton au passé différent.

Le dispositif expérimental utilisé a été mis au point dans le cadre du projet II. Une première série de mesures a montré que seule une double stimulation (c.à.d. thermique et optique) donne un signal exoémissif caractérisé par un pic situé à 240°C lors d'un chauffage lent (0,25°C) (figure 1).

Les principales constatations effectuées sont les suivantes:
*le signal n'apparait qu'en double stimulation(D.S)(3).
*le passé de l'échantillon influence le signal, en particulier les contraintes mécaniques subies. Certains échantillons présentent une très forte triboémission.
*la dispersion des mesures due à cette dernière est particulièrement importante (± 200%).
*l'influence des agents extérieurs sur la rèponse (humidité, air, etc...) ne sont pas significatifs.

Nous avons également constaté que la réponse èlectronique croît avec l'énergie des photons de stimulation.

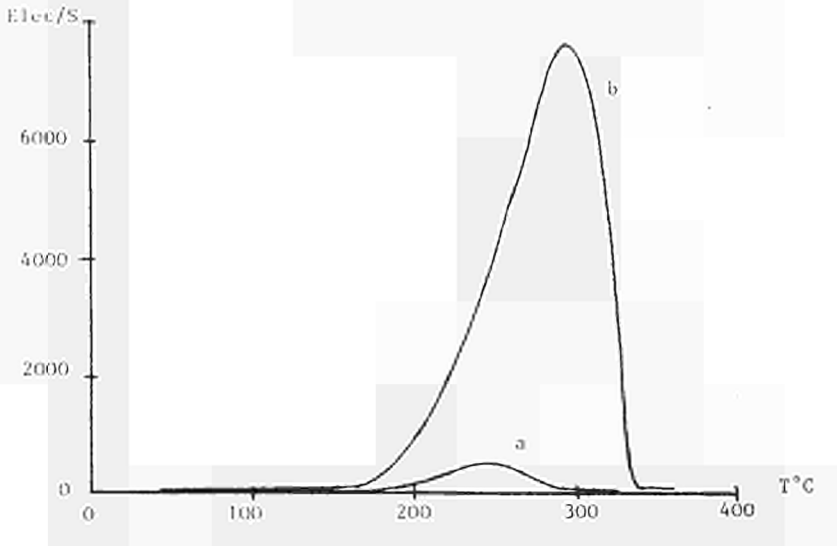
A l'issue de ces résultats un certain nombre d'observations, en particulier la vitesse de chauffage, montrent que l'émision èlectronique n'est pas une émission exoélectronique au sens conventionnel du terme, mais que la double stimulation faisant intervenir deux processus simultanés thermique et optique, agit plutôt comme un révélateur des modifications de la structure interne naturelles ou engendrées par l'irradiation ou les contraintes mècaniques. La trop forte dispersion des mesures effectuées jusqu'à ce jour ne nous a pas permis de corréler dans tous les cas l'intensité de l'émission et la dose pour des doses faibles < 25Gy (apparition d'un pic supplémentaire).

(1) BARTHE J., PETEL M. et PORTAL G.:
Perspectives de l'émission exoélectronique en microdosimétrie 8th Symposium on Microdosimetry, 27 Sept.-1 Oct. Julich (1982).

(2) FREYTAG R.:
"Communication personnelle" (1982),
Centre de Recherches Textiles de Mulhouse (France).

(3) BARTHE J., IACCONI P., LAPRAZ D., PETEL M., PORTAL G. et KELLER P.: " Electron emission of cotton fibers ",
VIIth Inter. Sympos. on Exoelec. Emission and Applications, March 16-18 (1983) Strasbourg FRANCE (à paraître).

Figure 1 - Emission par double stimulation d'un tissu de coton blanc :
a : non froissé b : froissé



List of publications in 1982

I Publications in scientific journals, monographies, proceedings

II Short communications, Theses, Internals Reports, Patents...

BARTHE J.,IACCONI P.,LAPRAZ D.,PETEL M.,PORTAL G.,KELLER P.:
" Electron emission of cotton fibers "
VIIth. Inter. Symp. on Exoelec. Emission,
March 16-18, Strasbourg, (1982), France, (à paraitre).

COMBY G.,PETEL M.,QUIDORT J. et BARTHE J.
" Utilisation d'un compteur multipointes à focalisation cathodique pour la détection de l'émission exoélectronique "
VIIth. Inter. Symp. on Exoelec. Emission,
March 16-18, Strasbourg, (1982), France, (à paraitre).

Title of project n° 2 DOSIMETRIE EN CAS D'IRRADIATION GAMMA A PARTIR DE VETEMENTS.

Head of project and scientific staff: J.BARTHE, M.PETEL, H.EL AJOUZ .

Le travail réalisé cette année a essentiellement porté sur la fabrication d'un lecteur d'exoélectrons à double stimulation (DS). La technologie utilisée découle des compteurs à pointes réalisés au laboratoire par Pétel (1). Les paramètres qui ont été pris en compte pour sa réalisation ont été les suivants:

- Compteur à pointe à circulation de méthane.
- Support à très faible taux de photoionisation.
- Chauffage programmable de l'ambiance à 400°C.
- Stimulation optique de 200 à 1000 nm.
- Interface d'acquisition et de pilotage de l'ensemble du système par ordinateur.
- Programmes de développement.
- Irradiations in situ ou in vitro (béta, X, gamma).

Les échantillons utilisés se présentent sous la forme de disques de tissus (100% coton) extraits de plusieurs sources différentes ayant 17mm de diamètres. La température de stimulation varie de façon linéaire (0,1 à 2°C/S), la stimulation optique est réalisée à l'aide d'une lampe à halogène dont le spectre d'émission est limité par des filtres "passe-haut", la coupure basse variant entre 250 et 400nm.

Les essais du compteur ont été réalisés à partir de matériaux bien connus comme l'alumine et l'oxyde de béryllium (2). La stabilité se révèle être meilleure que $\pm$ 2%, le bruit de fond, de quelques centaines d'évènements pour l'oxyde de béryllium est moins d'une dizaine pour un échantillon inerte tel que la silice vitreuse mesurée dans des conditions analogue au tissu de coton (3).

La figure 1 présente une vue en coupe du compteur à double stimulation.

Une attention particulière a été portée sur l'influence de la longueur d'onde de coupure basse sur la réponse de l'échantillon. Une relation croissante entre l'intensité du signal électronique et l'énergie du rayonnement de stimulation a été mise en évidence, il s'agit d'une relation à caractère exponentiel de la forme : $N = No*\exp(k.E)$ où $k = 1,44$ (figure 2).

La relation entre l'intensité du signal et la dose n'a pu être correctement établie par suite de la dispersion des mesures elle-même due en grande partie au passé de l'échan-

tillon. On peut, en froissant plus ou moins l'échantillon avant la mesure, augmenter d'un facteur 3 à 4 l'amplitude du signal mesuré (4). Dès maintenant un certain nombre d'essais sont envisagés pour éliminer cette triboémission dont on estime que l'origine aléatoire est en partie due aux charges électriques créées à l'occasion des diverses manipulations de l'échantillon. La résolution de ce point est primordiale.

(1) PETEL M.:
"Recherches sur la dosimétrie par émission exoélectroniques stimulées", Rapport CEA-R-4754 (1976).
(2) GUILHOT B., PETEL M., ROGER A.M. et THOMAS G.:
"Etude de l'excitation mécanique d'une alumine alpha par émission exoélectronique thermostimulée"
Microscopic aspects of adhesion and lubrification, pp 507-520 . Elsever Scient. Publish. Comp. (1982) .
(3) RAKOTOMALA R.:
Thèse de 3°Cycle, n°2607, Toulouse (FRANCE), (1982).
(4) BARTHE J., IACCONI P., LAPRAZ D., PETEL M., PORTAL G. et KELLER P.:
" Electron emission of cotton fibers "
VIIth Inter. Symp. on Exoelec. Emission,
March 16-18, Strasbourg, (1983), FRANCE, (à paraitre).

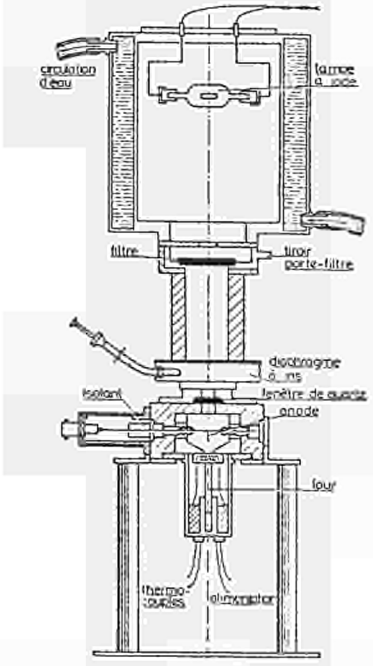


Figure 1 - Schéma du compteur à double stimulation optique et thermique -

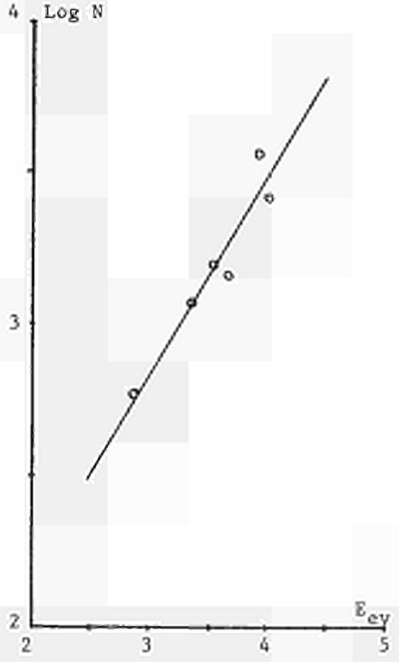


Figure 2 - Relation entre le logarithme nombre d'électrons et l'énergie des photons de stimulation -

List of publications in 1982

I Publications in scientific journals, monographies, proceedings

II Short communications, Theses, Internals Reports, Patents...

BARTHE J.,IACCONI P.,LAPRAZ D.,PETEL M.,PORTAL G.,KELLER P.:
" Electron emission of cotton fibers "
VIIth. Inter. Symp. on Exoelec. Emission,
March 16-18, Strasbourg, (1982), France, (à paraitre).

COMBY G.,PETEL M.,QUIDORT J. et BARTHE J.
" Utilisation d'un compteur multipointes à focalisation cathodique pour la détection de l'émission exoélectronique "
VIIth. Inter. Symp. on Exoelec. Emission,
March 16-18, Strasbourg, (1982), France, (à paraitre).

Progress Report
1982

Contractor:

Commissariat à l'Energie
Atomique, CEA
CEN de Fontenay-aux-Roses
B.P. n° 6
F-92260 Fontenay-aux-Roses

Contract no.: BIO-A-505-82-F

Head(s) of research team(s):

Dr. G. Portal
Serv.Techn.d'Equipt.de Prot.
CEA - CEN FAR
B.P. n° 6
F-92260 Fontenay-aux-Roses

General subject of the contract:

Individual soft-beta rays dosimetry using exoelectronic emission.

List of projects:

1. Individual soft-beta rays dosimetry using exoelectronic emission.

Title of project n° 1 DOSIMETRIE INDIVIDUELLE DES RAYONNEMENTS BETA DE FAIBLE ENERGIE PAR EMISSION EXOELECTRONIQUE

Head of project and scientific staff : M.PETEL, J.BARTHE
C.WIETERS .

Notre laboratoire s'est, depuis plusieurs années, spécialisé dans la détection de l'émission exoélectronique thermo et photostimulée (1). Les dosimètres à émission exoélectronique peuvent être conçus " équivalent tissu " en utilisant un support adéquat. L'épaisseur de leur couche sensible, très mince, est comprise entre 1 et 10 nanomètres.

Certains matériaux comme le fluorure de lithium et l'oxyde de béryllium sont très sensibles et peuvent apporter une solution à la détection des rayonnements de faibles énergies. Ces deux matériaux ont été sélectionnés pour la réalisation de cette étude.

I) L'oxyde de béryllium.

Ces dosimètres sont étudiés au laboratoire du Professeur A.SCHARMANN de l'Université de Giessen (RFA) avec qui nous travaillons en étroite collaboration.
Ils sont préparés par évaporation sous vide partiel et sous atmosphère oxydante, d'une couche de beryllium métal déposée sur un support en graphite (2).

La couche sensible de BeO ainsi créée a une épaisseur de l'ordre d'une centaine de nanomètres. Ces dosimètres sont contrôlés systématiquement depuis quelques années. Ils se sont avérés stables. A titre d'exemple: un dosimètre irradié 60 fois à une dose de 40 uGy, présente un écart type des mesures de 2%. L'écart type des mesures faites sur un lot de détecteurs irradiés à la même dose est de 15%. La courbe caractéristique de l'émission exoélectronique thermostimulée (EETS) est représentée sur le thermogramme de la figure 1.

II) Le fluorure de lithium.

Ces dosimètres sont étudiés et mis au point en collaboration avec le Service de Protection contre les Radiations du Centre d'Etudes Nucléaires de Grenoble (3). Ils sont fabriqués par compression et frittage d'un mélange intime de fluorure de lithium et graphite (95% FLi et 5% C). Ils sont réalisés dans les dimensions suivantes:
Ø=7,5,3mm , épaisseur 0,4 à 0,5 mm.

Le fluorure de lithium choisi (PTL 717) est couramment utilisé dans notre laboratoire pour la dosimétrie du personnel par thermoluminescence (TL).

La reproductibilité des mesures effectuées sur un même échantillon irradié à une dose absorbée de 60 mGy avec les rayonnements béta du 90Sr + 90Y est comprise entre 0,9 et 1,7%.
La reproductibilité entre dosimètres est localisée autour d'un écart type de 3 à 4%.

Nous avons également étudié la réponse de ces détecteurs aux faibles doses allant de 0,1 à 1 mGy. A 0,1 mGy la reproductibilité est de 20%. A 1 mGy elle est de 10%.
La courbe caractéristique de l'EETS du fluorure de lithium est représentée sur la figure 2.
Nous avons étudié la réponse de ces 2 types de dosimètres en fonction de l'énergie du rayonnement béta en utilisant les sources suivantes:

147Pm	Emax = 0,225 MeV
204Tl	Emax = 0,763 MeV
90Sr + 90Y	Emax = 2,27 MeV.

Nous avons normalisé nos mesures pour une épaisseur de tissu égale à 7 mg / cm2.
Dans cette gamme d'énergie la réponse des 2 dosimètres est indépendante de l'énergie du rayonnement béta.

L'ensemble de ces mesures a été réalisé en utilisant le dispositif de lecture d'exoélectrons développé dans notre laboratoire. Cet appareil est actuellement en cours de commercialisation (4).

Afin d'effectuer des mesures de routine plus rapide, nous avons étudié un appareillage de lecture semi-automatique. La construction est terminée. Il comprend un système de transfert du dosimètre sur un four de préchauffage maintenu à une température constante T1. Ce système est destiné à éliminer la partie des pics du thermogramme inutilisable en dosimétrie. Le dosimètre est ensuite transféré sur le deuxième four réglé à une température plus élvée T2 sur lequel est disposée la tête de lecture (compteur). Cette phase permet la lecture du détecteur. Les essais de cet appareil seront réalisés au cours de cette année. Nous étudions parallèlement un nouveau modèle de compteur à faible temps mort adapté aux mesures rapides (5).

(1) PETEL M.:
Rapport CEA-R-4754 (1976).

(2) KRIEGSEIS W.:
6th Internat.Symp. on Exoemis. Ahrenshoop RDA (1979).

(3) TATAH B.:
Rapport CEA-R-5161 (1982).

(4) HERBAUT Y., TATAH B., PETEL M. et PORTAL G.:
Radioprotection Vol. 17 n°4 (1982) pp 269-272.

(5) COMBY G.,PETEL M.,QUIDORT J. et BARTHE J.:
"Utilisation d'un compteur multipointes à focalisation cathodique pour la détection de l'émission exoélectronique"
VIIth Inter. Symp. on Exoelec. Emission
March 16-18, Strasbourg (1983), FRANCE.

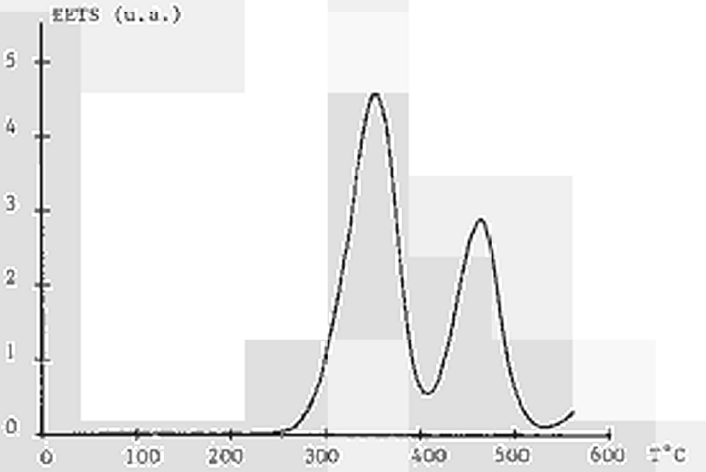


Figure 1 - Thermogramme du BeO.

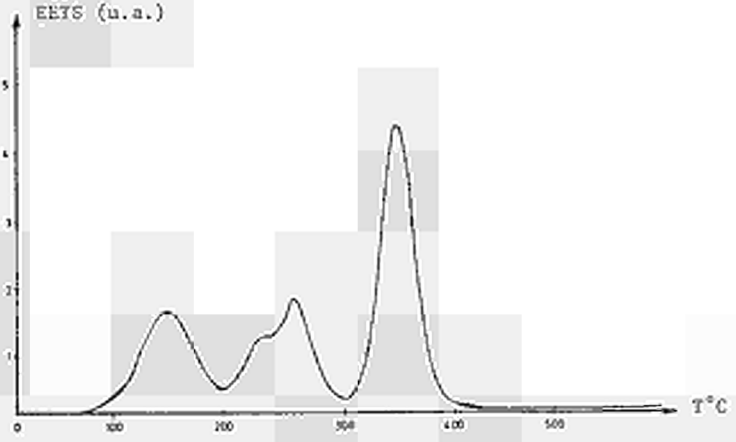


Figure 2 - Thermogramme du LiF.

List of publications in 1982

I Publications in scientific journals, monographies, proceedings

II Short communications, Theses, Internals Reports, Patents...

COMBY G.,PETEL M.,QUIDORT J. et BARTHE J.
" Utilisation d'un compteur multipointes à focalisation cathodique pour la détection de l'émission exoélectronique "
VIIth. Inter. Symp. on Exoelec. Emission,
March 16-18, Strasbourg, (1982), France, (à paraitre).

HERBAUT Y.,TATAH B.,PETEL M. et PORTAL G.:
Radioprotection Vol. 17 n° 4 (1982) pp. 269-272.

Progress Report
1982

Contractor:
Université Louis Pasteur
Rue Humann 11
F-67085 Strasbourg

Contract no.: BIO-A-294-81-F

Head(s) of research team(s):
Dr. R.V. Rechenmann
LBRM - INSERM U.220
Université Louis Pasteur
Rue Humann 11
F-67085 Strasbourg

General subject of the contract:

Theoretical and experimental analysis of the ionizing track pattern in dense tissue-like media.

List of projects:

1. Theoretical and experimental analysis of the ionizing track pattern in dense tissue-like media - Consequences for radiation protection.

Titre du projet N°1 : Theoretical and experimental analysis of the ionizing track pattern in dense tissuelike media - Consequences for radiation protection.

Chef du projet et collaborateurs scientifiques : R.V. RECHENMANN, E. WITTENDORP-RECHENMANN, B. SENGER.

I. SECONDARY ELECTRONS.

In the previous annual report (1981), we have proposed a mixed treatment (DDCS-MT) which allows to calculate double differential ionization cross-sections (DDCS) in the case of heavy charged particles crossing tissuelike media. This treatment led to a very satisfactory reproduction of the experimental results obtained in ionographic detectors (considered as models of biological tissues) of various compositions bombarded by 4- to 12 MeV α particles, as well as in noble gases (helium, argon) traversed by protons and α particles; furthermore, promising results could already be obtained in the case of molecules (H_2O, CH_4).

The applicability of the treatment proposed has been improved by introducing the actual molecular binding energies as given by Siegbahn (1). The increase of the binding energies owing to the presence of the energy dependent supplementary positive charge introduced by the passing ion (2,3) has also been taken into account. On Fig. 1-4, DDCS measured in water vapour (4) are compared with DDCS calculated by means of the formalism proposed for two energies of the incoming protons, 0.3 and 1.5 MeV. It appears that the agreement between calculated and experimental data stays acceptable for ejection energies T as low as $\sim$ 10 eV, considering the experimental uncertainties (4). The results are still improved at the small ejection angles θ if we take into account the final state interaction between the ejected electron and the proton.

In collaboration with a group of the Centre de Recherches Nucléaires of Strasbourg, a study has been undertaken in order to interprete the light emission of an anthracen crystal irradiated by heavy charged particles, notably by the application of the DDCS-MT to the evaluation of the spatial distribution of the energy deposited by the δ-rays.

II. STRONG IONIZING EVENTS.

Like already mentionned in the last year's report (EUR 7800-1981), we have restarted the study of strong ionizing events (S.I.E.) on the basis of a revised experimental approach, and of particularly rigorous measurement procedures. Let us recall that these S.I.E.'s can be recognized as thick

branches, discriminated from secondary electrons by the usual ionographic criteria allowing to characterize stronger ionizing processes.

The determination of the eventual energy lost in one event could be carried out by referring to a range-energy curve which we have established in the codified experimental conditions applied to our determinations (Fig. 5).

The presence of S.I.E.'s on α tracks has a significant influence on the first and second order moments of range. As far as the 13.6 MeV α particles submitted to the measurements considered in this report are concerned, we have measured a mean range $\overline{R}$ = 92.49 μm with a second order moment of range σ = 1.85 μm. The mean range of a statistically equivalent (∿ 300 tracks) sample of tracks with one or more S.I.E.'s corresponds to $\overline{R}_{S.I.E.}$ = 90.72 μm with a second order moment of range σ = 3.80 μm. The statistical significance of the difference $\overline{\Delta R} = \overline{R} - \overline{R}_{S.I.E.}$ = 1.77 μm has been verified by a hypothesis test.

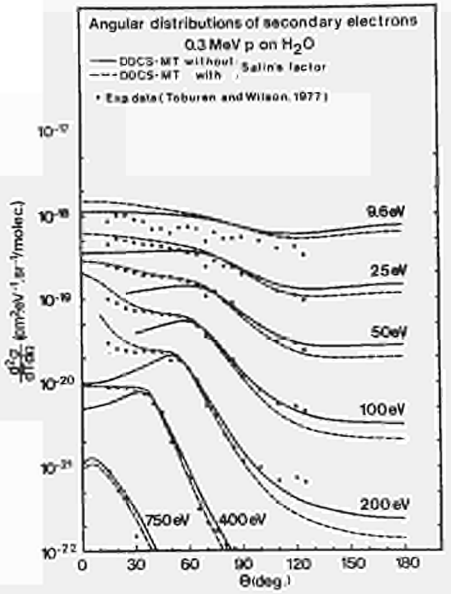
Large samples of 13.6 MeV α particle tracks have been scanned in order to count and localise S.I.E.'s. Preliminary results obtained on 2400 primaries are illustrated by Fig. 6. In the α energy interval considered, the number of S.I.E.'s increases with the primary's energy and their production is relatively important considering the quite large detection thresholds. The solid curve has been calculated by means of the following parametric formula:

$$N/5\ \mu m \simeq 375\ (e^{0.2 E_\alpha} - 1)\ e^{-9.08 r_0}$$

Considering that part of these protuberances are certainly short tracks of recoil ions/nuclei ejected by elastic scattering, we have tentatively taken into account the influence of the nuclear potential on the production of heavy secondaries by adapting an optical model code (5) to experimental data of the literature. In the case of the ejection of H-nuclei, a preliminary study by means of the optical model code indicates an increase in the number of energetic recoils, which is especially marked for the higher energies of the secondaries.

It appears that a part of the observed S.I.E.'s can be described in terms of classical Coulomb scattering (Fig. 7) and of total elastic scattering, where the action of the nuclear forces is taken into account, and eventually inelastic scattering processes which still have to be prospected.

Références : 1)Siegbahn K. et al., ESCA applied to free molecules, North Holland, Amsterdam (1969). 2)Basbas G., Brandt W., Laubert R., Phys. Rev.A7(1973)983. 3)Brandt W., Lapicki G., Phys. Rev.A10(1974)474. 4)Toburen L.H., Wilson W.E., J.Chem.Phys.66(1977)5202. 5)Bonneaud G., Bing O., Senger B. and Magnac-Valette D., Conf. on Nucl. Structure, Manchester(1973).



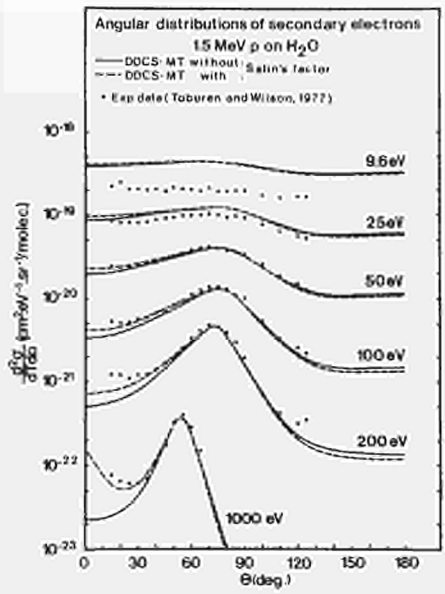
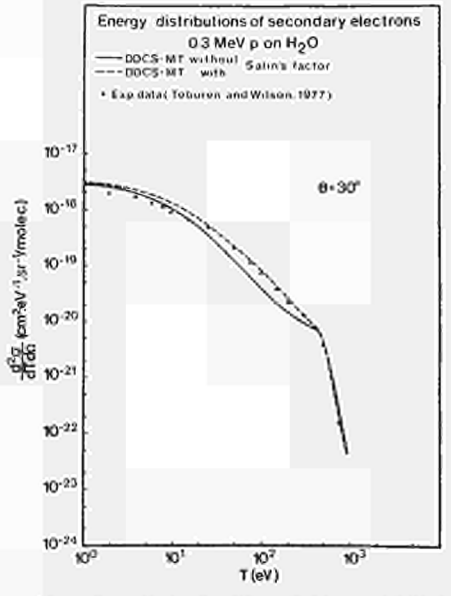


Fig. 1 and 2: Double differential ionization cross-sections vs. ejection angle for different ejection energies T, experimental (4) and calculated (DDCS-MT without and with Salin's factor).



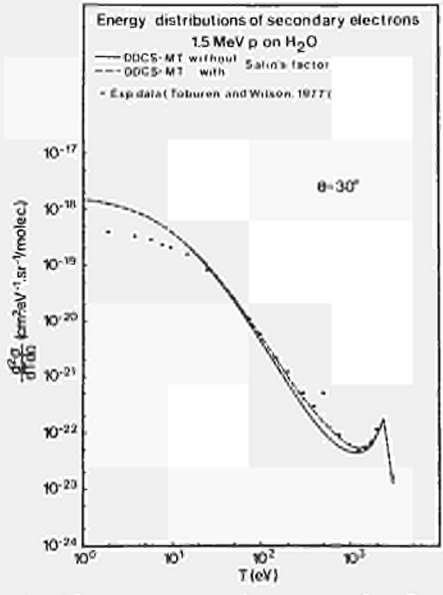


Fig. 3 and 4: Double differential ionization cross-sections vs. ejection energy for the ejection angle θ = 30°, experimental (4) and calculated (DDCS-MT without and with Salin's factor).

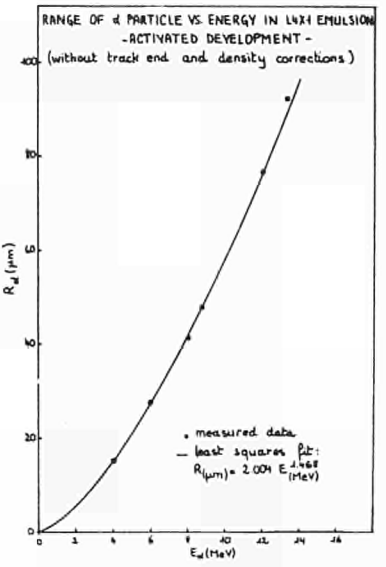


Fig. 5: Experimental range-energy relation of α particles in L4x1 emulsion.

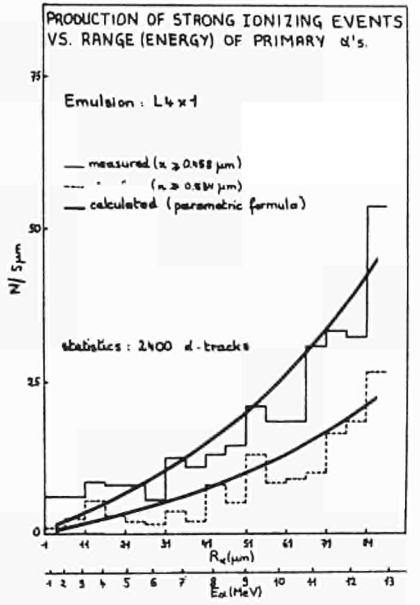


Fig. 6: Number of S.I.E.'s per 5 μm (projection on the focal plane) as a function of α particle range (energy) for two radial detection thresholds.

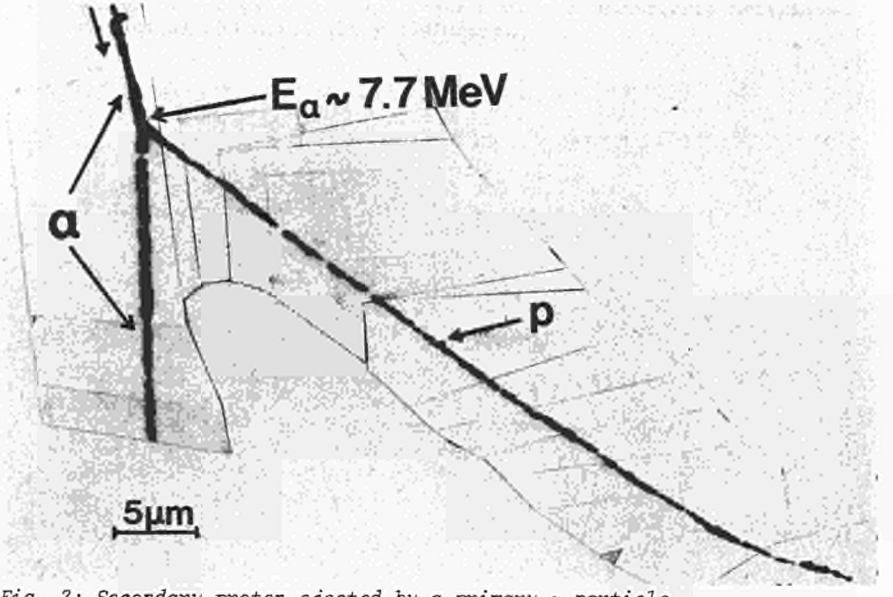


Fig. 7: Secondary proton ejected by a primary α particle. ΔE_α = 2500 keV ± 150 keV. The proton energy determined by referring to a range-energy relation is of the order of 2700 keV (Photo-mosaic assembly).

PUBLICATIONS 1982

- B. SENGER, E. WITTENDORP-RECHENMANN, R.V. RECHENMANN.
  Ionization cross-sections in the case of medium and low energy heavy charged particles crossing complex media.
  Nucl. Instr. and Meth., 194(1982), 437-441.

- E. WITTENDORP-RECHENMANN, B. SENGER, R.V. RECHENMANN.
  Strong ionizing events produced by medium energy α particles crossing nuclear emulsion.
  VIIIth Symp. on Microdosimetry, Jülich, sept. 1982.

- B. SENGER, E. WITTENDORP-RECHENMANN, R.V. RECHENMANN.
  A double differential cross-section mixed treatment. Comparison with experiments in nuclear emulsions.
  VIIIth Symp. on Microdosimetry, Jülich, sept. 1982.

- R.V. RECHENMANN, E. WITTENDORP-RECHENMANN, B. SENGER.
  Secondaires lourds énergétiques éjectés par des particules α (E_α < 12 MeV) traversant des milieux denses.
  9^{e} Colloque sur la Physique des Collisions Atomiques et Electroniques, Nice (1982).

- B. SENGER.
  Approche théorique de l'éjection de rayons δ par des protons et des particules α.
  9^{e} Colloque sur la Physique des Collisions Atomiques et Electroniques, Nice (1982).

Progress Report
1982

Contractor:

The National Radiological
Protection Board, NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

Contract no.: BIO-A-309-81-UK

Head(s) of research team(s):

Dr. J.A. Reissland
Physics Department, NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

General subject of the contract:

Solid state physics underlying the properties of luminophors used in luminescence dosimetry.

List of projects:

1. The implementation of thermo luminescence techniques, including reassessment of absorbed dose, to dose measurement under operational conditions.
2. Studies of the practical application of lyoluminescence materials to clinical and criticality dosimetry.

Solid state physics underlying the properties of luminophors used in luminescence dosimetry

Contract No. BIO-A-309-81-UK

Project 1 The implementation of thermoluminescence techniques, including reassessment of absorbed dose, to dose measurement under operational conditions

Head of Project and Scientific Staff: Dr. J.A. Reissland
Dr. A.F. McKinlay
Dr. C.M.H. Driscoll
Mr. M.J. Whillock

Lithium fluoride (LiF) is one of the most commonly used thermoluminescent materials for environmental gamma measurements mainly because of its relatively flat photon energy response. However, it is generally not sensitive enough for short-term environmental monitoring. The requirement to develop a dosemeter for short-term monitoring (<1 month) has led us to pursue two main areas of experimental research.

(i) to investigate the properties of energy discriminating shields for use with existing high sensitivity thermoluminescent materials such as calcium fluoride (CaF_2) chips which over-respond at low photon energies.

(ii) to investigate the properties of new materials with higher sensitivities than LiF and relatively flat photon energy responses.

(iii) To investigate the low temperature thermoluminescence behaviour of LiF with particular regard to photo-transfer and LET effects. The results of a preliminary study related to the first area of research have been presented in an internal report (Elliot et al 1982 - see references). A number of metal planar shields and combinations of shields of various thicknesses were used. The most encouraging results in reducing the over-response of CaF_2 chips were obtained using a combination shield of 0.5 mm tin and 0.5 mm aluminium. This combination reduces the over-response to 25% of its peak without a significant loss of sensitivity. The slight over-response of LiF chips can be effectively removed with 0.5 mm of aluminium.

Various forms of preparations of lithium borate ($Li_2B_4O_7$) and Yugoslavian magnesium borate (MgB_4O_7) pellets have been studied and compared with LiF chips. The results of these studies have been presented in internal reports and in the scientific literature (Wall et al 1982, Furetta, Padovani et al 1982, Furetta, Driscoll et al 1982). Thermoluminescence sensitivity, backgrounds, repeatability, dose-response, photon energy response, and fading have all been investigated. MgB_4O_7 is the most sensitive of the materials investigated (3 to 7 times LiF). However, variations within a batch and significant light induced fading still indicate it to be unsuitable for large-scale routine applications. Of the powder forms of $Li_2B_4O_7$, the Japanese copper doped material has combined a good tissue equivalence with a sensitivity of about 3 times that of LiF. $Li_2B_4O_7$ pellets (Swedish and Yugoslavian) were not as sensitive as LiF.

LiF chips irradiated with ultra violet radiation (254 nm) at about 80K exhibit a glow peak around 194K. Difficulties have been encountered with background effects due to ultra violet induced phosophorescence in the adhesive used to fix the chip to the heating element. Chips subject to irradiation ($\sim$0.2 Gy) at room temperature which have been subsequently read-out to give the normal peak 5 (210^{o}C) readout, cooled to 80K and irradiated with UV, have exhibited the capability of 'normal' reassessment at 210^{o}C. The number of measurements are small but provide enough evidence to encourage further investigation. The results suggest that a higher ultra violet irradiance will be required to produce a substantial low temperature phototransfer signal. It is intended to replace the UV source now in use with a more powerful one.

Contract No. BIO-A-309-81-UK

List of publications

Wall, B.F., Driscoll, C.M.H., Strong, J.C. and Fisher, E.S. (1982) 'The suitability of different preparations of thermoluminescent lithium borate for medical dosimetry'. Phys. Med. Biol. 27, 1023.

Driscoll, C.M.H. (1982), Comments on 'Particle size effects in LiF'. Phys. Med. Biol. 27, 156.

Furetta, C., Driscoll, C.M.H., Richards, D.J. (1982) 'Preliminary results on a new batch of MgB_4O_7 TL material' NRPB internal report PDTM 17(82).

Furetta, C., Padovani, R., Richards, D.J., Driscoll, C.M.H. (1982) 'The TL properties of $Li_2B_4O_7$ dosemeters'. NRPB internal report PDTM 40(82).

Elliot, J.M., Richards, D.J., Driscoll, C.M.H. (1982) 'Photon energy response studies of high sensitivity TL materials'. NRPB internal report PDTM 35(82).

Solid state physics underlying the properties of luminophors used in luminescence dosimetry

Contract No. BIO-A-309-81-UK

Project 2 Studies of the practical application of lyoluminescence materials to clinical and criticality dosimetry

Head of Project and Scientific Staff: Dr. J.A. Reissland
Dr. A.F. McKinlay
Mr. J.B. O'Hagan

A detailed study has been made on the use of Diphenylisobenzofuran (DPBF) as a chemical signal enhancer for lyoluminescence. The enhancement as a function of solution storage time has been studied and the solution remains at maximum effectiveness for about 2 hours. This falls to about 50% at 3 hours, 10% at 5 hours and no enhancement is found for solutions prepared more than 24 hours before use. Eleven different batches of BDH mannose have been irradiated with 147 keV ISO High X-rays over a range of doses from 0.1 to 1 Gray. Readouts in both water and DPBF show a factor of four variation among batches; the relative responses (in counts per milligram of mannose per Gy) ranging from 40 to 170 in water and 190 to 870 in DPBF. The minimum detectable dose (three standard deviations above background) using 30 mg samples is between 80 and 20 mGy in water and 40 and 10 mGy in DPBF.

An intercomparison study has been started with the Western Infirmary, Glasgow. Lyo-luminophors are being irradiated along with thermoluminescence dosimeters. Samples of mannose in a perspex/water phantom have been given calibrated doses of cobalt-60 photons. The dose-response curve over the range 0.1 to 20 Gy has a dose to the power 1.2 dependence.

Samples of the lyo-luminophors mannose and sucrose have been successfully recrystalised. Large crystals of sucrose have been grown with the intention of studying the surface-bulk processes responsible for lyoluminescence. Sucrose crystals have been grown from solutions containing the chemical enhancer luminol. This is to be extended to incorporating DPBF in mannose crystals.

The lyoluminescence readout apparatus has been partly automated to improve efficiency and precision. Counts, as a function of time, are recorded on a pulse height analyser operating in multi-channel scaling mode. These are then transferred, on magnetic tape, to a remote microcomputer for analysis.

Progress Report
1982

Contractor:

Physikalisch-Technische
Bundesanstalt, PTB
Bundesallee 100
D-3300 Braunschweig

Contract no.: BIO-A-291-81-D

Head(s) of research team(s):

Prof.Dr. V.Siegel
PTB - Reaktorstrahlung
Bundesallee 100
D-3300 Braunschweig

General subject of the contract:

Development, investigation and application of reference beams of monoenergetic neutrons.

List of projects:

1. Reference beams of thermal, 2 keV and 24 keV neutrons for the calibration of neutron dosemeters to be used in radiation protection.

Title of project: Reference beams of thermal, 2 keV and 24 keV neutrons for the calibration of neutron dosemeters to be used in radiation protection

Head of project and scientific staff: W.G. Alberts
E. Dietz
H. Kluge
K. Knauf

For the calibration of neutron dosemeters with reference neutron beams the "true" neutron flux density $\phi = \int \phi_E(E)\, dE$ must be known. In the case of the slow neutron beams at the PTB reactor (thermal neutrons and bismuth-filtered neutrons) the flux density is generally measured by gold foil activation yielding the conventional flux density of slow neutrons $\phi_o = \int \sqrt{E_o/E}\ \phi_E(E)\, dE$ (E neutron energy, E_o = 0.0253 eV reference energy for which the detector cross section σ_o is usually quoted, ϕ_E spectral neutron flux density). The true flux density can only be obtained from the activation measurement if the neutron spectrum is known. Therefore the spectral distribution in the two reference beams were determined using the time-of-flight method. The starting signal was generated by a chopper consisting of a slitted disc of boron-loaded polyethylene, the neutrons were detected after 3.0 and 7.9 m flight-path with ^{3}He proportional counters or ^{6}LiI scintillators. The figures show the resulting spectral distributions: In Fig. 1 the spectral flux density in the thermal neutron beam is compared with a Maxwellian distribution (temperature parameter 63 ^oC obtained by fitting the distributions above 63 meV). Fig. 2 shows the spectrum when a 200 mm thick bismuth filter is inserted which reduces fast-neutron and photon components in the beam. The ratio ϕ/ϕ_o can be calculated by numerical integration. The present results are given in the following table:

	ϕ/ϕ_o
thermal beam	1.32
thermal beam (earlier measurement with a Cd chopper)	1.29
bismuth-filtered beam	0.8
Maxwellian distribution (63 ^oC)	1.21

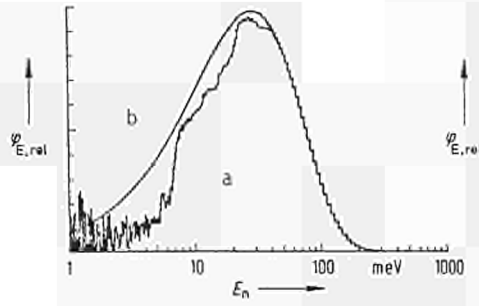


Fig. 1. Neutron spectrum in the thermal-neutron beam (a) and Maxwellian distribution with T = 336 K. (b)

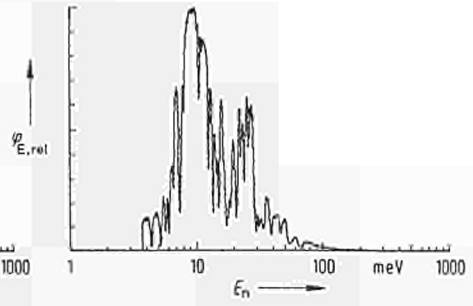


Fig. 2. Neutron spectrum in the bismuth-filtered beam.

$\phi_{E,rel}$ relative spectral neutron flux density, E_n neutron energy

These two beams provide the possibility of investigating the response of neutron dosemeters at different "thermal" energies. Because of it's low photon and epicadmium-neutron contamination the bismuth-filtered beam is especially suited for the investigation of slow-neutron influence on photon dosemeters.

The development of the recoil-proton proportional counter system for the investigation of the filtered neutron beams was continued in three directions: (1) Since the measurement of high-energy "background" in the filtered beams requires long counting times, responses and gas amplification of the counters must be stable over a long period. This goal was reached by improvements in sealing the counter and by closing the filling tube through "cold welding". (2) For neutron spectrum unfolding purposes the response functions of the counter must be known. A theoretical calculation which gives these functions for the special collimated-beam geometry was tested by measurements at different gas pressures using the silicon-filtered 144 keV neutron beam. The agreement between measurement and calculation is not yet satisfactory and the studies will be continued. (3) In order to improve the neutron-gamma pulse shape discrimination, fast electronics (time constant in the order of 10ns) are to be introduced. Test measurements with electronic modules from other experiments were performed using the multi-line iron-filtered beam (24 keV neutrons and above).

List of publications in 1982

1. W.G. Alberts and H. Lesiecki
   Neutron flux density measurements and rem counter calibration at the 24.5 keV filtered beam of the FMRB.
   Rad. Prot. Dosimetry 2 (1982) 241 - 244

2. Internal:
   W.G. Alberts, E. Dietz, H. Kluge, W. Sosaat
   Messung der spektralen Neutronenflußdichte am Referenzstrahl thermischer Neutronen
   Physikalisch-Technische Bundesanstalt, Jahresbericht 1982, Braunschweig 1983.

Progress Report
1982

Contractor:
CENDOS
Radiobiological Institute TNO
P.O. Box 5815
NL-2280 HV Rijswijk

Contract no.: BIO-A-311-81-NL

Head(s) of research team(s):
CENDOS Committee
Radiobiological Institute TNO
P.O. Box 5815
NL-2280 HV Rijswijk

General subject of the contract:
Collection and evaluation of neutron dosimetry data.

List of projects:

1. Collection and evaluation of neutron dosimetry data (CENDOS).

Cooperative European Research Project coordinated by:
D.K. BEWLEY, J.J. BROERSE (Chairman), G. BURGER (Vice-chairman), M. COPPOLA (Secretary), J.A.B. GIBSON, N. PARMENTIER, W. POHLIT, and G. PORTAL.

Title of project nr. 1:
Collection and evaluation of neutron dosimetry data (CENDOS).

Head of project and scientific staff:
CENDOS Committee J.J. Broerse (chairman), G. Burger (vice-chairman) and M. Coppola (secretary).

A number of new cooperative projects have been started during the past contract year and several on-going programs have shown considerable progress.

The working group on ion recoil dosimetry studied the response of track detectors in different neutron beams. The intercomparison was performed with monoenergetic neutrons produced by a van de Graaff accelerator, a bare californium source and a moderated fission spectrum. Various dosimeters were used: CR39 with polyethylene converter (proton detection), CN85 with $Li_2B_4O_7$ converter (α detection) and NTA film. The NTA film appears to be sensitive to fast neutrons only, for neutron energies in excess of 2 MeV. The CN85 can be used as an albedo dosimeter; its response is mainly due to (n,α) reactions in boron and lithium and its sensitivity decreases with increasing neutron energy. The CR39 is the best dosimeter for fast neutrons when associated with a polyethylene converter; its sensitivity varies from $1x10^5$ to $6x10^5$ tracks.cm^{-2}.Sv^{-1} for neutron energies between 126 keV to 15 MeV. A complete report of these results will be prepared by Medioni, Harrison and Lembo.

The response of thermoluminescent materials is presently investigated by several groups cooperating within the CENDOS framework. Furetta and coworkers calculated the activation energies for $CaSO_4$: Dy (TLD-900) material as evaluated by a decomposition into single components of the experimental glow-curve. Gibson and Douglas are presently reviewing all experimental and theoretical data concerning the response of thermoluminescent dosimeters to neutrons of different energies. One of the objectives of the evaluation is to arrive at recommendations for the k_U as a function of neutron energy. In a cooperative study between Essen and Rijswijk, Rassow and coworkers investigated the possibilities of

TLD-300 detectors (CaF_2:Tm) for simultaneous and separate determination of total absorbed dose and gamma absorbed dose in mixed neutron-photon beams. The evaluation procedure is based on mathematical analysis of the LET dependence of the two main glow curve peaks. For mean neutron energies of 5 MeV produced by the d(14)+Be and the d(2.3)+Be reactions the two peaks differ considerably, however, for 14.8 MeV neutrons produced by the d(0.5)+T reaction the two peaks are nearly equal. This implies that the method will probably not be applicable for these higher energy neutrons.

Reports on W values for the individual charged particles resulting from neutron interactions and on calculations of dose and dose equivalent in anthropomorphic phantoms are in preparation.

List of publications:

B.J. Mijnheer, S. Guldbakke, V.E. Lewis, J.J. Broerse.
Comparison of the fast-neutron sensitivity of a Geiger-Müller counter using different techniques.
Phys.Med.Biol. 1982, 27, 91.

J. Rassow, A. Temme, F. Hensley, J. Zoetelief, J.J. Broerse.
Applicability of TLD-300 detectors for simultaneous and separate measurement of total and gamma absorbed dose distributions for monoenergetic 5.3 and 14.8 MeV neutrons and d(14)+Be-neutrons.
Presented at the Conf. 50th Anniversary of the Discovery of the Neutron, Cambridge, 1982.

Progress Report
1982

Contractor:

International Commission
on Radiation Units
and Measurements, ICRU
7910 Woodmont Av.,Suite 1016
USA-Bethesda, MD 20814

Contract no.: BIO-A-312-81-US

Head(s) of research team(s):

Dr. H. O. Wyckoff
ICRU
7910 Woodmont Avenue,
Suite 1016
USA-Bethesda, MD 20814,

General subject of the contract:

Quantities, units and measurements techniques for ionizing radiation.

List of projects:

1. Quantities, units and measurements techniques for ionizing radiation.

Title of project nr 1: Quantities, units and measurement techniques for ionizing radiation

Head of project and scientific staff: Dr. Harold O. Wyckoff

During 1982, the International Commission on Radiation Units and Measurements published one report and completed the review of two reports.

The Published report is ICRU Report 34, The Dosimetry of Pulsed Radiation. The Report provides guidance on the special procedures to be followed in measuring the radiation dose from sources such as linear accelerators, betatrons, synchrotrons, or field-emission impulse generators. These sources deliver their output impulses within the range 10^{-9} to 10^{-6} seconds, spaced at least a few milliseconds apart. Condenser discharge machines with field-emission cathodes deliver much larger pulses, usually singly or at very low frequency, and with a pulse duration much less than a microsecond. The Report provides information on certain precautions and the selection of calibration constants needed to permit the use of methods of dosimetry employed for measuring continuous radiation from constant potential x-ray sources or from gamma-ray sources. Treated are measurements using ionization chambers, chemical dosimeters, calorimeters and solid state devices. The aim of the report is to guide those who have to measure pulsed radiation to the most convenient and accurate system for their particular problem.

The ICRU met in September 1982 at Schloss Reisensburg, Germany. At that meeting, the ICRU completed review of a draft report, "Stopping Powers for Electrons and Positrons." The ICRU received reports indicating the revision work on the draft report, "Microdosimetry" has been completed, and thus, with the review of these two reports completed, the preparation of printer's manuscripts is expected to begin very soon. At its meeting, the ICRU also received a report indicating that revision of the draft report on high energy electron beam dosimetry is continuing with the intent that one further review by the members of the ICRU would be appropriate when this work is completed.

At the 1982 meeting, the ICRU reviewed in some detail the work of the Report Committee on Practical Determination of Dose Equivalent Index. The Committee is preparing a report based on the following outline:

(1) Summary
(2) Introduction
(3) Concepts and Definitions
(4) Physical Data
(5) Quantities
(6) Conversion Factors
(7) Appendices

The draft report is to be submitted to the ICRU for review at the 1983 meeting.

The ICRU, at the 1982 meeting, reviewed the plans for new work concerned with tissue equivalent materials. The report to be prepared is to:

(1) Evaluate the acceptability of existing tissue substitutes and materials for dosimetric studies using photons, electrons, neutrons and heavy charged particles.
(2) Evaluate solid, liquid, gel and gaseous substitutes.
(3) Highlight deficiencies of available materials and illustrate variations in experimental results (for example, depth dose curves) when different substitutes are used purporting to simulate the same tissue.
(4) Discuss the important biological tissues requiring simulation and give the most up-to-date elemental composition for each tissue and organ.
(5) List recommended, useful substitutes to help the experimentalist select the best product for their particular application.
(6) Suggest further research and development into categories of substitutes thought to require improvement.

At the 1982 meeting, the ICRU determined to undertake further work on stopping powers, and plans are now being drawn for a phased effort aimed at the development of reports treating particles heavier than electrons.

The ICRU also examined the status of work underway in the many active report committees and recognized that at least one of these is likely to be submitted to the ICRU for review in 1983 (in addition to the report on practical determination of dose equivalent index). The ICRU scheduled the next meeting during the period 25 June - 2 July 1983 in Paris.

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

ICRU Report 34, The Dosimetry of Pulsed Radiation

II Short Communications, Theses, Internal Reports, Patents...

III B

VERHALTEN UND KONTROLLE DER RADIONUKLIDE IN DER UMWELT

BEHAVIOUR AND CONTROL OF RADIONUCLIDES IN THE ENVIRONMENT

COMPORTEMENT ET CONTROLE DES RADIONUCLEIDES DANS L'ENVIRONNEMENT

Weitere Forschungsarbeiten zu diesen Themen werden auch in folgenden Tätigkeitsberichten beschrieben :

Further research work on these subjects is also described in the following progress reports :

D'autres travaux sur ces thèmes de recherche sont également décrits dans les rapports suivants :

III A. ICRU	Bethesda	BIO 312 US
III C. Apelgot, S.	Inst. Curie Paris	BIO 349 F
III C. Dumont, J.E.	Univ. Bruxelles	BIO 360 B
III C. Taaffe, J.K./Malone, J.F.	Coll. Technol. Dublin	BIO 364 EIR
III D. Chalabreysse, J.	CEA, CEN Pierrelatte	BIO 372 F
III D. Hogeweg, B./Barendsen, G.W.	TNO Rijswijk	BIO 301 NL
III D. Kriegel, H.	GSF Neuherberg	BIO 365 D
III D. Lafuma, J.	CEA, CEN Fontenay-aux-R.	BIO 370 F
III D. Palmer, G.H./Ramsden, D.	UKAEA Winfrith	BIO 380 UK
III D. Smith, H.	NRPB Chilton	BIO 388 UK
III D. Taylor, D.M.	KFZ Karlsruhe	BIO 367 D
III D. Vanderborght, O.	SCK, CEN Mol	BIO 377 D
III E. Delpoux, M.	Univ. Toulouse	BIO 430 F
III F. Coulon, R.	CEDHYS Fontenay-aux-R.	BIO 320 F
III F. Goddard, A.J.H.	ICST London	BIO 499 UK

Progress Report
1982

Contractor:

Contract no.: BIO-B-339-81-DK

Risø National Laboratory

DK-4000 Roskilde

Head(s) of research team(s):

Dr. A. Aarkrog
Health Physics Department
Risø National Laboratory
DK-4000 Roskilde

General subject of the contract:

Radioecological studies in temperate and arctic waters of the North Atlantic region with emphasis on transuranic elements.

List of projects:

1. Uptake and loss of certain transuranic-, fission- and activation - nuclides by Mytilus and Fucus with special emphasis on physicochemical form and the effect of low temperatures.
2. Studies of transuranic elements, radiocaesium, tritium and cobalt-60 in seawater, sediments, seaplants and mussels.
3. Environmental studies of plutonium and americium at Thule, Greenland.

In this study the North Atlantic region comprises the waters arour Greenland, the North Atlantic Ocean between the Faroe Islands and Green land, and the North and Baltic Seas (including the Danish Straits). I this region the salinity ranges from ocean water to brackish water, th climate from arctic to temperate.

As a whole the fallout nuclides from nuclear weapons testing ar still the main sources of man-made radioactivity in the North Atlanti region. However, in local waters other sources may prevail. For example the North Sea most of the radiocesium comes from reprocessing plants fc nuclear fuel in Western Europe. In the inner Danish waters a reactor-prc duced corrosion product such as ^{60}Co is measurable, and at Thule, Green land a local contamination with Pu and Am has increased the levels c transuranics.

In recent years the discharges from the UK reprocessing plant a Sellafield have not only been detectable in the North Sea, but als in the Danish Straits, the Baltic Sea, along the west coast of Norway at Spitzbergen, in 1982 even in East Greenland, and probably also in Wes Greenland waters. Radiocesium is the important tracer for this activity but now ^{99}Tc has appeared to be another possible indicator for the dis charges from Sellafield. As an important supplement to the field studie the uptake of transuranics and other radionuclides in Fucus and Mytilus ar examined experimentally.

of project no. 1:
e and loss of certain transuranium-, fission-, and activa-
nuclides by Mytilus and Fucus with special emphasis on
co-chemical form and the effect of low temperatures.
of project:
cient. Henning Dahlgaard

---

isotopes

Due to unexpected problems some of the radionuclides ordered
ril 1981 were not ready for use until July 1982. However, now
xperiments are conducted with the following nuclides simul-
usly: ^{239}Pu, ^{241}Am, ^{237}Np, ^{244}Cm, ^{155}Eu, ^{144}Ce, ^{60}Co, ^{65}Zn
^{34}Cs. The 1 mg of ^{239}Pu in the stock solution are under in-
tional safeguard control! Till now experiments have been
cted only with the delivered chemical forms: Pu(IV), Am
, Np(V) and Cm(III).

tical methodology

The multielement actinide analysis introduced at our labora-
in 1981 is now used routinely. The spiked samples are sepa-
from bulk elements by ion-exchange and electroplated on
less steel discs, and measured with a high-resolution
ce-barrier detector. The 4 actinides in the experiment
e measured simultaneously with ^{243}Am- and ^{242}Pu-spikes,
ugh the complex energy distribution of ^{237}Np-α-particles
ts in interference with ^{242}Pu. Very high chemical yields
btained for all four actinides.

ter and particulate matter

After addition to seawater, the actinides behave different-
u, Am, and Cm associates quickly with particulate matter
he activity in aquarium water including suspended particles
ases with time. Typically 10-20% was filterable on 0.4 μm
rs in the first experiments. Neptunium, however, is not
l in significant amounts in particulate matter, and the de-
e in water activity with time is far less pronounced.

Mytilus edulis experiments

For mussels, only results from γ-emitters and ^{241}Am and ^{244}Cm are available. Am and Cm seem to behave exactly identically. Although individual animals varied in concentration ratio over an order of magnitude, the Am to Cm ratio was the same. The great variation in the initial rate of accumulation is probably explained by the importance of particulate activity and variation in filtration rate between animals.

Fucus vesiculosus

The effect of salinity, temperature, and light on the rate of accumulation of the nuclides mentioned was studied in a series of short-term experiments with growing Fucus tips. The first results indicate significant differences in the initial rate of accumulation of the actinides. As compared to Pu, the rate of accumulation of Np is only ~ 10%, whereas Am and Cm apparently are accumulated 3 times faster. The low accumulation of Np is in accordance with data from the Irish Sea (MAFF, Lowestoft), whereas Danish field data shows no Am-accumulation in excess of Pu.

The reproducibility of these experiments is being tested by repeating certain factor combinations at different times.

The long-term loss rate of all the nuclides mentioned from whole, growing Fucus plants is under investigation. Young plants on stones or fixed to synthetic ropes accumulated the radionuclides in the laboratory (November 1982), and the loss rate is now measured in cages in a Danish fjord. The α-emitters are measured in plants to be sampled during one year, whereas the γ-emitters in addition are traced by repeated "whole-body" countings. During the first two months of loss, Eu, which might approximately trace Am and Cm, was lost faster than Co and Zn and even Cs. Caesium is expected to show a seasonal variation, and the relatively quick Eu-loss might be explained by the loss of surface "contamination".

Title of project no. 2

Studies of transuranic elements, radiocesium, tritium and cobalt-60 in seawater, sediments, seaplants, and mussels.

Head of project: Dr. Asker Aarkrog

Other scientific staff: Drs. Henning Dahlgaard, Heinz Hansen, Søren Boelskifte and Elis Holm (University of Lund, Sweden).

Sampling expeditions in 1982

Through the duration of this project in 1982 there have been seven sampling expeditions. From February 2 to March 1 R/V DANA (The Danish Institute for Fisheries and Marine Research, DFH) collected surface seawater in the North Sea at 8 locations between Jutland and England. From February 18 to February 27 HMS FYLLA from the Royal Danish Navy took surface seawater samples at 5 locations between the Shetland and Faroe Islands and at 5 locations in the Denmark Strait between Iceland and East Greenland. Lund University and Risø performed an expedition by car around the Scottish and a part of the English coastline from June 12 to 28. The Orkney and Shetland Islands were also included in the programme. Seawater samples, seaweed, mussels and other marine biota were collected. Precipitates with radiocesium and transuranics were taken from the water samples in the field. From July 15 to 19 Risø performed an expedition by normal air connections to the Angmagssalik District in East Greenland in order to collect seawater, seaweed, mussels, lichen, and moss. A new field technique for collecting activity from large volumes of seawater was successfully tested at this expedition. Plastic bags containing 100 ℓ of water were suspended beneath a normal photo tripod. After precipitation of the activity the water was siphoned off and the precipitate was collected in a small bottle, which could be carried back on the plane.

DANA from DFH collected surface seawater from July 17 to August 14 at nine locations from the Faroes to Kap Farvel in Greenland and at eleven locations along the west coast of Greenland up to 69°N.

With M/S NELLA DAN, one of the supply ships chartered by the Royal Greenland Trade Company, Risø and Lund University collected seawater and various marine biota along the east coast of Greenland fra 70°N to 75°N. Furtheremore, approximately 40 surface seawater samples were collected on the way between southern Norway and East Greenland. This expedition took place from August 1 to 27. The last expedition of the year was performed by DANA, DFH from Sept. 18 to 29, when 12 surface seawater samples were collected in the North Sea between Jutland and Scotland.

During 1982 under this programme we have collected more than 120 seawater samples from the North Atlantic region. Most of the samples have already been analysed for radiocesium. In the course of 1983 samples will also be analysed for ^{90}Sr, transuranics, and tritium. Some of the biological samples have been measured for γ-emitters and analysed for transuranics.

Preliminary results from the 1982 samplings

The most interesting result of the analysis performed hitherto o the 1982 samples has been the detection of ^{134}Cs at East Greenlan between 70°N and 75°N. Ten locations were sampled in this region. Tw hundred liters of surface seawater were collected at each location, an radiocesium was collected on AMP precipitates on board the ship. The fiv locations closest to the coast contained 5.43±0.19 (1SD) Bq ^{137}Cs m^{-} while the five locations farther away from land (approx. 4° east o the other locations) contained 6.70±0.84 Bq ^{137}Cs m^{-3}. The eastern lo cations thus yielded significantly higher ^{137}Cs concentrations than th western. The concentrations of ^{134}Cs were too low to be detected in single samples. It was therefore necessary to combine the 1 kg AMP fro the 10 samples representing 2 m^3 of seawater. However the AMP caugh some natural activity from the water, and the peaks of ^{137}Cs in th Ge(Li) spectra were disturbed by this activity. Hence we concentrate the radiocesium from the AMP on a precipitate of 1 g of Cs_2PtCl_6, whic carried 95% of the activity from the AMP. By this concentration it wa possible to measure the activity of ^{134}Cs in the 5 eastern station separately, and we found 0.056 Bq ^{134}Cs m^{-3} (±20% counting error) The inner stations contained only 0.0032 Bq ^{134}Cs m^{-3}, but this leve was not significantly different from zero activity. If we assume tha approximately 5 Bq ^{137}Cs m^{-3} represents the fallout background in Eas Greenland waters and that the remaining part of the activity comes fro Sellafield in the UK we may conclude that the five eastern station contain radiocesium discharged from Sellafield during the 1975-78 period The inner stations may show a small Sellafield contribution from th years 1970-73. We may imagine that we have two transport routes to thi region of the East Greenland waters: one crossing the Greenland Sea betwee Norway and Greenland and another making its way over Spitzbergen to th East Greenland Polar current.

Four stations at the west coast of Greenland collected between 62 and 64° N contained 6.33±0.41 Bq ^{137}Cs m^{-3}; this was nearly as hig as the concentration found on the east coast. We suppose that Sellafiel thus also contributed to the activity in West Greenland waters. The re latively high concentrations suggest short transit times of water fro the east to the west coast of Greenland.

Preliminary conclusions of our radiocesium studies in Greenland water are that waterborne pollution in the North Sea may be carried to Green land coastal waters in 3-5 years, and the dilution factor from the Nort Sea to Greenland waters is approximately 50-100.

Tc-99 analyses

Lund University has developed a suitable analytical procedure fo determining ^{99}Tc in algae and seawater. Due to the very high concentratio ratio between algae and seawater it will be possible to use algae as bioindicator over long distances from the release point of ^{99}Tc to th sea.

We expect that in the future ^{99}Tc will be an important supplement t the radiocesium in the tracing of the Sellafield discharges in the Nort Atlantic region.

: project no. 3:
nental studies of plutonium and americium at Thule,
ıd.
project:
ɜr Aarkrog
ɔientific staff: Henning Dahlgaard and Elis Holm, (Uni-
of Lund, Sweden).

ɜ analyses of biological samples collected in 1979 at
ave now been completed. The final determinations support
ɔlusion from earlier ones: Benthic organisms at Thule show
/^{239,240}Pu ratio, which is two times higher than that
ı the sediments where they are dwelling.
have experimentally determined the K_D value for Pu in
ediments, and found that the value varied with the amount
nents used in the extraction. If 50 mg (wet weight) sedi-
ere extracted by 1 ℓ of water for 24 hours the K_D was
Bq g^{-1} sediment (dry) per Bq g^{-1} water. For 0.5 g sedi-
t became $5.2 \cdot 10^5$ and for 5 g, $2.3 \cdot 10^5$. There was similar
e in K_D with increasing sediments contaminated by Sella-
ischarge. The K_D's for the Thule debris was probably
higher than those found for Sellafield plutonium. The
ments of K_D for Am in Thule sediments have not been
d, but they seem to be lower than those observed for Pu.
is true it provides an explanation for the difference
u ratios observed for benthos and sediments at Thule.

List of publications in 1982

I:

Aarkrog A., Dahlgaard H., Nilsson K. and Holm E.
"Further Studies of Plutonium and Americium at Thule, Greenland". Health Physics 1983 (in press).

Aarkrog A., Bøtter-Jensen L., Dahlgaard H., Hansen H., Lippert J., Nielsen S.P., and Nilsson K.
"Environmental Radioactivity in Denmark in 1981", Risø-R-469 (June 1982).

Aarkrog A., Dahlgaard H., Hallstadius L., Holm E., and Lippert J., "Environmental Radioactivity in the Faroes in 1981", Risø-R-470 (July 1982).

Aarkrog A., Dahlgaard H., Holm E., Hansen H., Lippert J., and Nilsson K.
"Environmental Radioactivity in Greenland in 1981", Risø-R-471 (July 1982).

Aarkrog A., Dahlgaard H., and Nilsson K.
"Studies on the Distribution of Transuranics in the Baltic Sea, the Danish Belts, the Kattegat and the North Sea", IAEA-TECDOC-265 p. 23-32 (1982).

Holm E., Persson B.
"Transuranic Cycling Behaviour in Marine Environment", IAEA-TECDOC 265 p. 105-110 (1982).

II:

Holm E.
"Release, distribution and pathways of radionuclides in the Baltic Sea", Communication at IAEA Research coordinating meeting in Vienna 5/9, 1982.

Aarkrog A. and Dahlgaard H.
"Sources of ^{137}Cs and ^{90}Sr in the Danish Straits", Communication at IAEA Research coordinating meeting in Vienna 5/9, 1982.

Progress Report
1982

Contractor:

Contract no.: BIO-B-439-81-UK

Natural Environment Research
Council
Polaris House
North Star Avenue
GB-Swindon SN2 1EU

Head(s) of research team(s):

Mr. S.E. Allen
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Merlewood Research Station
Grange-over-Sands
GB-Cumbria LA11 6JU

General subject of the contract:

Radionuclide pollutants in natural plant-soil ecosystems.

List of projects:

1. Radionuclide pollutants in natural plant- soil ecosystems.

Title of project nr

Radionuclide pollutants in natural plant-soil ecosystems

Head of project and scientific staff:

S.E. Allen with Dr. A.D. Horrill, Dr. B.J. Howard and J.A. Parkinson

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This research project is concerned with the fate of radionuclides that are deposited by air or water on vegetation and soils. Most of the experimental work is carried out in, or uses material from, the vicinity of the Sellafield Nuclear Fuel Reprocessing Plant in West Cumbria, U.K., as levels of radionuclides are higher in this area than elsewhere in the country. Initially, survey work was carried out to determine site distribution and variation in the higher activity saltmarsh localities but studies are now concentrating on particular aspects of the uptake of radionuclides by plants and animals.

1. The distribution of radionuclides in a saltmarsh

The variation in concentration and spatial patterns of radionuclides in an ungrazed saltmarsh bordering the Ravenglass estuary, Cumbria, U.K., has been examined. The technique of Indicator Species Analysis was used to construct a vegetation map of the plant communities on the marsh. These ranged from a grass turf of Puccinellia maritima on the sea-ward side, through a low bushy vegetation of Halimione portulacoides to a well defined strand-line at the landward edge.

As expected, the distribution patterns were dominated by tidal immersion, with which all elements showed positive correlations. The exceptions were nuclides with short half-lives, ^{95}Zr and ^{95}Nb, which had significant ($P<0.01$) negative correlations. Overall point to point variation was large, in the case of ^{241}Am ranging from 35 p Ci g^{-1} on the open mud flats to over 250 p Ci g^{-1} on the strand-line. The importance of vegetation in trapping material was strongly demonstrated, with levels in the vegetated areas being 3 times those on open mud. Trend surface analysis was successfully used to demonstrate the different distribution patterns of the radionuclides on the marsh, the main constrasts being between the long-lived nuclides in the upper marsh and the short-lived nuclides on the lower, frequently immersed areas. Similar work on a grazed marsh has shown less variability due to the lack of contrasting morphological features in the cropped vegetation. There is a graded diminution in levels with distance from the sea but there is also evidence of a strand-line deposition on the ungrazed marsh.

2. Plant uptake studies

Glasshouse experiments have been carried out to examine uptake from a contaminated growth media. This consisted of a mixture of silt, relatively high in radionuclides, from the Cumbrian coast and a horticultural loam. Plant species common in the U.K., with contrasting growth forms, were used in the experiment. These included a pasture grass (Lolium perenne), a legume (Trifolium repens), a shallow rooting plant (Ranunculus repens) and a deep rooting plant (Rumex obtusifolius). Turnip was grown as a root crop and maize as a potential silage plant. The dominant salt marsh species, (Puccinellia maritima), was also grown. Soil contamination was checked by monitoring titanium levels.

Total harvested biomass for each block was between 300 and 400 g dry weight. Results so far, show that significant amounts of ^{137}Cs are taken up by all the species, with the tissue concentrations ranging from 1 to 8 p Ci g^{-1} dry matter.

3. Transfer factors for radionuclide intake by sheep

This study has concentrated on a flock of sheep grazing an area of saltmarsh (similar and close to the marsh referred to above) and adjacent rough heath. The proportion of time spent on different parts of the range (divided into sub-areas according to radionuclide levels), at different times of the day and year and in different weather conditions has been observed. Regular collections of vegetation and faeces have been made on these sites. In this way it has been possible to estimate total radionuclide intake with reasonable confidence.

Initial measurements of the γ radionuclides in vegetation showed a sharp concentration decrease as the summer progressed. This demonstrates the fallacy of projecting results, obtained at one sampling period, to a full year. In order to measure the digestibility of contaminated saltmarsh vegetation in sheep a separate feeding trial using tethered sheep was carried out. In this experiment 3 sheep were fed for 3 weeks, and samples of the food, faeces and urine were collected for the determination of α and γ emitters.

A detailed analysis (50 components) of a single ewe showed that ^{137}Cs was concentrated in the soft tissue. Levels in the muscles accounted for most of the ^{137}Cs because of their relatively large bulk. In contrast, most of the $^{239/240}Pu$ was found in the bone, liver and lungs. A number of ewes and lambs from the main study flock were slaughtered at intervals during the study year and key tissue and bone samples are being analysed for α and γ emitters. A control ewe, from a Yorkshire farm which has supplied sheep to the study flock, is also being examined.

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

Horrill, A.D. (1983). Concentrations and Spatial Distribution of Radioactivity in an Ungrazed Saltmarsh. Proceedings, Ecological Significance of Radionuclides, British Ecological Society, April 1982.

Progress Report
1982

Contractor: Contract no.: BIO-B-487-82-D

Technische Hochschule Darmstadt

D-6100 Darmstadt

Head(s) of research team(s):

Prof. Dr. K. Bächmann
Fachbereich für Anorganische
Chemie und Kernchemie
Technische Hochschule Darmstadt
D-6100 Darmstadt

General subject of the contract:

Development of a new method for the determination of lead-210 and study of the air-plant and soil-plant transfer pathways in uranium mining areas.

List of projects:

1. Development of a new method for the determination of lead-210 and study of the air-plant and soil-plant transfer pathways in uranium mining areas.

Projektnr. BIO-B-487-82-D

EINE NEUE METHODE ZUR BESTIMMUNG VON Pb-210 IN NATORLICHEN PROBEN SOWIE UNTERSUCHUNGEN ZU DEN TRANSPORTPFADEN LUFT/PFLANZE UND BODEN/PFLANZE SOWIE URANERZBERGWERKVORFLUTER

Prof. Dr. K. Bächmann
Fachbereich Anorganische Chemie und Kernchemie
Technische Hochschule Darmstadt, 6100 Darmstadt/FRG

Die Bestimmung von Blei-210 in radioökologischen Untersuchungen ist durch die radioaktiven Eigenschaften des Blei-210 kompliziert und zeitaufwendig. Dies liegt daran, daß Pb-210 nur eine schwache β-Strahlung aussendet mit der Ausnahme einer γ-Strahlung von 48 KeV mit niedriger Übergangswahrscheinlichkeit. Durch das Fehlen charakteristischer Strahlung ist es entweder notwendig, die Bestimmung über die höher energetische β-Strahlung des Bi-210 durchzuführen oder über die α-Strahlung des Po-210. In beiden Fällen muß zunächst eine Abtrennung erfolgen, um dann die nachwachsenden Produkte zu bestimmen. Dies erfordert nicht nur zeitaufwendige Abtrennung, sondern auch das jeweilige Abwarten der radioaktiven Gleichgewichte. Da Blei-210 in der neuen Strahlenschutznorm ein wesentlich höheres Gewicht eingeräumt wird, so daß es etwa dem Radium-226 an Toxizität gleichgesetzt werden kann, erscheint es notwendig, eine wesentlich einfachere Analyse dieses Nuklids zu entwickeln.

Unsere Methode beruht auf einem sogenannten Fällungsaustausch, wie er z.T. in der Spurenelementanalyse angewandt wird. Zunächst wird ein frischer Niederschlag von Zinksulfid hergestellt, und dann werden die Probenlösungen bei einem pH-Wert von 3,6 durchgesaugt. Bei höheren Werten wird Eisen ebenfalls als Hydroxid niedergeschlagen und dann Uran mitgefällt. Unter diesen Bedingungen wird von den Radionukliden lediglich Bi-210 mit ausgetauscht, so daß die Gleichgewichtseinstellung abgewartet werden muß, wenn nicht schon Gleichgewicht herrscht. Die Konzentration des natürlichen Bleis ist ohne jeden Einfluß. Da das Blei auf einer sehr dünnen Zinksulfidschicht sich befindet, läßt sich sehr leicht eine Messung mit einem dünnen Plastik-Szintillator vornehmen. Dadurch wird die Selektivität gegenüber anderen, höher energetischen β-Strahlern noch einmal erhöht. In Tab. 1 sind verschiedene Verfahren zusammengestellt und miteinander verglichen.

Die Methode kann sowohl auf biologische Proben als auch auf Bodenproben Aerosole in Luft und auf Wasserproben angewandt werden. Es ist lediglich notwendig, für die Festproben einen Aufschluß durchzuführen. Wir haben inzwischen diese Methode auf etwa 400 Proben in Feldexperimenten angewandt und dabei sowohl das Verhalten in Uranerzbergwerkvorflutern untersucht als auch die Immissionsbelastung in der Nähe eines Uranerzbergwerks. Da wir gleichzeitig Ra-226 bestimmt haben, läßt sich sagen, daß das Verhalten von Pb-210 wesentlich komplizierter ist. Dies hängt damit zusammen, daß Pb-210 in jede Matrix über zwei Wege kommt, nämlich einerseits direkt aus dem Zerfall des Ra-226, andererseits aber auch über das Tochterprodukt Rn-222. Durch die Entstehung aus dem Zwischenglied Rn-222, das sich als Edelgas schnell verbreitet und auf andere Art und Weise als das Blei selbst, wird die Deutung der einzelnen Experimente kompliziert. Dies ergibt sich auch daraus, daß die Verhältnisse von Pb-210 zu natürlichem Blei sehr unterschiedlich sind. Interessante Phänomene bei den Transportpfaden des Pb-210 entstehen auch dadurch, daß z.B. das gesamte Pb-210 durch Regen aus der Luft genommen wird und auf die Vegetation gelangt. Die Versuche sollen insbesondere in dieser Richtung fortgesetzt werden.

Tabelle 1: Vergleich verschiedener Nachweismethoden

	γ-spectroscopy	liquid scintillation	methane flow counter	plastic scintillator
limit of detection	10 pCi	3 pCi	0.06 pCi	0.3 pCi
time for the measurement	72 h	2 h	15 h	15 h
time between separation and measurement	no	20 d	4 d	no

Veröffentlichung 1982:

K. Bächmann, A. Olkis, H. Klenk
A new method for the determination of Pb-210 in natural samples
Health Physics (eingereicht)

Progress Report
1982

Contractor: Contract no.: BIO-B-465-81-F

Commissariat à l'Energie
Atomique, CEA
CEN de Fontenay-aux-Roses
B.P. n°6
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Head(s) of research team(s):

Dr. G. Baudin
Service d'Etudes Analytiques
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General subject of the contract:

Development of a method of determining traces of long-lived beta-emitters by opto-galvanic spectroscopy and assisted fluorescence.

List of projects:

1. Development of a method of determining traces of long-lived beta-emitters, such as I-129 and Tc-99, by opto-galvanic spectroscopy and assisted fluorescence.

PROGRAMME RADIOPROTECTION CONTRACT N° BIO-B-465 PL-F

DEVELOPMENT OF A METHOD OF DETERMINING TRACES OF LONG-LIVED BETA-EMITTERS BY OPTO-GALVANIC SPECTROSCOPY AND ASSISTED FLUORESCENCE

Dr G. BAUDIN - Dr Th. BERTHOUD

a) Détection d'iode

Les études préliminaires (Cf Progress report n°2) nous ont conduit à retenir la technique de fluorescence extra-cavité avec la raie 514,5nm du laser à argon comme source d'excitation. Une cellule intracavité permet d'assurer la sélection isotopique de I_2^{129} jusqu'à un rapport I_2^{129}/I_2^{127} de 1%. Nous étudions les possibilités d'amélioration de ce rapport par une variation des conditions de pression de la cellule intra-cavité. La spécificité spectrale de la fluorescence de I_2^{129} est également un moyen de sélectivité isotopique dans un rapport I_2^{129}/I_2^{127} de 1/°°. Dans l'état actuel de notre appareillage, nous avons donc une sélectivité isotopique dans un rapport I_2^{129}/I_2^{127} de 10^{-5} avec une limite de détection de l'ordre de 10^{11} molécules de I_2^{129} par cm^3 d'air ramené à pression atmosphérique.

Les études en cours portent sur les interférences crées par l'éventuel polluant de l'air (oxyde d'azote en particulier)

b) Détection du technétium 99

On rencontre principalement le Tc^{99} dans l'environnement sous forme de Tc à la valence VII dans l'eau. Afin d'éviter au maximum les problèmes de manipulation d'échantillons contenant de faibles traces, nous étudions les possibilités d'application de la technique du Thermal Lensing qui pourrait s'appliquer directement sur les liquides à analyser. Les résultats préliminaires semblent encourageants, mais ils nécessitent un appareillage relativement complexe.

LIST OF PUBLICATIONS IN 1982

I. Publications in Scientific Journal :

Fluorescence de l'iode stimulée par laser, Proceedings de la 3ème Réunion franco-italienne de spectroscopie atomique - Florence 4-6 octobre 1982
T. BERTHOUD, B. REMY

II. Rapport interne :

Fluorescence de l'iode stimulée par laser - Application au dosage de l'iode 129 dans les effluents gazeux.
Rapport technique SEA/82-478
T. BERTHOUD, B. REMY

Progress Report
1982

Contractor:

Commissariat à l'Energie
Atomique, CEA
CEN de Cadarache
B.P. n° 1
F-13115 Saint-Paul-lez-Durance

Contract no.: BIO-B-340-81-F

Head(s) of research team(s):

Dr. Y. Belot
SERE-DPR
CEA - CEN de Fontenay-aux-Roses
B.P. n°6
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General subject of the contract:

Simulation of tritium transfer in the environment; study of organic tritium transfer in the aquatic environment, the soil-plant and the plant-mammal system.

List of projects:

1. Development of a tritium behaviour model.

Title of project nr 1 : DEVELOPPEMENT D'UN MODELE DE COMPORTEMENT DU TRITIUM

Head of project and scientific staff : Yves BELOT, Jacques DELFORGE, Claude CAPUT, Jean GUENOT

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1) Amélioration du modèle

Le modèle précédemment développé a fait l'objet d'améliorations en 1982. Les efforts principaux ont porté sur le sous-modèle de transfert de HTO entre l'atmosphère et le sol. Dans ce modèle la tranche de sol considérée est subdivisée en n couches superposées. Le contenu en tritium de chaque couche est décrit par une équation linéaire du premier ordre à coefficients variables. Ces coefficients sont fonction de la diffusion de HTO à l'interface sol-atmosphère, de la diffusion de HTO dans le sol et de l'écoulement gravitaire de l'eau. Les valeurs de ces coefficients sont estimées d'après les résultats expérimentaux les plus récents.

Il est apparu que le nombre de couches ne pouvait être choisi arbitrairement. L'épaisseur d'une couche doit être choisie de telle sorte que, durant le pas de temps Δt, les atomes de tritium se distribuent de manière homogène à travers la couche. Pour un pas de temps de 1 heure et les valeurs standards des paramètres, l'épaisseur de chaque couche ne peut dépasser 3×10^{-3}m. Compte tenu de cette valeur, il apparaît nécessaire d'effectuer le calcul sur plusieurs centaines de couches superposées. Ce nombre élevé n'entraîne pas de temps de calcul prohibitif, car le modèle reste assez simple.

2) Simulation d'un rejet continu de vapeur d'eau tritiée

Un rejet continu de vapeur d'eau tritiée se traduit pour des végétaux situés en un point donné, par des expositions répétées discontinues, dont la fréquence est fonction des conditions météorologiques. Une simulation a été faite en admettant que les végétaux étaient exposés pendant 10% du temps. On a supposé que les périodes d'exposition étaient réparties de manière aléatoire et qu'elles avaient elles-mêmes une durée aléatoire comprise entre 1 et 8 heures. Cette simulation représente schématiquement le cas d'une arrivée discontinue de vapeur d'eau tritiée, soumise aux aléas

de la direction du vent.

Il apparaît que l'activité moyenne de l'eau du sol dans la couche explorée par les racines, nulle au temps zéro, augmente progressivement avec le temps pour atteindre une valeur d'équilibre au bout de quelques dizaines de semaines. L'activité spécifique de l'eau du sol, à l'équilibre, est égale à l'activité spécifique de la vapeur d'eau atmosphérique multipliée par l'humidité relative moyenne de l'atmosphère. On atteint 50% de la valeur d'équilibre au bout de 10 semaines environ.

Il en résulte que, dans le cas d'un rejet continu qui se prolonge pendant plusieurs années, on ne doit pas négliger l'activité spécifique de l'eau du sol, qui est d'autant plus proche de l'activité spécifique de l'eau atmosphérique, que le climat est plus humide.

3) Etudes expérimentales complémentaires

Dans le but d'aider à la modélisation, des expériences complémentaires ont été effectuées portant sur l'incorporation du tritium dans la matière organique des végétaux, et sur le rôle de la photosynthèse et des réactions d'échange isotopique. On a mis en évidence trois formes distinctes de tritium : le tritium aqueux, le tritium organique échangeable et le tritium organique non-échangeable. Les deux premières formes ont une durée de vie relativement courte, la troisième forme ne décroît que très lentement, et peut devenir à terme prépondérante devant les deux autres formes.

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

Y. BELOT et J. DELFORGE. "Modélisation du transfert de HTO entre l'atmosphère et le sol". Annales de l'Association Belge de Radioprotection, 7, 183-198 (1982).

J. GUENOT, C. CAPUT, Y. BELOT et F. BOURDEAU. "Rôle des réactions de photosynthèse et d'échange dans l'incorporation du tritium dans la matière organique des végétaux". Annales de l'Association Belge de Radioprotection, 7, 293-306 (1982).

Y. BELOT, C. CAPUT, J. GUENOT et F. BOURDEAU. "Incorporation of Tritium into organic matter of terrestrial plants exposed to tritiated water releases of short duration". Health Physics (sous presse).

II. Short Communications, Theses, Internal Reports, Patents ...

Y. BELOT. "Transfer of tritiated water into the plants : a review". European Seminar of the Risks from Tritium Exposure. Mol (Belgium), 22-24 Nov. 1982.

Progress Report
1982

Contractor:

Com.Naz.per la Ricerca e
per lo Sviluppo dell'Energia Nucl.
e delle Energie Alternative, ENEA
Viale Regina Margherita 125
I-00198 Roma

Contract no.: BIO-B-324-81-I

Head(s) of research team(s):

Dr. G. Busuoli
Coordinatore Attività PAS Bologna
ENEA
Via Mazzini 2
I-40138 Bologna

General subject of the contract:

Characterization of particulate contamination of the air and its deposition in the airways.

List of projects:

1. Characterization of particulate contamination in the air and its deposition in the airways, with particular regard to alpha emitting aerosols in special working environments.

Title of project nr. 1: Characterization of particulate contamination of the air and its deposition in the airways

Head of project and scientific staff: Prodi V.
Melandri C.
Tarroni G.
Lembo L.
Civolani O.

The measurement system composed of an inertial spectrometer and a screen-type diffusion battery, both equipped with CR 39 track etch detectors has been used in dwellings to measure the distribution of alpha activity over the aerosol particle size.

The measurements have made clear the importance of particles in the ultrafine range (size smaller than 0,1 μm) for the assessment of the lung dose due to the natural alpha activity. The size range 0,2 to 0,7 μm is also very important since the aerodynamic and diffusive mechanisms superimpose: for the two instruments, the superimposition is still insufficient for a detailed characterisation and should be improved.

The study on the influence of electrostatic charges carried by aerosol particles on the airways total deposition has been completed. The relevant results concerning the long contamination can be summarized as follows:

- for each particle size there is a threshold charge below which no effect is detactable since the particles are captured any way by other mechanisms (settling and diffusion)
- the deposition increase above the threshold is a unique function of the mechanical mobility times the charge squared
- in many practical situations the deposition increase is remarkable and it is not negligible for the assessment of the lung burden
- the increased deposition in the size range investigated (0.3 through 1.0 μm) takes place in the same alveolar region where diffusion and settling act
- an intersubject variability of the electrostatic deposition has been found of the same magnitude of the variability with neutral particles.

The experiments have been performed at a charge concentration where image forces prevail on the electrostic scattering.

List of publications in 1982

elandri, G. Tarroni, V. Prodi, T. De Zaiacomo, M. Formignani, C.C.
ardi
sition of charged particles in the human airways.
Meeting of the GAeF "Aerosols in Science, Medicine and Technology"
gna, 14-17 September, 1982.

rodi, C.C. Lombardi, T. De Zaiacomo, C. Melandri, M. Formignani,
arroni
distribution measurements of natural alpha active aerosols.
Meeting of the GAeF "Aerosols in Science, Medicine and Technology"
gna, 14-17 September, 1982. To be published on: J.Aerosol Sci.

Progress Report
1982

Contractor:

Commissariat à l'Energie
Atomique, CEA
CEN de Cadarache
B.P. n° 1
F-13115 Saint-Paul-lez-Durance

Contract no.: BIO-B-466-81-F

Head(s) of research team(s):

Dr. C. Caput
SERE-DPR-IPSN
CEA-CEN de Fontenay-aux-Roses
B.P. n° 6
F-92260 Fontenay-aux-Roses

General subject of the contract:

Resuspension of radioactive nuclides released from the ocean surface.

List of projects:

1. Resuspension of radioactive nuclides released from the ocean surface.

Title of project nr

Resuspension de nucléides radioactifs à partir de la surface des océans

BIO-B-466-81-F

Head of project and scientific staff :

C. CAPUT

Y. BELOT

D. GAUTHIER

I Introduction.

Les phénomènes de resuspension à partir des sédiments et de la surface des océans n'ont retenu que depuis peu l'attention de quelques laboratoires. Au Royaume Uni, la division des Sciences Médicales et de l'Environnement de Harweld a effectué des prélèvements atmosphériques sur la côte au voisinage de l'usine de Windscale et dosé les actinides démontrant que les embruns marins constituent une voie de transfert des actinides. Ceux-ci, de même que d'autres radionucléides, peuvent ensuite s'accumuler sur certains végétaux de la frange littorale, comme le montre un travail récent présenté par le laboratoire de Radioécologie Marine de la Hague du C.E.A. (Fr.82). D'autre part notre laboratoire à montré (Be.82), aux cours d'expériences de simulation effectuées ces deux dernières années, que l'aérosols marins engendré par éclatement de bulles à la surface de l'eau de mer pouvait être enrichi en americium par rapport à l'eau d'un facteur supérieur à 1000. Une collaboration à été amorçée cette année avec les chercheurs de Harwell travaillant in situ dans ce domaine (Environmental and Medical Sciences Division). En ce qui nous concerne, nous menons parallelement deux types de recherches, les unes par simulation en laboratoire, les autres à partir de prélèvements effectués in situ.

II Expérimentations en laboratoire.

Le dispositif d e simulation permettant d'engendrer un aérosol par éclatement de bulles à la surface de l'eau de mer a subi en 1982 différentes améliorations: Il est maintenant possible de faire varier la hauteur de la couche d'eau de mer traversée par les bulles, ce qui était nécessaire à la compréhension du mécanisme d'enrichissement : trois hypothèses sont en effet avancées : le phénomène se produisant soit au cours de l'ascension des bulles, soit seulement à la surface, la bulle isolant avant d'éclater une micro-couche superficielle, soit enfin par la conjonction de ces deux mécanismes. Par ailleurs les performances de l'appareil ont été améliorées, autorisant la génération d'un flux d'aérosol plus important, donc une meilleure sensibilité de détection. Enfin une méthodologie a été mise au point pour étudier l'influence de la matière organique et de la matière particulaire sur l'enrichissement de l'aérosol.

III. Expérimentations in situ.

Une nouvelle technique de prélèvement, inspirée de celle imaginée à Harwell, va être mise en oeuvre, consistant à tendre une surface de mousseline en bordure de mer perpendiculairement au vent. Cette technique permet de prélever en quelques heures les embruns contenus dans un grand volume d'air en évitant de piéger les particules fines représentatives des retombées. Quelques essais ont été effectués pour tester ce mode de prélèvement à proximité de l'usine de La Hague. Alors que les prélèvements effectués jusqu'ici par filtration classique de l'air sont caractéristiques des retombées et de la radioactivité naturelle (présence de Be.7), les spectrogrammes gamma obtenus avec ces nouvelles techniques sont caractéristiques des rejets en mer (présence de Rh 106, Cs 137, Ce 144, Co 60). Ceci confirme bien que la mousseline piège sélectivement les grosses particules, essentiellement par impaction, ce qui permet d'éliminer la composante fine de l'aérosol atmosphérique. Le potassium 40 permet d'évaluer la quantité d'eau de mer représentée par les embruns. Dans le cas d'une vitesse de vent de 10 mètres seconde et d'une durée d'échantillonnage de 5 heures, ce qui correspond au piégeage des embruns contenus dans un million de m^3 d'air, cette quantité d'eau est de l'ordre de 2 litres. Dans l'avenir, des analyses alpha seront également effectuées, d'une part sur de l'eau prélevée sur le même site, d'autre part sur la mousseline ayant collecté les aérosols.

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

Y. BELOT, C. CAPUT et D. GAUTHIER "Transfer of americium from sea water to atmosphere by bubble bursting" - Atm. Env. 16, n°6 pp 1463-1466, 1982.

A. FRAIZIER, Y. BARON "Données nouvelles sur les sources et transferts de radioactivité au continent par aérosols et embruns marins" Journée d'étude sur les pollutions marines, Cannes, 2-4 Déc. 1982.

Progress Report
1982

Contractor:

Contract no.: BIO-B-322-81-I

Com.Naz.per la Ricerca e
per lo Sviluppo dell'Energia Nucl.
e delle Energie Alternative, ENEA
Viale Regina Margherita 125
I-00198 Roma

Head(s) of research team(s):

Prof. A. Cigna
Centro St. Amb. Mar., ENEA
S. Teresa
Casella Postale 316
I-19100 La Spezia

General subject of the contract:

Environmental and health protection implications from nuclear plants discharging into coastal marine ecosystems.

List of projects:

1. Description and classification of typical marine coastal ecosystems.
2. Laboratory studies on the behaviour of long-lived radionuclides in the marine environment.

Title of project nr. 1 - "Description and classification of typical marine coastal ecosystems"

Head of project and scientific staff: R. Boniforti, G. Buffoni, A. Cigna, P. Marri, C. Peroni, A. Zattera, G. Zuccaro Labellarte G. Zurlini

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A greater emphasis was put on the in sea activity with respect to the experimental one on account of the movement of the Laboratory to the new premises of Forte di Santa Teresa and problems related to the supply of the laboratory's equipment and furniture. The new center offers additional facilities not previously available (e.g., a clean room for the analysis of trace metals in environmental samples, a new computer system, new laboratories for experiments with radionuclides etc.) which greatly improve the possibility of research work. Therefore, 1982 may be considered as an innovative year as far as the possibility of marine research by ENEA is concerned.

The larger effort devoted to in sea work permitted to carry out a 2-month campaign along the coast of the Puglie region. This campaign was aimed at an initial description and classification of that environment which will be interested by the installation of large electric power plants.

The analysis of radionuclides in sediments and various biological matrices and of trace metals in water, particulate matter and sediments collected in 1981 from the Garigliano area has been concluded. As traced by ^{60}Co, the coastal zone interested by the discharge of the nuclear power plant covers an area of some 1700 km^2. The highest concentration of ^{60}Co was found in the sediments (maximum value of 500 pCi/kg dry wt.). Some differences in trace metal concentration in samples collected from the Garigliano area and the Ligurian Sea are attributed mainly to differences in lithology and mineralogy of the catchment basins of the rivers flowing into the two zones.

Additional data relevant to the Garigliano site and concerning physical biogeochemical, and benthic parameters are being evaluated. These data will also help to prepare a sedimentological chart of the Italian coastal environment.

A spectrophotometric method for the determination of the aerobic and facultative anaerobic activities based on the reduction of resazurin by dehydragenase in microorganisms has been implemented.

The modification consisted in separating the dye from the sediment by filtration, whereas in the original method the separation was performed by extraction by amyl-alcohol under controlled pH conditions. Presently, the method is being modified by performing the manipulation under aneorobic conditions. This would permit to evaluate also the activity of strictly anaerobic microorganisms and that of chemical compounds.
A new CTD-O_2-pH-pE probe has been tested in both estuarine and seawaters during a campaign in the North Sea carried out in cooperation with the Deutsches Hydrographisches Institut.
Very low level radioactivity measurements on fallout and environmental samples collected along the Italian coasts have been regularly carried out. The radioactivity of fallout is steadily decreasing. By assuming ^{137}Cs as representative of the trend, the average values for samples collected e.g. at La Spezia in 1981 and 1982 are 0.05 and 0.03 mCi km^{-2}, respectively.
CDT and currentmeter data for the zone between La Spezia and Sestri Levante have been analysed and used for an analytical model of the circulation in the Ligurian Sea. The model shows that the circulation of the Ligurian Sea responds to atmospheric motions with a 5-day periodicity. At this periodicity, the average current speed is about 3 cm s^{-1}. The shelf circulation seems forced by the deep basin circulation and a local wind.
A radially symmetrical model for the determination of the turbulent diffusion coefficient has been developed. The model, whose maximum efficiency is shown to be reached in calm sea stretches far away from the main circulation line, has been applied to the Bay of La Maddalena Island.

Title of project nr. 2 - "Laboratory studies on the behaviour of technetium in the marine environment"

Head of project and scientific staff: P. Scoppa, E.H. Schulte

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Laboratory facilities have not been available during the whole period covered by this report.
Therefore, the main activities have been:

- the preparation of oral communications and publications on the basis of results obtained in 1981;
- the implementation of mathematical models for calculating the transfer of technetium between the various compartments of our experi-

mental systems.

Only minor progresses in experimental activities can be mentioned:

- preliminary experiments on accumulation and release kinetics of technetium-95m, added as pertechnetate to seawater, have been carried out on the crab Pachygrapsus marmoratus.
  Concentration factor for technetium in this species is very low ($\sim$ 10). After 30 days of accumulation, approximately 70% of the whole body radioactivity is localized in the digestive system (hepato-pancreas, stomach and intestine) and released with the biological half-life of $\sim$ 24 days.
- Binding of the anion pertechnetate to albumin has been studied at pH 7.4 and aionic strength 0.16 using diafiltration techniques. Wash-in experiments provided equilibrium constants at different temperatures, from which free energy changes have been calculated. On the basis of these results, the standard enthalpy variation has been evaluated in the range of temperature between 5 and 37°C. The reaction is characterized by negative changes of both standard free energy and enthalpy. Therefore, reversible binding of pertechnetate to albumin can occur spontaneously and it is favoured by low temperatures.

LIST OF PUBLICATIONS AND ORAL COMMUNICATIONS

ANON. - Report of the radioecological survey near the Latina's nuclear power plant. CNEN RT/DISP (81) 9 (In Italian).

ANSELMI, B., A. BRONDI, O. FERRETTI, C. PAPUCCI - Archipealgo of La Maddalena. Sedimentology and its correlation with the distribution of some radionuclides. Paper presented at the 4th Congress of the Italian Association of Oceanology and Limnology. Stresa, May, 1982 (In Italian) In Press.

Astraldi, M., A. BRUSCHI, G. MANZELLA, R. MELONI, R. PURINI - The radially symmetrical model in defining the diffusion coefficient: An application to the Bay of La Maddalena Island. Arch. Met. Geoph. Biokl., Ser. A (1982) Vol. 31, pp. 137-146.

BAUDOUIN, M.F., P. SCOPPA - Influenza della temperatura e della concentrazione idrogenionica sulla tossicità acuta dei metalli pesanti per lo zooplancton. Atti della 50ma Assemblea della Società Italiana di Biologia Sperimentale, p. 21 (1981).

BO, F., R. BONIFORTI - Elemental analysis of sediments by emission and absorption spectrometry. Paper presented at the 20th Seminar of the Italian Association of Metallurgy, 3rd Italian-French Meeting on Atomic Spectrometry (In Italian) In Press.

BONIFORTI, R., A. MOAURO - Comparison of trace element content in marine organisms collected from La Maddalena Archipelago and other Mediterranean and Pacific ocean Sites. ENEA RT/CHI (82) In Press.

BONIFORTI, R., A. BRUSCHI, P. FRIGIERI - Concentration of Ni, Co, Mn, Cr, and Sr in marine, riverine and lacustrine waters. Paper presented at the 4th Congress of the Italian Association of Oceanology and Limnology (In Italian) In Press.

BONIFORTI, R., R. FERRAROLI, P. FRIGIERI - Analytical problems in the analysis of trace metals in seawater. Poster Paper presented at the 20th Seminar of the Italian Association of Metallurgy; 3rd Italian-French Meeting on Atomic Spectrometry (In Italian).

BONIFORTI, R., M. MADARO, A. MOAURO, R. RUGGIERO, B. ZURLINI - Instrumental determination by NAA of trace elements in marine particulate matter: Canonical correlation analysis. Paper presented at the 4th Congress of the Italian Association of Oceanology and Limnology (In Italian) In Press.

BONIFORTI, R., R. RUGGIERO, F. BO, J.C. TOUSSAINT - Chemical and mineralogical analysis of marine, riverine and lacustrine sediments collected from the area delimited by Massaciuccoli lake and Mesco Cape (In Italian) ENEA RT/CHI (82) In Press.

BONIFORTI, R., M. MADARO, A. MOAURO - Trace Element determination in suspended particulate matter from natural waters by instrumental neutron activation analysis. ENEA RT/CHI (82) In Press.

BONIFORTI, R.,R. FERRAROLI, P. FRIGIERI - Determination of trace metals in the Ligurian and central Tyrrhenian Seas. Submitted for publication in "Mar. Poll. Bull.".

BRUSCHI, A;, C. PAPUCCI, G. ZURLINI - Results on a radiological and environmental study carried out at the La Maddalena Archipelago. Paper presented at the 1st Congress of the Italian Society of Ecology (In Italian) In Press.

BRUSCHI, A., O. LAVARELLO, C. PAPUCCI, G. RASO, M. RICCOMINI, S. SGORBINI, G. ZURLINI - Distribution of radionuclides in the marine environment near the Garigliano nuclear power plant. Paper presented at the 22nd Congress of the Italian Association for Radioprotection-Gardone Riviera, 1981 (In Italian) In Press.

ESPOSITO, A., G. MANZELLA - Current circulation in the Ligurian Sea. Hydrodynamics of semy-enclosed seas (1982) pp. 187-203, Elsevier Sci. Publ. Co.

PERONI, C., G. ROSSI - Estimation of the microbiological activity in marine sediments by resazurina dye reduction. Paper presented at the 4th Congress of the Italian Association of Oceanology and Limnology, Stresa, May, 1982 (In Italian) In Press.

SCHULTE, E.H., A. SECONDINI - Thermal tolerance of the Palaemonid shrimp Palaemon elegans(L.). Bollettino della Società Italiana di Biologia Sperimentale, In Press.

SCHULTE, E.H., P. SCOPPA, A. SECONDINI - Accumulo e rilascio del tecnezio da parte di alcuni organismi marini: I) Palaemon elegans . Bollettino della Società Italiana di Biologia Sperimentale, In Press.

SCHULTE, E.H.,P. SCOPPA, A. SECONDINI - Comportamento geobiochimico del tecnezio nell'ambiente marino. Atti del 4° Convegno Nazionale sull'attività di ricerca nei settori della radiochimica e della chimica nucleare, delle radiazioni e dei radioelementi. In Press.

SCOPPA, P. - Lo studio della radioattività nell'ambiente marino: recenti orientamenti ai fini della protezione radiologica. Atti del 12° Congresso della Associazione Italiana della Radiobiologia Medica, pp. 391-394. (1981).

SCOPPA ,P. - Complessazione di metalli pesanti da parte di sostanze umiche presenti nelle acque naturali. Atti del 1° Convegno Nazionale di Ecologia.

SCOPPA, P. - Impiego della diafiltrazione nello studio della interazione fra tecnezio ($^{95m}TcO_4^-$) ed albumina. Bollettino della Società Italiana di Biologia Sperimentale. In Press.

SCOPPA, P. - Il ruolo del chimico nello studio del comportamento ambientale dei radionuclidi. Conferenza tenuta all'ordine dei chimici della Campania.

SCOPPA, P., M.F. BAUDOUIN - La contaminazione radioattiva delle catene alimentari marine: metodologie per la valutazione dell'impatto radiologico. Atti della 14° Riunione della Società Italiana di Nutrizione Umana, p. 191 (1981).

SCOPPA, P., C. MYTTENAERE - Gli studi di radioecologia nell'ambito del programma di radioprotezione della Commissione delle Comunità Europee. Atti del 4° Convegno Nazionale sull'attività di ricerca nei settori della radiochimica e della chimica nucleare, delle radiazioni e dei radioelementi. In Press.

SCOPPA, P., E.H. SCHULTE, A. SECONDINI - Laboratory studies on the behaviour of technetium in the marine environment: progress report 1981. Oral communication at the 2nd Meeting of the Working Group on the Metabolism of Technetium, La Boule, Febr. 1982.

SCOPPA, P., E.H. SCHULTE, A. SECONDINI - Chemical form and behaviour of technetium in the marine environment. Communication at the International Symposium on Technetium in Chemistry and Nuclear Medicine.

SCOPPA, P., A. SECONDINI, E.H. SCHULTE - Indagini sulla stabilità dell'anione pertecnetato nell'ambiente marino. Atti del 22nd Congresso della Associazione Italiana di Protezione contro le radiazioni. In Press.

ZURLINI, G., A. BRUSCHI, C. PAPUCCI, A. BRONDI - Proposal for a biotypological classification of the marine environments of the Italian coasts. Paper presented at the 1st Congress of the Italian Society of Ecology (In Italian) In Press.

ZURLINI, G., A. ZATTERA, A. BRUSCHI - Structural analysis of phytoplankton communities variation in the Archipelago of La Maddalena. J. of Exp. Mar. Biol. Ecol. In Press.

Other publications not strictly related to the projects.

BERNHARD, M. - Toxic metals in the Mediterranean. A Review. Atti del XXVIII Congresso dell'Assemblea Plenaria del CIESM, Dic. 1982.

BERNHARD, M., G. BUFFONI - Mercury in the Mediterranean. An overview. Proceedings of the International Conference on Environmental Pollution, Thessaloniki, Greece, 1981, pp. 458-484.

BUFFONI, G., M. BERNHARD - Mercury in Mediterranean Tuna. Why is their level higher than in Atlantic Tuna? A model. Atti del VII International Symposium "Chemistry of the Mediterranean", Primosten, Yugoslavia, May, 1982. To be published in Thalassia Jugoslavica.

CIGNA, A. - Carsismo e inquinamento. Atti del 1° Convegno sull'Ecologia dei Territori Carsici, Castelnuovo di Sagrado, Aprile, 1979 (published in 1982).

CIGNA, A. - Behaviour of tritium in the marine environment. Paper presented at the European Seminar on the Risks from Tritium Exposure, 1982.

CIGNA, A. - Pollution problems in border karst regions. Proceedings of the International Symposium on the Protection of Karst (to be published).

CIGNA, A. - Il problema degli inquinanti persistenti. Estratto da: "Tutela e valorizzazione del patrimonio storico-artistico, culturale ed ambientale della regione Piemonte", Italia Nostra, Torino, (1979), pp. 185-208. ENEA RT/PROT (82) 12.

CIGNA, A. - Alcuni problemi sulla localizzazione di impianti nucleari in aree carsiche. Atti del Simposio Internazionale "Utilizzazione delle Aree Carsiche", 1982.

Progress Report
1982

Contractor: Contract no.: BIO-B-323-81-I

Com.Naz.per la Ricerca e
per lo Sviluppo dell'Energia Nucl.
e delle Energie Alternative, ENEA
Viale Regina Margherita 125
I-00198 Roma

Head(s) of research team(s):

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Divisione Scienze Ambientali
CSN della Casaccia, ENEA
C.P. 2400
I-00100 Roma

General subject of the contract:

Biological and environmental aspects of toxicology of plutonium.

List of projects:

1. Late effects and metabolic behaviour of plutonium in mice.
2. Fallout plutonium content in the italian diet and in members of the general public.
3. Behaviour of fallout plutonium in italian soils and sediments.

Title of project n. 1 - "Late effects and metabolic behaviour of plutonium in mice".

Head of project and scientific staff: U. Andreozzi, G.F. Clemente G. Ingrao, V. Covelli, B. Bassani, B. Di Caterino, G. Santori.

The experiment under way comprises six groups of animals intravenou injected with monomeric ^{239}Pu. Plutonium activity ranges from 30 pC mouse (about 500 animals) to 3000 pCi/mouse (about 300 animals), wi age matched uninjected controls for each experimental group. A tot of about 2500 mice has been treated between the end of 1978 and 198 At death, animals are autopsied and various organs are taken for histopatoloty, for Pu retention measurements and for dosimetric studies; an X-ray picture is taken of the whole skeleton.
Numbers of dead and surviving animals, as of December 1982, are reported in table 1. In the lowest contamination group and its cont all animals are dead. A significant number of dead animals is also available in the next two highest exposure groups. The cumulative mortality (fig. 1) shows no appreciable differences between the 30 pCi group and its matched control, while there could be some life shortening for the 150 and 300 pCi groups though the corrisponding control data are not yet available.
A fraction of 10% of all dead animals is randomly selected for Pu retention measurements in femour, liver, testis and kidneys. Fig. 2 shows the data for liver where up to now the % retention is in reasonable agreement with a retention curve previously determined after about a 100-fold higher contamination level. The values of the % retention in the femour at various times after contamination are also in close agreement with the retention values determined in a previous experiment after an injected ^{239}Pu dose of 5000 pCi/mouse.

Histopathological analysis is still very preliminary. Some osteosarcomas have been diagnosed in various experimental groups, and many more suspected cases have been seen radiologically. In 1983 the histopathological part of the experiment will be given thourough attention.

Microdistribution analysis in bone with the Quantiment image analyser has not started yet, but the equipment has been installed and it is expected to be working soon.

Experimental Group	Injected activity pCi/mouse	Number of animals per group	Dead mice	
			Total number	%
Control	0	783	330	42
LEP-1	30	472	472	100
LEP-2	150	491	365	74
LEP-1	300	397	249	62
LEP-4	750	400	51	12
LEP-5	1500	398	53	13
LEP-6	3000	300	24	8
TOTAL		3341	1544	46

Tab. 1 - Current status of the plutonium late effect experiment on mice as of December 1982.

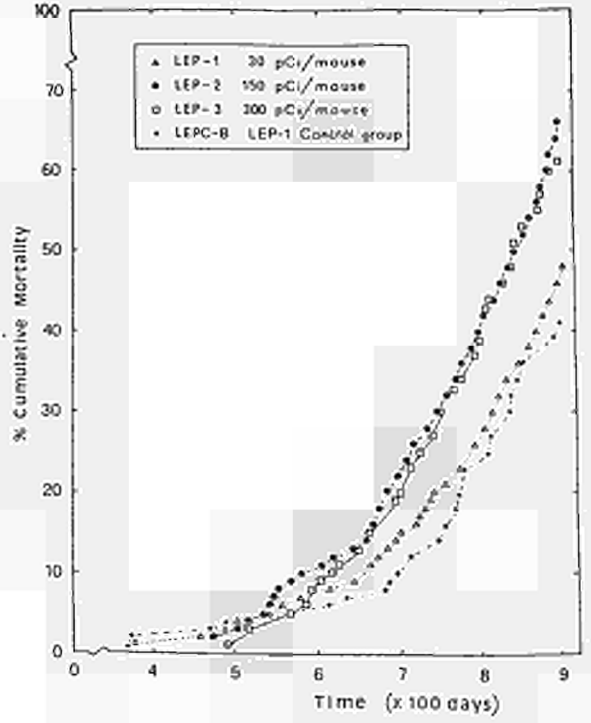

Fig. 1

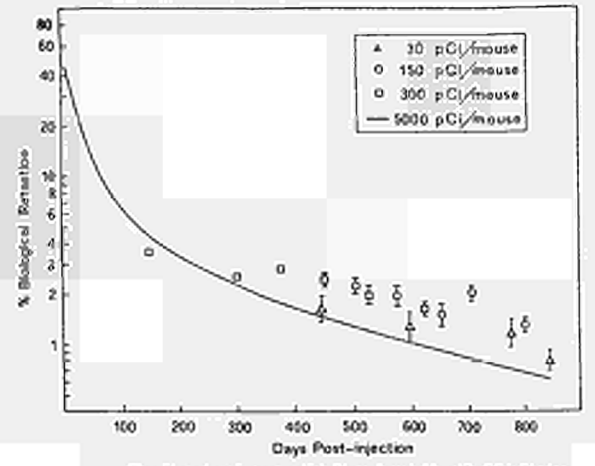

Fig. 2

The Biological Retention Of Plutonium In Liver Of CB3 F1 mice

Title of project n. 2 - "Fallout plutonium content in the italian diet and in members of the general public".

Head of project and scientific staff: G. Santori, G.F. Clemente

The collection of autopsy samples has been prosecuted during 1982 in collaboration with the Institute of Legal Medicine of the University of Rome.

More than 50 plutonium determinations have been performed in autopsy samples during 1982. The mean $^{239,240}Pu$ concentrations ± S.E. in all analyzed samples are the following: 10.1 ± 3.6 mBq/kg in bone, 23.2 ± 7.0 mBq/kg in liver, 2.0 ± 0.4 mBq/kg in lungs, 2.0 ± 1.2 mBq/kg in kidneys and 3.8 ± 1.1 in spleen.

All the concentration data are reported for kg fresh weight.

The average plutonium body burden in the ten analyzed subjects is of the order of 100 mBq.

During 1982 the plutonium concentration has been also measured in about 10 different food items representative of both the environment and the dietary habits of Italy. The mean $^{239,240}Pu$ concentrations ± S.E. given for kg fresh weight are the following: 0.0046 ± 0.0200 mBq/kg in flour, 0.025 ± 0.015 mBq/kg in fruity vegetables (five different kinds of fruity vegetables have been analyzed), 0.183 ± 0.030 mBq/kg in leafy vegetables, 0.250 ± 0.070 mBq/kg in fishes (three different kinds of edible mediterranean fishes have been considered) and 2.50 ± 0.26 in shrimps caught along the coast of the south of Italy. The ^{238}Pu has been also measured in some food samples (fishes and shrimps). Such preliminary data seem to indicate a reasonable agreement with similar data reported for USA terrestrial food items. On the other hand the plutonium concentration seems significantly higher in marine foods originated from the Mediterranea sea than in those originated from the italian terrestrial environment.

Title of project n. 3 - "Behaviour of fallout plutonium in Italian soils and sediments".

Head of project and scientific staff: L.Cigna Rossi,
G. Zuccaro-Labellarte,
A. Brondi

The sample collection in Piemonte and Lazio regions initiated during 1981 has been completed during 1982; a new campaign has been then performed in Veneto region and about 25 samples have been collected.

Some preliminary samples from Toscana region were also collected and processed. A total of more than 80 Pu measurements have been carried out during 1982 and more than 100 values of Pu concentration in italian soil samples, collected in 76 sampling points, are available up to now.

As planned in the 1982 programme of work, for each soil type the ^{239,240}Pu concentration and the ^{137}Cs/Pu, ^{90}Sr/Pu concentration ratios in correlation with the soil parameters were obtained. The vertical distribution of Pu in many sampling points has been also determined.

The most relevant data are summarized in fig. 1 (concerning cultivated soils) and fig. 2 (concerning undisturbed soils).

For the cultivated soils the average integrated ^{137}Cs and ^{239,240}Pu contents (±2σ) (referred to a surface of 1 m^2 and a thickness of 30 cm) are 4220±1030 Bq and 70±22 Bq for ^{137}Cs and ^{239,240}Pu respectively .

Such results are in good agreement with the evaluated average cumulative fallout deposition for Italy which is 4000 Bq/m^2 and 62 Bq/m^2 for ^{137}Cs and ^{239,240}Pu respectively (1).

In undisturbed soils the vertical migration of ^{137}Cs, ^{239,240}Pu and ^{90}Sr have been determined. As an index of the vertical migration the half-concentration depth (HCD) has been used (1).

The HCD values calculated by means of the formula given in (1) are

respectively:

^{137}Cs = 1.7 cm (clayey soils); 4.1 cm (sandy soils)

$^{239, 240}Pu$ = 1.9 cm " " ; 11 cm " "

^{90}Sr = 7.7 cm " " ; -

It is evident that both ^{137}Cs and $^{239,240}Pu$ migrate more deeply in sandy soils (sample 39 PI in fig. 2) than in clayey soils (sample 3 TO in fig. 2).

In undisturbed soils the vertical transport of fallout radionuclides is in some way influenced by the physical properties of the soil: the future investigations will be directed to identify the main parameters upon which the transport depends.

Reference

1. A. Brondi, L.Cigna Rossi, A. Perini, P. Scalvenzo and G. Zuccaro Labellarte. ^{137}Cs, ^{90}Sr and $^{239,240}Pu$ distributions in Italian soils. Proc. 3 rd Int. Symp of Soc. Radiological Protection, Inverness June 1982, vol 1: 00/105.

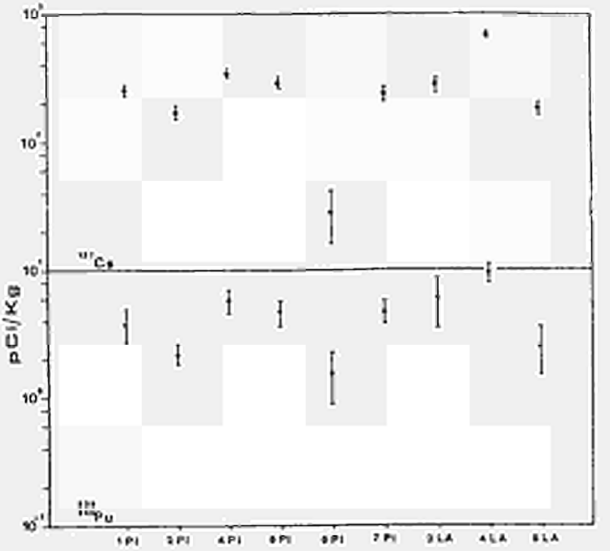

Fig. 1 - Cultivated soils

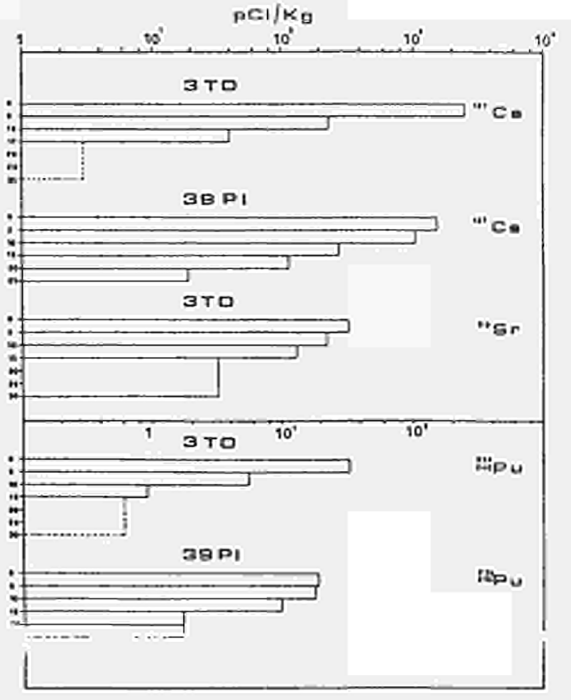

Fig. 2 - Undisturbed soils

List of publications in 1982

1. Publications in Scientific journal, Monographs, Proceedings

U. Andreozzi, G.F. Clemente et. al.:Long Term ^{238}Pu and ^{239}Pu retention and organ distribution in mice at low doses. Health Phys. (in press).

P. Belloni, G.F. Clemente et al.: La determinazione di plutonio e tritio in campioni autoptici, Proc. of the XX Spectrochemical Seminar, Florence, 4-6 october 1982,

A. Brondi, L. Cigna Rossi et al.: ^{137}Cs, ^{90}Sr and $^{239,240}Pu$ distribution in Italian soils. Proc. 3 rd. Int. Symp. of Soc. Radiological Protection, vol. 1, p. 99-105 (1982).

P. Belloni, G.F. Clemente et al.: Tritium and plutonium content in human tissues of the general population in Italy. Proc. 3 rd Int. Symp. of Soc. Radiological Protection, vol. 2 p. 518-524 (1982).

Progress Report
1982

Contractor:

Delta Institute for
Hydrobiological Research
Vierstraat 28
NL-4401 EA Yerseke

Contract no.: BIO-B-326-81-NL

Head(s) of research team(s):

Dr. E.K. Duursma
Delta Institute
Vierstraat 28
NL-4401 EA Yerseke

Dr. M. J. Frissel *
Dr. J. M. Martin **

General subject of the contract:

Differential migration of plutonium in the delta estuaries of Rhine, Meuse and Scheldt.

List of projects:

1. Differential migration of artificial radionuclides in the delta estuaries of Rhine, Meuse and Scheldt

Joint proposal of three laboratories.
* Association EURATOM-ITAL, postbus 48, 6700 AA Wageningen.
** Lab. Géologie, Ecole Normale Supérieure, Rue d'Ulm 46, F-75230 Paris.

ARTIFICIAL RADIONUCLIDES IN RHINE-MEUSE-SCHELDT DELTA

I. INTRODUCTION

The studies on the distribution of plutonium isotopes and various gamma emittors in the Rhine-Meuse-Scheldt delta of the south-west Netherlands were continued. Taking into account the limitation caused by the restricted number of plutonium analysis, due to long counting times, only selected samples were analysed. These are chosen on the basis of earlier results and derived hypothesis of distribution patterns.

In former progress reports, various results were presented and discussed on the distribution of radionuclides in the region and related to the type of sample: sediment, suspended matter, mussels, plants and lichens. (Euratom, 1979, 1980, 1981). Here the additional achievements will be presented for each studied radioisotope, and well for Cs-137, Co-60, Pu-238, 239, Sb-125, Ru-106 and Ce-144.

II. RADIOISOTOPE BEHAVIOUR

In general the behaviour of the detected radioisotopes has been investigated by relating their ratios to stable potassium, with the environmental circumstances. Potassium (determined by K-40) is indicative for the clay fractions and most radionuclides are proportionally present in these fractions. Instead of potassium also aluminium can be taken, which is equally related to the clay fractions.

II.1 Cs-137/K and Cs-134/Cs-137

The two available isotopes of cesium, Cs-134 ($t_{\frac{1}{2}}$=2.2 yr) and Cs-137 ($t_{\frac{1}{2}}$=30 yr) have been investigated. Cs-134 does not exist in fallout, thus the ratio Cs-134/Cs-137 is an indication of local contamination by nuclear installations. The results for suspended matter and top-layer sediment from Rhine, Meuse, Delta region, Scheldt and Southern North Sea towards Boulogne, showed Cs-137 levels of 0.1 - 0.5 pCi/g per % K. The Cs-134/Cs-137 ratios in % were about 5-10%.

In general there could not be distinguished a clear pattern for the Delta region, since the levels are relatively constant, while little variation was observed from river to sea. No clear increase was detectable close to the nuclear power stations Doel and Borssele.

II.2 Co-60

The concentrations of Co-60 ($t_{\frac{1}{2}}$=5.24 yr) ranged, equally for top-layer sediments and suspended matter, from 6 to 430 fCi/g per % K The highest values were detected for the Channel (Calais) and Scheldt, while the lowest concentrations were found in the Grevelingen. Thus Co-60 is a clear indicator for inputs from nuclear installations like Doel (Scheldt), and Gravelines (Channel) and those of the English Coast.

II.3 Pu-238, 239 (+240)

The suspended matter and sediment values for Pu-239 ($t_{\frac{1}{2}}$=2.4 x 10^4 yr) mixed with Pu-240 ($t_{\frac{1}{2}}$=6.6 x 10^3 yr) ranged in the Delta region from 0.9 to 43 fCi/g Pu-239,240/%K, with the highest values in the Southern North Sea (26-43 fCi/g/%) with North Sea water. No influences of Doel and Borssele could be detected, probably the surplus Pu-239,240 above fallout is explained from releases of the Windscale and/or La Hague reprocessing plants.

The Pu-238/Pu-239,240 ratios (Pu-238, $t_{\frac{1}{2}}$=86 yr) ranged from 4 to 54%, with again the heighest values (19-43%) in the Southern North Sea,

but also in the upper Scheldt (29-54%). The explanation is difficult to give for the Scheldt values, since the average source ratios are 35% for La Hague, 0.2% for Windscale and 5% for fallout. Abnormal high ratios also have been found in sediments of French rivers downstream of nuclear facilities (Loire, Thomas, pers. comm.) but also in rivers only exposed to fallout (Var-river, Ballestra, 1980 and Gironde, Jeandel, 1981).

II.4 Sb-125

Antimony-125 ($t_{\frac{1}{2}}$=2.8 yr) attached to sedimentary material is mainly from marine origin and in fact from the La Hague effluents. Values in the Channel of 330-475 fCi/g Sb-125/%K were found, descending to 15 fCi/g/%K in the upper Scheldt and 36-37 fCi/g/%K in the Rhine and Meuse.

III.5 Ru-106

An identical picture as Sb-125 was shown by Ru-106 ($t_{\frac{1}{2}}$=1.0 yr). High values in the Channel were found for both suspended matter and top-layer sediment of 1300-5700 fCi/g Ru-106/%K. In the mouth of the Western Scheldt these values were reduced to about 1000-1100, while the river concentrations of Rhine, Meuse and Scheldt were between 60 and 230 fCi/g/%K.

III.6 Ce-144

The Ce-144 ($t_{\frac{1}{2}}$=0.78 yr) data, determined in a similar way as the other gamma emittors, are not very reliable. The values are too close to the detection limit. Approximately 50 fCi/g/%K in the rivers and 100-2500 fCi/g/% in the Channel. Probably the source is equally the La Hague reprocessing plant.

IV. CONCLUSIONS

The observed patterns of the various radionuclide distribution in the Dutch Delta Region become more clear, in particular for radionuclides attached to suspended matter and top-layer sediment. In particular the presentation of the radioactivity per % K gives the opportunity to understand the transport of these radionuclides in the environment as related to their sources.

Additional measurements are proceeding on mussels, as filter-feeders of suspended matter. For this investigation the sampling area will be extended from the Channel and the Delta region to the Dutch North Sea coast northwards.

The lichen studies are completed, while the salt-marsh plant analysis revealed too low concentrations at the level of the detection limits (Euratom, 1980, 1981).

Some core samples are still under investigation in order to get a better view on the fallout maximum of the years 1958-1963.

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Ballestra, S., 1980. Radioactivité artificielle et environment marin. Etude relative aux transuraniens en Méditerranée. Thèse Un. Nice. 220 pp.

Euratom, 1979, 1980, 1981. Plutonium in Rhine-Meuse-Scheldt delta. Progress Reports CEC Progr. Radiat. Prot., Eur, 6766, 211-218, Eur. 7169, 285-290, Eur. 7800, 211-216.

Jeandel, C., J.M. Martin, A.J. Thomas, 1981. Les radionucléides artificielles dans les estuaires français. IAEA Vienne SM/284-123,15-32.

Progress Report
1982

Contractor:

Contract no.: BIO-B-325-81-NL

Stichting ITAL
Postbus 48
NL-6700 AA Wageningen

Head(s) of research team(s):

Dr. M. J. Frissel
Association Euratom - Ital
Postbus 48
NL-6700 AA Wageningen

General subject of the contract:

Evaluation of long-term behaviour of radiocontaminants - transuranics and activated corrosion and fission products - in different soils and vegetation.

List of projects:

1. Investigation of the behaviour in soils and uptake by plants of transuranics (Ra included) for different European Soil types.
2. Evaluation of the long-term behaviour of radiocontaminants in terrestrial ecosystems: the soil-plant transfer.

Title of project nr 1

Investigation of the behaviour in soils and uptake by plants of transuranics (Ra included) for different European soil types.

Head of project and scientific staff:

M.J. Frissel, R. Pennders, A. Ringoet, A.W. de Ruijter, N. v.d. Klugt

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Main activities were determination of man made radio nuclides in natural soils and their uptake by the vegatation, as well leaching experiments and transport studies of transuranics in soil columns in the laboratory.

For the measurements in nature, the sampling of twelve representative European soils is almost completed; only two soils (Italy and Greece) will still have to be sampled in 1983.
The analyses of plutonium, americium, and the gamma emitters Ac-228 (Th-232), Bi-214 (Ra-226), Cs-137 and K-40 in the soils and their corresponding grass samples are almost finished. The uranium analyses are in progress, typical transfer coefficients are: Humic gleysol (Hooglanderveen) 0.011, Calcaric fluvisol (Finsterwolde) 0.005, Distric histosol (Schoonebeek) 0.005, Eutvic histosol (Groot Ammers) 0.006, Dystric cambisol (Ardennen) 0.007, Orthic luvisol (Amiens) 0.028, Rhodic ferrasol (Giessen) 0.002, Rendzina (Kingston Lisle) 0.016. Unit: Bq per fresh weight (standardized moisture content 14%)/Bq per kg air dry soil. The variation is higher than expected. Therefore a second grass sampling of some sample-places was carried out at the end of the summer and, depending on the results, a third one may follow early in 1983. The results of the soil analyses so far, show that still about 80 percent of the Pu-239/240, Am-241 and Cs-137 is in the upper 10 centimeters of the soil.
Differences between the migration of plutonium, americium and cesium in different soils are small. This might indicate the same type of transport mode, i.c. particle mediated transport. Different soils show different migration patterns.

To verify the predictive model for plutonium and americium transport in soil some old samples dating from 1967, 1972, 1973 and 1978 were analysed for Pu and Am. The data obtained support the assumption that the predictive model can be used for a first estimate of the behaviour of Pu and Am in soils.

As already described last year in the laboratory the migration is measured with 24 columns filled with resp. sand, sea clay and peat. The columns are treated with artificially rainwater, what, before contacting the soil, leaches a sediment containing plutonium. Half of the experiments are terminated and are in progress for plutonium and cesium analyse.

Differences between columns are small. There is a slight indication that Pu migrates somewhat faster than Cs. This observation is in agreement with our observations in nature.

layer	Hooglanderveen						Ardennen						Rendzina					
	MBq . km^{-2}			ratio			MBq . km^{-2}			ratio			MBq . km^{-2}			ratio		
cm	Pu	Am	Cs	Pu	Am	Cs	Pu	Am	Cs	Pu	Am	Cs	Pu	Am	Cs	Pu	Am	Cs
0- 5	15.1	6.4	1020	1	0.42	68	12.6	5.5	1010	1	0.44	80	15.3	6.4	1165	1	0.45	76
5-10	20.0	9.1	1302	1	0.46	65	13.4	6.3	969	1	0.47	72	15.7	7.6	1157	1	0.48	74
10-15	10.3	5.0	947	1	0.49	97	7.8	4.7	525	1	0.60	67	10.1	4.9	711	1	0.49	70
15-20	0.9	1.2	120	1	*	*	4.3	7.0	270	1	*	*	2.8	1.7	203	1	*	*

* Absolute values too low to allow reliable ratio determinations.

Table 1 - Some representative results of soil analysis

	1967			1972		1973		1978	
layer cm	Prediction	Obs HVL	Obs GRAM	Pred.	Obs HVL	Pred.	Obs GRAM	Pred.	Obs HVL
0- 5	26.1	34.0	28.9	26.1	33.3	18.6	24.5	12.8	15.1
5-10	6.4	3.6	8.7	12.2	5.2	13.3	13.4	16.5	20.0
10-15	0	1.6	2.2	1.5	0.7	1.7	2.9	4.6	10.3
15-20	0	0.3	0.7	0	0.4	0	0.7	0.9	0.9

HVL = Hooglanderveen (Humic gleysol); GRAM = Groot Ammers (Eutric histosol)

Table 2 - Some predicted Pu values (residence time 3 γ-cm^{-1}) together with some presently determined Pu values of old samples (MBq-km^{-2}).

	1967			1978	
Layer cm	Prediction	Obs HVL	Obs GRAM	Prediction	Obs HVL
0- 5	13.1	19.4	15.8	6.4	6.4
5-10	3.2	2.8	4.8	8.5	9.1
10-15	0	1.8	2.5	2.3	5.0
15-20	0	1.2	0.4	0.5	1.2

Table 3 : Some predicted Am values (residence time 3 γ-cm^{-1}) together with some presently determined Am values of old samples (MBq-km^{-2}).

Title of project nr 2
Evaluation of the long term behaviour of radio contaminants in terrestrial ecosystems.

Head of project and scientific staff:
J.F. Stoutjesdijk, G. Desmet, M.J. Frissel, N. van der Klugt, H.P. Leenhouts, A. Ringoet, A.W. de Ruijter, J. Sinnaeve.

Determinations of these transfer factors should be performed under circumstances as near as possible to natural conditions. Therefore experiments are performed in a lysimeter installation with 32 large containers, of which 16 have been filled with löss, 12 with sandy soil and 4 with river clay. (For details see annual report 1981.) In 1981 the soil of the first four containers has been labelled with ^{54}Mn, ^{60}Co, ^{65}Zn, ^{90}Sr and ^{137}Cs and cultivated with grass. In 1982 the measurements of the uptake of the radionuclides by the grass have been continued: the results are given in table 1. It is shown that the grass takes up ^{54}Mn and ^{64}Zn much better than ^{60}Co and ^{137}Cs and that large variations may occur between different harvests. The uptake from sandy soil is much higher for ^{54}Mn, ^{60}Co and ^{137}Cs than that from löss. From the soil with a plough layer contamination the uptake is about two times higher than from the soil of which only the surface is contaminated for ^{54}Mn and ^{65}Zn.

In 1982 twelve other soils of the lysimeter were labelled with the same radionuclides. They were cultivated with potatoes, maize, barley and vegetables. Results are shown in table 2. Also for these crops the highest transfers were found for ^{54}Mn and ^{65}Zn. The highest values were found for spinach for all radionuclides, except for ^{65}Zn, which was taken up by potatoes most readily.

The complexation of radionuclides by root originating ligauds was determined. To assess fresh root material maize and wheat plants were grown in a $[^{14}C]O_2$ environment.

Extracts of the intact root-soil system were made after 0, 4 and 6 weeks growth by a mild percolation technique.

Crop	Soil type	Contamination type	Date		Dry mat. %	^{54}Mn	^{60}Co	^{65}Zn	^{137}Cs
			contamination	harvest					
Grass	löss	plough layer cont.	Aug.'81	Oct.'81	15	0.019	0.0057	0.14	0.0050
				June'82		0.032	0.0020	0.11	0.0018
				July'82		0.034	0.0018	0.26	0.0018
				Aug.'82		0.017	0.0003	0.071	0.0003
				Oct.'82		0.082	0.0036	0.28	0.0017
Grass	löss	surface cont.	Aug.'81	Oct.'81	15	0.027	0.0088	0.14	0.0079
				June'82		0.017	0.0007	0.049	0.0007
				July'82		0.016	0.0011	0.10	0.0008
				Aug.'82		0.020	0.0003	0.045	0.0003
				Oct.'82		0.055	0.0030	0.13	0.0018
Grass	sandy soil	plough layer cont.	Aug.'81	Oct.'81	15	0.18	0.023	0.18	0.026
				June'82		0.19	0.012	0.24	0.011
				July'82		0.15	0.010	0.32	0.014
				Aug.'82		0.084	0.0025	0.092	0.0062
				Oct.'82		0.15	0.0082	0.28	0.020
Grass	sandy soil	surface cont.	Aug.'81	Oct.'81	15	0.11	0.059	0.14	0.53
				June'82		0.040	0.0012	0.041	0.0026
				July'82		0.077	0.0073	0.21	0.0093
				Aug.'82		0.068	0.0025	0.066	0.0041
				Oct.'82		0.13	0.0073	0.19	0.011

Table 1 - Transfer factors for grass (Bq/kg in dry plant material / Bq/kg oven dried soil)
Activity concentration of the soil in a layer of 15 cm (plough layer contamination) or 5 cm (surface contamination).

Crop	Soil type	Contamination type	Date		Dry mat. %	^{54}Mn	^{60}Co	^{65}Zn	^{137}Cs
			conta-mination	harvest					
Potatoes	löss löss clay sandy soil	surface contamination	March 1982	Aug. 1982	25.2 23.5 25.4 24.6	0.0025 0.0035 0.0046 0.0049	0.0014 0.0017 0.0016 0.0012	0.030 0.035 0.15 0.024	0.0018 0.0013 0.0022 0.0069
Barley	löss löss clay sandy soil	idem	March 1982	Sept. 1982	87.6 86.8 87.4 86.4	0.017 0.016 0.0055 0.034	0.00016 - 0.00018 0.00021	0.25 0.23 0.37 0.18	0.00075 0.00034 0.0015 0.0030
Beans	löss sandy soil	idem	March 1982	July 1982	9.8 9.8	0.0061 0.014	0.0014 0.0014	0.014 0.025	0.00035 0.0010
Maize	löss sandy soil	idem	March 1982	Oct. 1982	33.2 32.0	0.0039 0.022	0.0006 0.0006	0.038 0.051	0.0013 0.0064
Spinach	löss sandy soil	idem	March 1982	Oct. 1982	5.0 5.0	0.011 0.016	0.0029 0.0044	0.12 0.21	0.0018 0.0034

Table 2 - Transfer factors for various crops (Bq/kg in dry plant material / Bq/kg oven dried soil). Activity concentration of the soil in a layer of 5 cm.

Addition of ^{57}Co, ^{65}Zn, ^{54}Mn and ^{59}Fe to these extracts and subsequent molecular sieving on Sephader G-15 allowed separation of ionic species from higher molecular weight ones. The percentage of metals present in higher molecular weight forms showed a significant shift from ionic forms to complexed ones. They were accompanied by an increase of ^{14}C, the complexing agents are therefore assumed to be produced by the roots. A comparison of the ^{57}Co distribution in a 4 weeks extract with a 6 weeks extract, showed that this ^{57}Co distribution was shifted by the ^{14}C increasement. This indicates that within two weeks, sufficient ^{14}C material is being released into the root environment to compete with nature soil organic matter.

The results obtained demonstrate that the availability of radionuclides which can form complexes with root originating material may in the root-soil interface, be quite different from the ones determined by standard bulk soil chemistry extractions.

With the aim of better understanding the behaviour of Technetium in löss and sandy soils an experiment was done to determine its extractability. The Technetium absorption on löss soils was clearly very weak since after as little as one hour of extraction with water and DTPA, Technetium was completely recovered. The extraction of sandy soils with the same solvents was slower, and it was never completed, even not after 25 hours of extraction.

An attempt to determine whether Technetium was chemically transformed in those soils, by means of gelfiltration techniques failed, Technetium extracted from the soils was clearly not bound to the soil organic matter and remained TcO_4^-.

In the lysimeter spinach plants were grown on the löss and the sandy soil; surface soil and plough layer contamination with Tc was applied. Several harvests were done in order to determine possible growth dilution phenomena. Besides, water extractable Tc was determined. Total Tc was determined by extraction with NaClO.

Migration into deeper layers was investigated by sampling layers of 5 cm from 0 to 25 cm of depth.

In order to determine the threshold of TcO_4^- toxicity spinach seedlings were grown on nutrient solutions containing different TcO_4^- concentrations. One can say that plants surely suffer from the contamination when more than 2.10^{-6} mol l^{-1} is present. A closer look at the distribution of Technetium in the different spinach leaves shows that every TcO_4^- concentration in the nutrient solution the first leaves contained more Technetium than the second leaves.
Further attention has been given to the determination of possible Tc-bio-organic complexes in the spinach leaves. Therefore, a special separation technique was developed by filtration of a crude cell suspension through a fractor gel column (a kind of gelfiltration). Although this technique appears to be successful data on the binding of Technetium with defined cell organcells are not yet available.

List of publications in 1982

FRISSEL, M.J., R.M.J. PENNDERS. Models for the migration of ^{90}Sr, ^{137}Cs, $^{239,240}Pu$ and ^{241}Am in the top layer of soils, in Environmental migration of long-lived radionuclides. IAEA, Vienna, 1982, p. 689-694.

FRISSEL, M.J. Gedrag van radionucliden in bodem en gewassen. Chem. Magazine 1982, juli/aug. 424-429.

STOUTJESDIJK, J.K. Recente ontwikkelingen in de vloeistof-scintillatie meettechniek. Para Medica 2 (1982), 12-14.

FRISSEL, M.J. and R.M.J. PENNDERS. Models for the accumulation and migration of ^{90}Sr, ^{137}Cs, $^{239,240}Pu$ and ^{241}Am in the upper layers of soils. British Ecological Soc. Special Publications Series. Blackwell (in Press).

STOUTJESDIJK, J.L., J. SINNAEVE, J.H. VAN GINKEL, R.M.J. PENNDERS, R. SIBBEL and G.M. DESMET. Determinations of soil-plant transfer factors at ITAL in "Report of a workshop on the transfer of radionuclides from Soil to Plant" IUR-Association Euratom-ITAL, Wageningen. Accepted for publication.

Progress Report
1982

Contractor:

Commissariat à l'Energie
Atomique, CEA
CEN de Cadarache
B.P. n° 1
F-13115 Saint-Paul-lez-Durance

Contract no.: BIO-B-317-81-F

Head(s) of research team(s):

Dr. A. Grauby
SERE-DPR-IPSN
CEA-CEN de Cadarache
B.P. n° 1
F-13115 Saint-Paul-lez-Durance

General subject of the contract:

Behaviour of freshwater entrained radionuclides in the event of contact with seawater.

List of projects:

1. Behaviour of freshwater entrained radionuclides in the event of contact with seawater

Title of project nr

BEHAVIOUR OF FRESHWATER ENTRAINED RADIONUCLIDES IN THE EVENT OF CONTACT WITH SEAWATER.

Head of project and scientific staff :

Head of project : M.GRAUBY

Scientific staff : MM BADIE, PERES

Description of results

But de l'expérimentation ;

Cette expérimentation a pour but d'apprécier l'importance de divers paramètres sur le devenir des ^{60}Co, ^{65}Zn, ^{22}Na, ^{137}Cs, ^{54}Mn et ^{51}Cr introduits à partir d'un fleuve dans le milieu marin. Pour cela, nous étudions la distribution des radionucléides entre les phases particulaire et soluble. Les paramètres abordés sont les suivants : la salinité, la charge en matière en suspension, la granulométrie des particules en suspension, la minéralogie des particules et la matière organique.

Méthode expérimentale :
Nous utilisons des eaux naturelles ou synthétiques préalablement filtrées et stérilisées.
Les eaux naturelles sont prélevées au large de TOULON (salinité : 38 ‰) et dans le rhône (salinité : 0 ‰).
Nous constituons des eaux de salinités variables, soit par mélanges d'eaux naturelles, soit par addition de sels dans une eau distillée. Ces solutions sont tracées et mises au contact de sédiments.
Pour cela, nous utilisons différentes quantités et qualités de sédiments :
- sédiments bruts ou débarrassés de matières organiques et de carbonates
- sédiments représentant quatre classes granulométriques (0-5 µm, 5-15 µm, 15-30 µm et 30-50 µm).
- argiles pures : Kaolinite, Illite et Montmorillonite.

Ainsi, nous disposons de 120 milieux différents introduits dans des flacons en plastique. Ceux-ci sont agités durant 5 jours à une température de 15°C. Pour chacun de ces milieux, nous exprimons la distribution des différents radionucléides entre la phase dissoute et particulaire par l'intermédiaire du coefficient de distribution (Kd) Kd en $ml.g^{-1}$:

$$Kd = \frac{\text{Activité fixée sur un gramme de suspension}}{\text{Activité restant dans un millilitre de solution}}$$

Résultats et discussion :
L'augmentation de la salinité permet une remise en solution de tous les radic éléments sauf du chrome qui semble au contraire précipiter. Il est à noter que dans tous les cas, les modifications importantes des Kd enregistrées interviennent surtout aux basses salinités (de 0 à 7 ‰).

Enfin, la salinité semble masquer dans une grande partie l'influence d'autres paramètres sur le comportement des radioéléments. Cependant, nous notons que :

- Plus la concentration en matières en suspension est élevée, plus le pourcentage d'activité fixée par les particules est élevé. Par contre, l'activité fixée rapportée à une unité de masse (Kd augmente quand la charge en matières en suspension diminue).
- La présence de matières organiques sur des particules semble favoriser la fixation des radionucléides.
- Les sédiments des classes granulométriques les plus fines ont une capacité de fixation des radioéléments plus élevée. Il est à noter que la classe granulométrique de 0 à 5 µm correspond surtout à des argiles.
- La fixation augmente en présence d'argiles à capacité d'échange cationique élevée, nous observons en effet un Kd élevé pour la montmorillonite, moyen pour l'illite et faible pour la kaolinite.
  Ce phénomène est surtout visible pour les salinités faibles ou nulles.
- Les Kd sont plus forts dans les milieux naturels que dans les milieux artificiels. Ces résultats soulignent l'importance des matières organiques dissoutes, qui, aux faibles salinités favorisent, la fixation des radionucléides en les complexant.

CONCLUSION :

Lors d'une augmentation de salinité, nous constatons une désorption des radionucléides ^{137}Cs, ^{54}Mn, ^{65}Zn, ^{60}Co et ^{22}Na. Cette mise en solution s'explique par la compétition ionique qu'introduit un électrolyte fort tel que l'eau salée. Par contre, le phénomène s'inverse pour le chrome, dans ce cas, le radioélement semble plutôt soumis à un processus de floculation.

Notons aussi que si la fixation de radionucléides sur des particules de granulométrie fine est forte, phénomène imputable au fait que le rapport surface sur volume augmente avec la diminution du diamètre des particules. De plus est liée aux classes granulométriques fines une teneur en argile importante, or l'argile s'avère un bon fixateur de radioéléments. Par contre, la capacité d'échange cationique ne semble pas dans le milieu marin représenter une priorité importante.

Enfin, nous proposons dans l'avenir :

- d'apprécier l'importance de l'évolution de la matière organique sur le comportement des radionucléides.
- mettre en évidence les phénomènes de floculation qui semblent affecter certains éléments.

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

II. Short Communications, Theses, Internal Reports, Patents...

"Comportement des radionucléides vis-à-vis des argiles en milieu estuarien".
Thèse de 3ème cycle - Juin 1983 - Faculté de NICE.

"Comportement de certains nucléides en milieu estuarien"
Article à paraître (RIOM) - NICE.

Progress Report
1982

Contractor:

Commissariat à l'Energie
Atomique, CEA
CEN de Cadarache
B.P. n° 1
F-13115 Saint-Paul-lez-Durance

Contract no.: BIO-B-318-81-F

Head(s) of research team(s):

Dr. A. Grauby
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CEA-CEN de Cadarache
B.P. n°1
F-13115 Saint-Paul-lez-Durance

General subject of the contract:

Physico-chemical changes of transuranic elements in the environment : transfer through the food chain towards man.

List of projects:

1. Synergistic effects between transuranic elements, uranium matrix and soil parameters and effects on soil-plant transfer.
2. Influence of the deposited quantity of transuranic elements on the soil-plant transfer coefficient.
3. Field study of soil-plant-animal transfer; practical aspects of methods of cultivation and fertilization.
4. Soil-plant transfer with the uranium-sodium-transuranic matrix and with the glass leaching solution matrix from a vitrification plant or waste storage area.
5. Effect of the macro-and micro-constituents of reprocessed waste effluents on physico-chemical evolution, soil mobility and soil-plant transfer of transuranics.

Title of project nr

PHYSICO-CHEMICAL CHANGES OF TRANSURANIC ELEMENTS IN THE ENVIRONMENT TRANSFER THROUGH THE FOOD CHAIN TOWARDS MAN.

Head of project and scientific staff :
Dr A. GRAUBY
SERE-DPr-IPSN
CEA - CEN CADARACHE
B.P. N° 1
13115 SAINT PAUL LEZ DURANCE

Les travaux entrepris en 1982 peuvent se classer en deux chapitres distincts :
- Etude d'une méthodologie de traitement des échantillons de l'environnement.
- Influence de la forme chimique sur le facteur de transfert sol-plante.

Ce rapport fait également le point des travaux menés en 1982 et dont les résultats ne seront disponibles que dans le courant de l'année 1983.

1) Etude d'une méthodologie de traitement des échantillons de l'environnement

Les critères relevés pour la mise au point d'une méthode sont d'une part la possibilité d'utilisation sur l'ensemble des échantillons en provenance du milieu naturel (animaux, végétaux, sols, sédiments, eaux...), d'autre part la compatibilité des prises d'échantillons avec les faibles teneurs en transuraniens. Cette méthode de fractionnement illustrée par la figure 1, conduit à la séparation des transuraniens en quatre fractions électro-déposées (Np + Pu ; U + Po ; Th ; Am + Cm). Elle a été développée également dans le souci de satisfaire aux impératifs de la recherche de laboratoire (fortes activités avec des impuretés, petit nombre d'isotopes, réponse rapide). Dans ce cas particulier, des travaux expérimentaux, il est possible d'éliminer certaines étapes sans inconvénient pour le résultat final.

2) Influence de la forme chimique sur le facteur de transfert sol-plante

L'étude des transferts du plutonium en solution nutritive sous trois formes chimiques différentes : Nitrate, complexe citrate, complexe TBP a été abordée. Dans les feuilles et les racines, les formes nitrate et citrate se transfèrent de façon équivalente, alors que les formes citrates semblent plus disponibles que les formes nitrates au niveau des tiges (figures 2 a, b, c). La forte toxicité des TBP sur les végétaux n'a pas permis - à l'heure actuelle - d'obtenir des résultats significatifs des transferts du Pu dans les différents organes.

3) Travaux en cours

Des recherches sur les transferts de Neptunium du végétal (carotte) à l'animal (singe) sont en cours ; ainsi que des études de toxicité du Neptunium sur le haricot. Des essais sur l'effet du type de sol et l'effet de la masse de l'isotope sont en cours pour suivre le transfert sol-plante du Neptunium.

SEPARATION SEQUENTIELLE DES ACTINIDES

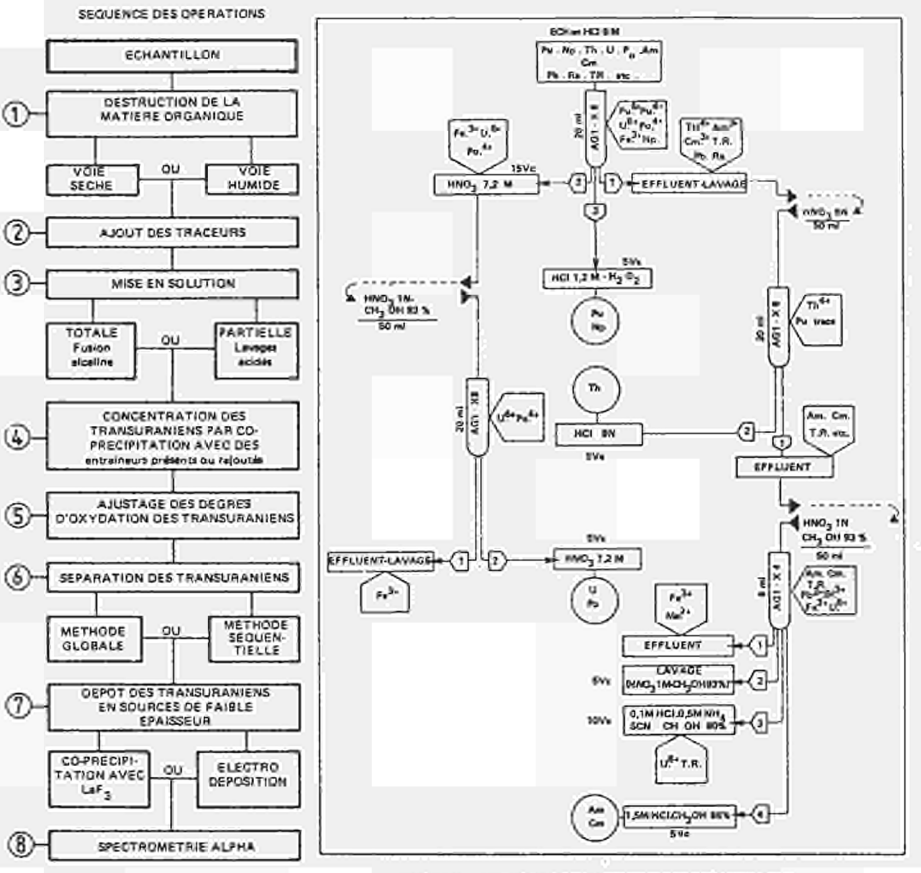

FIGURE 1 : METHODE DE TRAITEMENT DES ECHANTILLONS

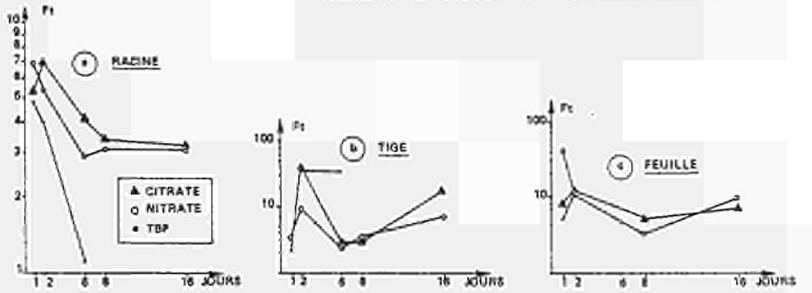

FIGURE 2 : TRANSFERTS DU PLUTONIUM AU HARICOT

List of publications in 1982

- Américium et Curium en milieu terrestre ; rétention et transfert sol-plante .
  Par A. SAAS - P. ROUCOUX.
  CEC meeting on the transfer of Am and Cm in the environment
  Monaco 12-13 Octobre 1982.

- Valeurs expérimentales de transfert sol-plante des isotopes obtenus par le laboratoire de Radioécologie Terrestre du CEN Cadarache (FRANCE).
  Par A. SAAS.
  Short communication ; wockshop of soil-plant transfer factors in CEC.
  Wageningen 7-10 Decembre 1982.

- "Comparaison de deux méthodes de détermination des transuraniens dans l'environnement".
  Par H. CAMUS - M. MORELLO - A. SAAS.
  4th symposium of the determination of radionuclides in environmental and biological materiels - A paraître ; 18-19 Avril 1983.
  Teddington (G.B.).

Progress Report
1982

Contractor:

Commissariat à l'Energie
Atomique, CEA
CEN de Cadarache
B.P. n°1
F-13115 Saint-Paul-lez-Durance

Contract no.: BIO-B-321-81-F

Head(s) of research team(s):

Dr. A. Grauby
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Dr. Ph. Picat
SERE-DPR-IPSN
CEA-CEN de Cadarache
B.P. n° 1
F-13115 Saint-Paul-lez-Durance

General subject of the contract:

Evaluation of the impact on the population of the dispersion of radium-226 in mining areas. In situ research on the radionuclide transfer in rivers, irrigated land and processed foodstuffs.

List of projects:

1. Study of the environmental impact of mining sites and processing plants of uranium.

Title of project nr BIO B 321 81 F

"EVALUATION DES CONSEQUENCES POUR LES POPULATIONS DE LA DISPERSION DU 226 Ra DANS L'ENVIRONNEMENT DES SITES MINIERS. RECHERCHES IN SITU DES TRANSFERTS DE CE RADIONUCLEIDE EN RIVIERE EN CULTURES IRRIGUEES DANS LES PRODUITS ALIMENTAIRES TRANSFORMES".

Head of project and scientific staff :

Head of Project : M. GRAUBY
Scientific staff : B. DESCAMPS, L. FOULQUIER, J. REAL, J. HUGON

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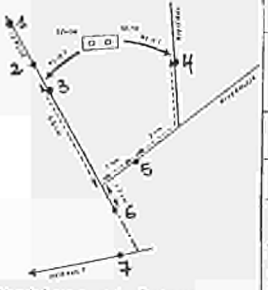
1) TRANSFERT EN RIVIERE

Nous avons suivi l'environnement aquatique du site minier de LODEVE (schéma et tableau ci-dessous). La mesure la plus précise possible des niveaux d'activité (U, Ra, Th, K), en particulier de l'eau, permet par les facteurs de concentration (F.C) et les coefficients de distribution (Kd) de quantifier les principales voies de transfert.



Stations et dates de Prélèvements

STATIONS	EAU 81	EAU 82	SEDIMENTS 81	SEDIMENTS 82	VEGETAUX 81	VEGETAUX 82	POISSONS 82
LODEVE 1		1 et 4					
LERGUE amont 2	5 (1)	1 et 4	5	4	5	6	
Aval rejet usine 3		1 et 4		4			4
Rivieral 4		1 et 4		1 et 4		4	
Rivernoux 5	5	1 et 4	5	4	5	4	4
Lerque aval 6		1 et 4	5	4	5	6	4
Herault 7			5	4			

(1) les chiffres correspondent au mois dans l'année.

La méthode spectrométrique répond à nos objectifs. Elle permet de connaître l'état des familles radioactives et leur situation d'équilibre ou non. Pour les échantillons de bas niveaux (cas de l'eau en général) on propose de mesurer l'uranium par fluorimétrie et le radium par émanation.

Pour l'eau, on constate à l'aval immédiat une augmentation, essentiellement dans la fraction dissoute, des teneurs en uranium et en radium dans une situation de non équilibre. L'uranium reste principalement sous forme anionique.

Le sédiment constitue le compartiment de stockage principal de l'uranium et du radium ; il est d'autant plus efficace que la granulométrie est fine

Pour les végétaux semi-aquatiques, l'augmentation des teneurs en uranium et radium constatée à l'aval immédiat est particulièrement visible au niveau des parties souterraines.
La légère élévation des teneurs en uranium et en radium des poissons à la station 5 est essentiellement due à l'activité des viscères. La chair consommable a les niveaux les plus faibles. Aux stations 2 et 5, pour les poissons entiers les facteurs de concentration sont voisins pour l'uranium (1 et 5) et pour le radium (75 et 55).

Dans l'état acutel de l'exploitation de la mine, l'impact des rejets n'est visible que sur une courte distance ; dès la station 6, la situation est la même qu'à l'amont. . Le tableau ci-dessus résume les résultats de la campagne d'avril 1982.

Exemple de résultats obtenus pour l'eau ($Bq.l^{-1}$), pour les végétaux et les sédiments ($Bq.kg^{-1}$ sec) et pour les poissons ($Bq.Kg^{-1}$ frais) en avril 1982.

STATIONS	EAU		SEDIMENTS		VEGETAUX		POISSONS	
	U	Ra	U	Ra	U	Ra	U	Ra
2	< 0,06	0,007	30	70	- (1) 18 (2)	0,4 22	0,7	0,7
5	6,3	0,06	1500	1500	55 470	37 300	7	4
6	0,15	0,01	60	60	5 55	4,5 52	0,7	0,7

(1) parties aériennes - (2) parties souterraines

2) TRANSFERT DANS LES CULTURES IRRIGUEES

Les ressources agricoles locales du site de LODEVE reposent essentiellement sur la vigne et l'olivier, les jardins familiaux sont rares et situés en bordure des ruisseaux souvent à sec l'été.
Au cours de la phase d'enquête locale, il est apparu rapidement deux voies de transfert ; la vigne et les légumes.
Les campagnes de mesures effectuées sur ces produits ainsi que sur les sols de culture nous ont permis de déterminer les facteurs de transfert sol-plante pour le Radium.

Salade $2,4.10^{-4}$ Kg/Kg
Tomate $1,4.10^{-4}$ Kg/Kg

Pour la vigne, le facteur de transfert global sol-vin est de :

$2,2.\ 10^{-3}$ Kg/l (irrigation par submersion)

Moyennant certaines hypothèses, relativement pessimistes, on a pu en déduire les doses annuelles délivrées à l'organe critique - l'os en l'occurrence - qui sont de :

5/100 DMAP par le vin
0,5/100 DMAP par les légumes

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

Colloque international sur la gestion des déchets provenant de l'extraction et du traitement du minerai d'uranium. ALBUQUERQUE-New-Mexique 10/14/ Mai 1982 - AIEA-SM-257/20.
"L'apport des mesures hydrobiologiques dans la surveillance radioécologique de sites français d'extraction et de traitement d'uranium.
B.DESCAMPS, L.FOULQUIER, Y.CARTIER, Y.BAUDIN-JAULENT - CEA/DPr/SERE/ St PAUL-LEZ-DURANCE.
Evaluation du cycle du radium dans l'environnement à partir d'observations in situ de son impact radiologique. J.HUGON - J.DELMAS - JC CARIES - CRA/SERE/DPr/CEA
Symposium Albuquerque 10-14 Mai 1982
IAEA - VIENNE 1982

II. Short Communications, Theses, Internal Reports, Patents. . .

- Etude bibliographique - comportement et transfert du radium dans les milieux aquatiques et terrestres.
  S.ROUSSEL - J.REAL - J.HUGON
  Laboratoire de Radioécologie Appliquée - SERE/DPr/IPSN, - Juin 1982.

- Recherche des transferts du 226 Ra en cultures irriguées autour du site minier de LODEVE. - Etat d'avancement des travaux.
  J.REAL, S.ROUSSEL, J.HUGON, J.C.CARIES
  Laboratoire de Radioécologie Appliquée SERE/DPr/IPSN - Juin 1982

Progress Report
1982

Contractor:

Commissariat à l'Energie
Atomique, CEA
CEN de Cadarache
B.P. n° 1
F-13115 Saint-Paul-lez-Durance

Contract no.: BIO-B-315-81-F

Head(s) of research team(s):

Dr. A. Grauby
SERE-DPR-IPSN
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F-13115 Saint-Paul-lez-Durance

Dr. A. Saas
SERE-DPR-IPSN
CEA-CEN de Cadarche
B.P. n° 1
F-13115 Saint-Paul-lez-Durance

General subject of the contract:

Transfer and evolution of certain long-lived beta emitters in terrestrial food chains leading to man.

List of projects:

1. Transfer of technetium in the terrestrial environment.
2. Transfer of iodine in the terrestrial environment.
3. Transfer of selenium and nickel in the terrestrial environment.

Title of project nr

TRANSFER AND EVOLUTION OF CERTAIN LONG-LIVED BETA EMITTERS IN TERRESTRIAL FOOD CHAINS LEADING TO MAN.

Head of project and scientific staff :

M.GRAUBY André
MM SAAS Arsène et CAMUS Henry

Les travaux entrepris en 1982 peuvent se résumer sur le thème suivant :
- Etude des variations des facteurs de transfert en fonction de la concentration du milieu en isotope ; observation des niveaux de toxicité chimique.

De plus, ce rapport comporte l'énoncé des travaux en cours menés en 1982 et dont les résultats ne pourront être obtenus que dans le courant de l'année 1983 ou ultérieurement.

Etude des variations des facteurs de transfert en fonction de la concentration du milieu en isotope ; observation des niveaux de toxicité chimique.

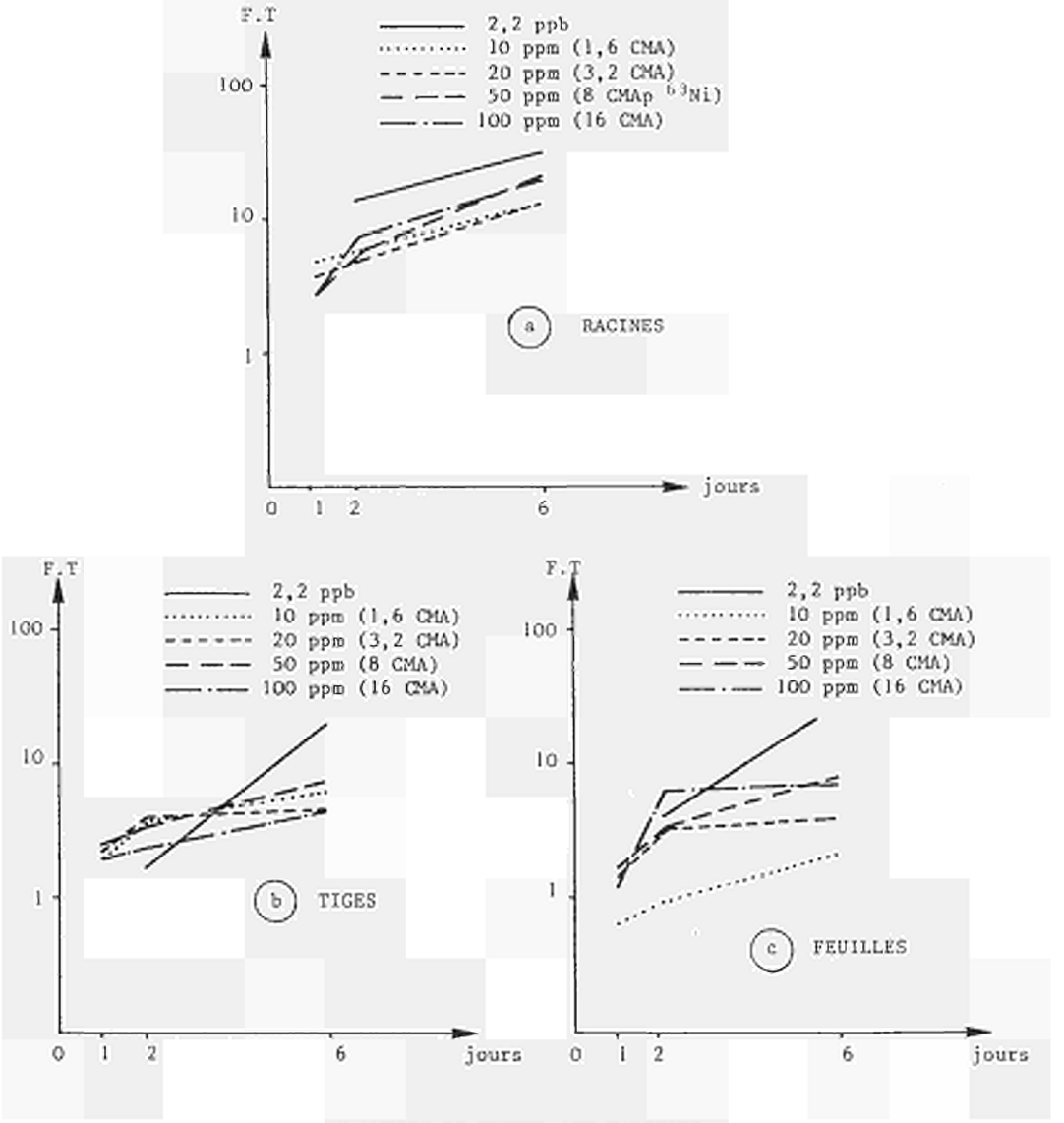
La cinétique d'absorption du ^{63}Ni et du ^{99}Tc a été effectuée. La gamme de concentrations en solution nutritive s'étage depuis les concentrations équivalentes aux CMA du ^{63}Ni et du ^{99}Tc jusqu'à des niveaux toxiques. Ces expériences sont menées sur des haricots cultivés en solution nutritive. Les travaux menés sur ^{99}Tc confirment les résultats de la bibliographie (constance du facteur de transfert (Ft $\simeq$ 3) acquis en 2 jours dans les tiges et les racines, valeurs environ 10 fois plus élevées dans les feuilles).

En ce qui concerne le nickel (c.f figures 1 (a, b, c) les variations en concentration ne modifient pas la cinétique de transfert aux racines. Au niveau des tiges et des feuilles, la toxicité (symptômes visibles au niveau des feuilles) se manifeste pour une concentration de 10 ppm par un ralentissement de l'adsorption. Les valeurs des facteurs de transfert oscillent entre 10 et 40 au niveau des racines, entre 2 et 7 au niveau des tiges et des feuilles, sauf pour une concentration faible (2,2 ppb) où on peut atteindre la valeur 20.

Travaux en cours

L'étude de la cinétique du facteur de transfert de l'iode en fonction de la concentration en isotope du milieu est en cours d'achèvement. Une tentative de mise au point de méthodes de séparation des isotopes considérés est en cours, à partir des étapes du protocole défini pour les éléments transuraniens (c.f contrat BIO-B 318-81-F).

On pourra disposer aussi d'une méthodologie unique de traitement des échantillons du milieu naturel susceptible de conduire à la détermination de leur contenu en transuraniens et en émetteurs β à vie longue.



TRANSFERT DU NICKEL AUX HARICOTS

Figures 1 (a, b, c)

List of publications in 1982

Publications in Scientific Journals, Monographs, Proceedings.

A.SAAS, T.NOMURA
"Volatilization and fixation of Selenium on two french soil"
Environmental and experimental Botany, 21, 1982.

A.SAAS, A.GRAUBY, C.COLLE, J.L.DENARDI, T.NOMURA, Ph.JOYER.
"Evolution physico-chimique du technetium 99 et du selenium 79 dans les sols" p.263-273 in : A.I.E.A. - Proceedings of International Symposium on Environmental Migration of Long Lived Radionuclides, Knoxville (USA), 27.31 July 1981. - Vienne : A.I.E.A, 1982. - 831 p.

Progress Report
1982

Contractor: Contract no.: BIO-B-316-81-F

Commissariat à l'Energie
Atomique, CEA
CEN de Cadarache
B.P. n°1
F- 13115 Saint-Paul-lez-Durance

Head(s) of research team(s):

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SERE-DPR-IPSN
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B.P. 270
F-50107 Cherbourg

General subject of the contract:

Actinides in the marine environment : study of their physico-chemical behaviour in seawater and marine sediments and their transfer between sediments and benthic species.

List of projects:

1. Sorption of actinides on different types of marine sediments and their transfer to molluscs.

Sorption of actinides on different types of marine sediments and their transfer to molluscs.

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A - BIOLOGIE (GERMAIN, P. et MASSON, M.)

En 1981 des études sur la biodisponibilité du Pu et de Am contenus dans des sédiments vaseux ont été menées pour trois espèces benthiques fouisseuses (arénicoles, scrobiculaires, corophium). Elles ont été poursuivies en 1982 pour une espèce Arenicola marina et 5 types de sédiments: sédiments des grands fonds - plaine du Cap Vert (très fin), de St Vaast - Cul de Loup (sable vaseux riche en matière organique), de St Vaast - Pont de Saire (sable vaseux), de Ouistreham (sable vaseux), de Nacqueville (sable fin). Les résultats sont du même ordre que ceux cités en 1981 (facteur de transfert compris entre 1.10^{-2} et 5.10^{-3}). Du fait de la faiblaisse des niveaux d'activité, nous estimons qu'il n'y a pas de différences significatives pour les facteurs de transfert entre les différents sédiments.

Les transferts du Pu et de Am entre un sédiment et le mollusque Cardium edule (coque : espèce suspensivore, consommée par l'homme) ont été étudiés. Les facteurs de transfert, pour la chair, sont de l'ordre de 5.10^{-3} pour Pu et 15.10^{-3} pour Am. Dans le protocole utilisé, l'étude des niveaux du ^{241}Am de l'eau interstitielle et les données des transferts eau-animaux conduisent à envisager un simple transfert eau interstitielle - espèces de cet élément préalablement fixé sur le sédiment. Par contre, pour les arénicoles, il y a confirmation de deux voies de transfert : eau interstitielle et désorption de Am des grains sédimentaires au cours du passage dans le tractus digestif.

Chez les coques la répartition de Am et du Pu dans les tissus, dans le cas des transferts sédiments-animaux, est identique à celle obtenue dans le cas des transferts eau de mer - animaux. Notons que dans le cas du transfert par l'eau de mer les chairs fixent plus Am (etCm) que la coquille, alors que chez de nombreux mollusques le contraire est observé.

Les études in situ de distribution des transuraniens dans les environs du cotentin montrent que les facteurs de concentration, les facteurs de transfert des espèces précitées sont en général plus élevés que ceux obtenus au laboratoire.

A la fin de l'année 1982 un protocole d'étude des transferts de radionucléides suspensions-moules a été étudié, lequel sera utilisé en 1983.

Enfin des essais ont été engagés afin de suivre une éventuelle évolution biogéochimique de Am sous l'influence d'une activité biologique. Ces expériences ont consisté à extraire périodiquement les formes physico-chimiques de Am lié aux particules sédimentaires, dans le cas où l'activité bactérienne est inhibée et dans le cas où elle est activée. Il n'apparaît pas d'évolution durant 6 mois pour un sédiment de la plaine du Cap Vert (fin, riche en carbonate, pauvre en matière organique).

B - GEOCHIMIE (GUEGUENIAT, P.)

L'objectif de la présente étude est de rechercher des éléments stables, naturellement présents dans le milieu, qui soient homologues des transuraniens. On a, dans ce but, suivi le comportement de l'américium et de deux terres rares : le cérium et l'europium. Tous sont à l'état trivalent dans l'eau de mer, les rayons ioniques (Ce^{3+}:1.034 A°) (Eu^{3+}: 0.950 A°) (Am^{3+}: 0.99 A°) et les produits de solubilité sont comparables.

Les études expérimentales ont consisté à comparer (à 15°C) les sorptions des ^{241}Am, ^{144}Ce, ^{152}Eu sur 32 sédiments de la Manche (résultats exprimés sous forme de K_D) pendant une période de 4 mois ; les radionucléides ont été au préalable laissés 24 heures (temps de vieillissement) dans l'eau de mer avant la contamination proprement dite. Dans le cas de l'europium, en outre, on a étudié l'influence du temps de vieillissement (TV : 1 j, 7 j, 21 j, 79 j - eau conservée à 4°C) sur les modalités de sorption. Dans la partie relative aux terres rares stables, on a analysé des suspensions et eaux de la Manche par activation neutronique; les comparaisons avec les études expérimentales ont été établies en considérant des sédiments possédant des propriétés géochimiques comparables.

1. Etudes expérimentales

a) Temps de vieillissement 1 jour. Temps de contact dans eau de mer 1 jour

- 32 échantillons analysés
  Corrélations Ce/Am R = 0.957
  Eu/Am R = 0.865
  Am/Am R = 0.905 (répétition de la même expérience)
  Eu/Eu R = 0.857 (" " ")

b) Influence du temps de vieillissement sur la sorption de l'europium

- 14 échantillons analysés. Evolution du coefficient de distribution moyen - après 1 jour de contact - en fonction du temps de vieillissement :

TV = 1 j K_D = 0,42 x 10^4 - TV = 7 j K_D = 0,55 x 10^4 - TV = 21 j K_D = 0,58 x 10^4 - TV = 79 j K_D = 0,26 x 10^4.

2. Comparaison entre le K_D des éléments stables et celui des radionucléides

		Am	Ce	Eu
Temps de vieillissement 1 jour	K_D	3.7 à 8.1x10^{5*}	1.4 à 4.2x10^{5*}	1.1 à 1.5x10^{5*}
Eléments à l'équilibre dans le milieu	KD	-	1.3 à 3.5x10^{5**}	1.8 x 10^{5**}

(* radionucléide - ** élément stable)

Discussion

Pour les terres rares étudiées il existe un bon accord entre les observations expérimentales et in situ. Il semble cependant lorsqu'on introduit dans l'eau de mer les radioisotopes, qu'il faille attendre environ 3 mois pour la mise en équilibre avec le milieu.

En ce qui concerne la recherche d'un homologue stable de l'américium il apparaîtrait que le cérium, contrairement à ce que nous pensions en considérant les formules électroniques, soit préférable à l'europium. Cette observation est intéressante car le cérium est présent dans le milieu naturel à des teneurs environ 20 fois supérieures à celles de l'europium, donc plus faciles à mesurer. Il faudra cependant poursuivre les recherches en faisant intervenir les conditions d'oxydo-réduction du milieu car le cérium et l'europium sont respectivement susceptibles de présenter les états de valence 4^+ et 2^+ alors que l'américium reste à la valence 3^+.

Publications

MIRAMAND, P., GERMAIN, P., CAMUS, H. 1982. Uptake of americium and plutonium from contamined sediments by three benthic species : Arenicola marina, Corophium volutator and Scrobicularia plana. Mar. Ecol. prog. Ser. Vol. 7, 59-65.

Progress Report

1982

Contractor:

Natural Environment
Research Council
Polaris House
North Star Avenue
GB-Swindon SN2 1EU

Contract no.: BIO-B-438-81-UK

Head(s) of research team(s):

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GB-Devon PL1 3DH

General subject of the contract:

Cellular biochemistry of uranium, plutonium, americium and curium in the common marine mussel Mytilus edulis L.

List of projects:

1. Organ and cellular concentrations and distribution of U, Pu, Am and Cm in Mytilus edulis.
2. Ecological aspects of alpha emitting radionuclides in aquatic environments; development of an assessment methodology.

In both projects (1 and 2) attention is being directed towards identifying stable element analogues for uranium, plutonium, americium and curium, together with other radionuclides present in the marine environment. An objective is to place uranium and the transuranium group of elements in perspective in terms of their behaviour in marine ecosystems, rather than consider them in isolation to other elements of the Periodic Table.

Cellular biochemistry of uranium, plutonium, americium and curium in the common marine mussel Mytilus edulis L.

Project 1. Organ and cellular concentrations and distributions of U, Pu, Am and Cm in Mytilus edulis L.

Project 2. Ecological aspects of alpha emitting radionuclides in aquatic environments; development of an assessment methodology.

Head of project: Dr E.I. Hamilton.

Staff: Mr R.J. Clifton and Miss H. Stevens.

PROJECT 1

Depuration Studies. The transplant-depuration study of mussels from the Esk (Cumbria) to Plymouth (Devon) has been completed. In reporting data for the concentration of radionuclides in mussel tissues there is a need to consider biological state of the animals; variations in total soft tissue weight with time of year results in apparent concentration differences of more than a factor 2. A major route of entry for U, Pu, Am and Cm is from "dissolved species" in seawater together with uptake of particulate phases by macrophage action. An apparent metallothionein-transuranide element association has been observed and confirmed by laboratory experiments using Cd. The availability of food to the animal alters the rate of uptake of Pu and Am; in the presence of food less Am and Pu is available for uptake presumably because of absorption of radionuclides onto food. We have identified the importance of chemical-physical form on uptake in the field compared with laboratory tracer studies. We have transplanted 2000 animals from the Esk to the Restronguet Creek (Cornwall), the water of which contains levels of Zn, Cu and Cd sufficient to induce metallothioneins in the mussel. We propose to study depuration rates over a period of cly, and compare the results with the Esk-Plymouth study. Supporting laboratory experiments are being designed to investigate chronic uptake of U, Pu, Am and Cm in the presence of metallothioneins. We consider that the apparent uptake in systems containing metallothioneins is by absorption, possibly on polymeric thioneins.

Organ distribution of U, Pu, Am and Cm. Evidence accumulates which indicates an active uptake of U, Pu, Am and Cm (together with stable element analogues) by byssus threads and the periostracum; active

excretion is via the pericardial gland and kidney with significant levels of activity associated with granules (eg tertiary lysosomes).

Sub-cellular distributions. A problem area has been identified in achieving an adequate separation after centrifugation of some forms of lysosomes and mitochrondia. Molecular binding studies have been started in order to identify the selectivity of radionuclide uptake as distinct from general protein labelling. Microchemical methods have been developed for the analysis of individual granules in order to determine the composition of those associated with the transuranics; methods used are thin layer chromatography and anodic stripping voltametry in association with electrophoresis and other methods of analysis.

Stable element - Radionuclide Studies. The concentration of c30 stable elements in 10 organs and tissues of the mussel have been determined for four different populations of UK animals. Preliminary data indicates the importance of water composition rather than local geochemical factors in explaining observed organ tissue concentrations.

Microsurgery. We continue to investigate techniques capable of allowing direct sampling of body fluids in vivo.

Measurements continue in determining the concentration of U, Pu, Am, ^{137}Cs and ^{106}Ru in native populations of mussels from SW England which are remote from influences of the nuclear industry and are exposed to general aerial fallout debris.

A first stage mussel model has been developed in order to examine the uptake-retention-loss dynamics. For purposes of monitoring the concentration of radionuclides in the mussel use of the pericardial gland and kidney seems useful; the byssus and periostracum are also useful, but less is known concerning the nature of the tanning process with which the radionuclides are associated. A technique has been developed for determining the total weight of a kidney free from occluded gonad and connective tissue.

A general observation from our investigations is that the physical form of radionuclides derived from the nuclear industry is an important factor which influences uptake and retention by marine organisms at different geographical sites.

PROJECT 2

A model which accounts for the rate of release of radionuclides by BNFL Sellafield, in relation to transport times and deposition in sediments of the Esk estuary, Cumbria has been completed. We identify the importance of transuranics in particulate form, representing fuel element debris transported to sediments. We identify the importance of low salinity water in the Esk in controlling deposition and retention of radionuclides. We confirm an urgent need to pay attention to the sediment/water interface as a site influencing retention and loss of radionuclides from the Esk. Data obtained in the laboratory on sediment cores, and other samples, has been verified by using a Li(Ge) MCA in the field. Surface radioactivity is high (September 1982) in relation to reduced levels of release by BNFL in 1981-2; we infer that only a small proportion of radionuclides which enter the Esk are retained.

Our completed study of sedimentation rates for the Esk indicates:
i) Based upon a consideration of general salt marsh development using vegetation - 0.6cm y^{-1}. ii) Data derived from fibre mats placed in situ to collect deposited sediment-1-4cm y^{-1} (corrected for variation in input of sediment with time of year). iii) Silts, using a complex array of radionuclides - 3.5cm y^{-1} and 1.2cm y^{-1} for muds. The presence of silts and muds in the estuary for the past c 50y have been controlled by bioturbation of the amphipod Corophium v. Data have been obtained which show that the type of sediment deposited in the Esk is influenced by major changes in the direction of water transport for the Irish Sea as a whole. The highest concentrations of radionuclides are found in anaerobic muds, and contain both conservative and non-conservative species; the aerobic silts contain predominantly non-conservative species. With the passage of time the deeper horizons of the muds become oxygenated by reaction with low salinity waters, and the conservative phases which remain, or are produced in situ as a result of redox changes, are lost from the sediments. The effect of radionuclides present in particulate forms is likely to be most significant at the end of the BNFL effluent pipeline in relation to deposition in sediment. As this involves expensive off-shore work it is more suitably considered by MAFF, U.K., but we are interested in the results of their studies in relation to ours from the Esk.

The geochemical behaviour of non-detrital uranium in the Esk has been described; from a consideration of isotopic composition the uranium in the

Esk is of natural origin. Some detrital uranium is derived from BNFL particulate debris, but in terms of the mass of uranium present in the system the quantity involved is negligible. We observe that the behaviour of non-detrital uranium in the Esk is also influenced by changes in direction of water movement in the Irish Sea. In the vicinity of Sellafield, for the first time, we observe the presence of ^{236}U in environmental samples which provides an unequivocal tracer for BNFL derived uranium.

Using CR-39 plates emplaced in Lake Ennerdale, together with measurements on bottom sediment core samples, we observe the presence of high levels of alpha emitters relative to other Cumbrian Lakes; L. Ennerdale is situated along the axis of the SW prevailing winds, and we propose to determine ^{236}U levels, if possible, in order to identify any influence on uranium concentrations through the presence of BNFL debris.

Data obtained in this project is being used in the development of a model concerned with estuarine deposition of radionuclides over long periods of time in relation to risk assessment.

Publications

R.J. Clifton and E.I. Hamilton. "The application of radioisotopes in the study of estuarine sedimentary processes. Est. Coastal Mar. Sci. 14, 433-446. (1982).

R.J. Clifton and E.I. Hamilton. "Concentration and depuration of some radionuclides present in a chronically exposed population of mussels (Mytilus edulis L.) from the Esk estuary Cumbria, England. Accepted for publication Mar. Ecol. Prog. S. (1982).

Progress Report
1982

Contractor:

Rijksuniversiteit Gent
Sint Pietersnieuwstraat 25
B-9000 Gent

Contract no.: BIO-B-327-81-B

Head(s) of research team(s):

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General subject of the contract:

Measurement and analysis of the evolution of the krypton-85 activity in the atmosphere and study of its synergistic action with chemical pollution.

List of projects:

1. Measurement and analysis of the evolution of the krypton-85 activity in the atmosphere and study of the synergistic action of krypton -85 and chemical pollution.

Title of project nr 1 : Measurement and analysis of the evolution of the krypton-85 activity in the atmosphere and study of the synergistic action with chemical pollution.

Head of project and scientific staff : Dr. A. Janssens, F. Baes, H. Vanmarcke

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1. Kr-85 measurements

The new set-up for measurement of the atmospheric kr-85 activity has been used since September 1982. The procedure is much simplified and is less time consuming. Due to the better purification of the Kr-gas fraction and the smaller collection volume the uncertainty on the mass of recovered krypton is very small. A new design is being tested for the screw cap of the scintillation vial to eliminate the small leakage through the septum.

As the measuring procedure with the old set-up was improved several times and a number of small corrections were introduced since 1979, a thorough examination of all the data was performed. The most variable factor was the pressure correction allowing for the presence of other gases in the recovered Kr-fraction. The eventual presence of other gases can be observed also through an enhanced pression after condensation of the gas. All data have been recalculated with a correction based on the measured condensation pressures. This revision resulted in a smaller spread of the data and in a small but observable increase of the background with time. The data for the measurements in the period 1979-1982 are plotted in Fig. 1. After application of a procedure of successive elimination of excess values a linear fit to the "background" data was made This fit indicates a constant increase of the global Kr-85 activity with 3 or 4% per year since 1979. This small rate could give an indication of th present world reprocessing activities.

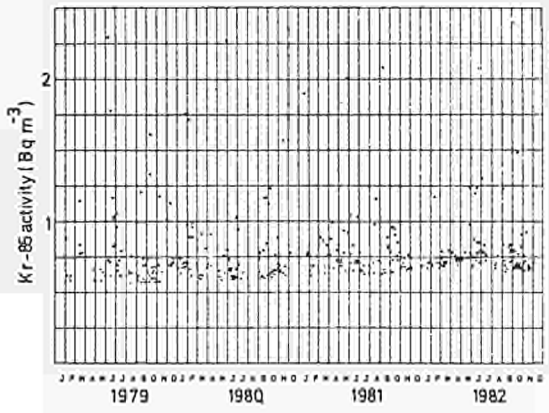

Fig. 1. - Kr-85 activities 1979-1982

2. Radiation effects on aerosols

The electrostatic classifier and CNC were thoroughly tested and compared with similar devices from other laboratories. A new type of diffusion screen battery was constructed for the measurements sub 0.01 µm.

To avoid a difficult interpretation of the measurements with the previous flowreactor (see report 1981) we decided to transform it into a reactor of completely mixed type.

The increase in aerosol volume due to ionizing radiation was measured for a fixed mean residence time (MRT) of 20 min as a function of the dose rate. The results (Fig. 1a & b) show that the aerosol volume, which can be traced back to the SO_2 transformation rate, may increase at dose rates even below the maximum permissible exposure (2.5 mrad/h or 7 nGy/s). The relative increase is most apparent in dry conditions.

The increase in aerosol number concentration was measured at a fixed dose rate as a function of the MRT. For long MRT's the increase is approximately the same as the aerosol volume increase at the same dose rate. For short MRT's the increase is a hundredfold (see Fig. 2). This can be explained by the classical theory of homogeneous and ion-induced nucleation. Numerical calculations are being performed.

The construction has started of twin smog chambers (2 x 200 l) for measurements in static conditions.

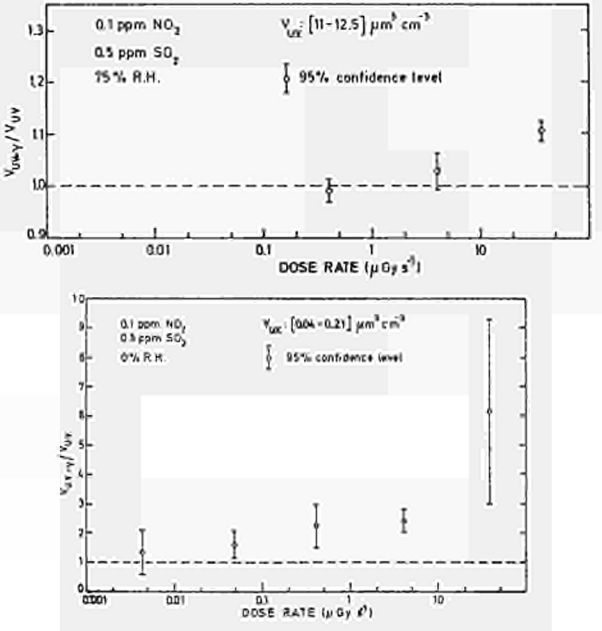

Fig. 1a & b
Relative aerosol volume under simultaneous UV and gamma radiation as a function of the dose rate.

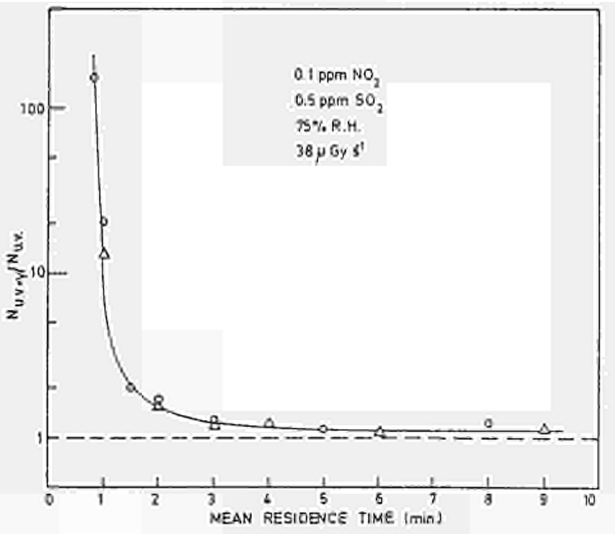

Fig. 2. - Relative number concentration under simultaneous UV and gamma radiation as a function of the mean residence time.

List of publications in 1982

I. *Publications in Scientific Journals, Monographs, Proceedings.*

F. Raes and A. Janssens,
Study of the combined effect of ultra violet and ionizing radiation on gas to particle conversion.
in Proc. GAeF symposium, Bologna 1982
to be published in J. Aerosol Sc.

F. Raes and A. Plomp,
Comparison of two condensation nucleous counters TSI model 3020; calibration of the photometric mode.
in Proc. GAeF symposium, Bologna 1982
to be published in J. Aerosol Sc.

II. *Short Communications, Theses, Internal Reports, Patents ...*

A. Janssens, F. Raes, E. Cottens,
Atmospheris ^{85}Kr activity; Radiation effects on aerosols,
in Annual Report Nucl. Phys. Lab. 1981, 65-81.

Progress Report
1982

Contractor: **Contract no.:** BIO-B-313-81-D

Bundesgesundheitsamt
Institut für Strahlenhygiene
Ingolstädter Landstrasse 1
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Head(s) of research team(s):

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Dr. G. N. Kistner
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General subject of the contract:

Distribution of tritium- and carbon-14-compounds over the aqueous and organic phases of various structures of aquatic and terrestrial food chains.

List of projects:

1. Distribution of tritium- and carbon -14- compounds over the aqueous and organic phases of various structures of aquatic and terrestrial food chains.

Title of project nr: Bio - B - 313 - 81 - D
Distribution of tritium- and carbon 14-compounds over the aqueous and organic phases of various structures of aquatic and terrestrial food chains.

Head of project and scientific staff:
Dr.rer.nat. G. Kistner, Dr.rer.nat. M. Brehm, Dr.-Ing. E. Clausen, Dr.-Ing. W. Leister, Dipl.-Biol. E. Nürnberger, Dipl.-Biol. u. Tierarzt G. Gauß, Dr. rer.nat. P. Török, Dr.agr. E. Wirth

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Investigations on the transfer of tritium- and carbon 14-compounds in the aquatic food chain have been continued. Incorporations of tritium in proteins and lipids of green algae were meseasured.

I. Incorporation of tritium into proteins:

Further experiments have shown that some of the amino acids of the extracted proteins of algae possess a relativ high and strong tritium label. The highest amounts were found with aspartate and glutamate and with the essential amino acids leucine and arginine.

Material and Methods:

Organism: Scenedesmus quadricauda ssp.subspicatus, a laboratory strain from the Institute for Water-, Soil- and Air Hygiene of the Federal Health Office in Berlin.

Cultivation was carried out in a batch-system of a 5 Liter fermenter under constant illumination. Temperature was 27°C and a culture time of 120 hours, with a modified Holm-Hansen (6) solution:
0,07% $NaNO_3$,0,006% K_2HPO_4,0,01% $MgSO_4.7H_2O$,0,004% $CaCl_2.2H_2O$, one aliquot $FeSO_4.7H_2O$/EDTA-solution and tracer elements.An addition of 30 mg/l ampicillin keeps the culture sterile;after autoclaving 3,5 g/l $NaHCO_3$ and 50 mCi tritium water and 5 µCi $NaH^{14}CO_3$ (sterile filtered) were added.

Protein extraction: From several possible methods (7) for cell wall desintegration, the supersonic method (5) was used combined with 0,1N NaOH and mercapto ehtanol. Protein was precipitated with 1 N HCl at pH 3,5, chlorophyll was removed by ethanol/ether treatment. The amino acid mixture yielded by HCl-hydrolysis (6 N HCl, 110°C, 24 h, addition of phenol and mercaptoethanol) (1) was subsequently fractionated by column chromatography.

Ion-exchange-chromatography: A highly acid cation exchanging substance was used and the individual amino acid fractions were eluated by different buffers. (Table 1).

buffers:		pH values:	eluated amino acids:
Citrat	+ 0,2 n NaOH	3,25	Asp,Thr,Ser,Glu,Pro,Gly
Citrat	+ 0,2 n NaOH	4,25	Ala,Val,Ile,Leu
Citrat/Borat	+ 0,2 n NaOH	7	Tyr,Phe
Citrat/Borat	+ 0,2 n NaOH	10	His,Lys,Arg

Table 1: pH-values of buffers for the eluation of different amino acids by ion-exchange chromatography.

Results: After 120 hours of growth in a contaminated medium the algal dry matter, yielded by centrifugation and lyophyllisation showed an incorporation of 0,02% tritium and 95% carbon 14. Since the discrimination of isotopes from C-14 to C-12 proved to be relatively low and negligible small in comparison to the tritium discrimination (2), the carbon 14 activity served as a reference for the tritium values,assuming that the measured amount of C-14 is equal to the actual concentration of carbon atoms of the examined substance.
Since in amino acids not all H-positions are stable - which may lead to a loss of tritium from the labeled molecules - the activity values measured had to be corrected according to loose and fast bounded tritium. Corrected figurs already have been published (8).
The distribution of tritium in the amino acid fractions is shown in Fig. 1. Leucine and arginine showed the highest activities per molecule in the examined protein fraction next to asparaginic- and glutamic acid.

T/C-14 : H_{ne}/C = Spezifische Tritium-Aktivität bezogen auf die spez. Aktivität von C-14 und die nicht austauschbaren H-Positionen

Asp, Glu, His : variable Anzahl von nicht austauschbaren H-Positionen

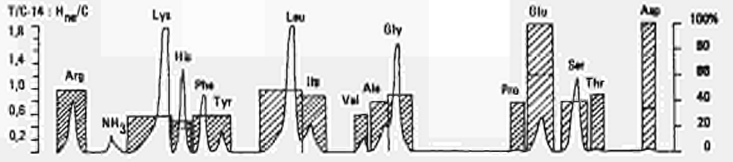

Fig. 1: Distribution of tritium in the amino acid fractions of hydrolysed algal proteins corrected for loose and fast bound tritium.

Discussion and Conclusion: A concentration of radioactivity may occur in molecules with very stable C-H-positions (3,4). The portion of tritium which is bound in the amino acids in a stable form seems to undergo no essential change after their incorporation into proteins (2) and thus is passed on, in the food chain. Therefore,in heterotrophic organisms which absorb these proteins as food, an increased radiation exposure may occure. This is of particular importance for the ingestion of essential amino acids and their metabolism in intermediare pathways.

Parts of the molecules of asparaginic and glutaminic acid are transfered by transamination-reactions. The amino-groups are excepted by ketoglutaric acid, which serves as a distributor for labeled parts of arginine,proline,histidine, glutamine and glutamate.Parts of aspartate and glutamate are introduced by biosynthetic pathways into purine and pyrimidine components of nucleinic acids. Via inosinic and orotic acid trtium maybe transfered into sensitive targets (DNA and RNA) of genetic material.

II. Incorporation of tritium into lipids:

Saturated and unsaturated fatty acids with 16 and 18 C-atoms can be found in many strains of algae also in Sc.quadricauda sp.(1) we used in our experiments.Investigations of Kanazawa et al.(2) with Chlorella pyr. revealed a very high variation in the ratio of bound tritium to unexchangeable hydrogen for the lipids of algae biomass in total. Diskrimination of C-14 during photosynthesis can be set as 4%, the ratio T/H (exchangeable and non exchangeable positions together) reaches levels of 0,89 to 1.13.Wether in the course of photosynthetic uptake of tritium radiation exposure specielly for the target "Algae" and also for subsequent links of the food chain will be lowered by diskrimination or be elevated by radionuclide accumulation can't be interpreted by these variable results.

To answer this question more precisely measurements of separate lipid components, as fatty acids and other in this course extractable compounds, are necessary.

Methods were worked out for

- Extraction of the cruded lipid-fraction from algal bio-mass
- Conversion into a form which is measurable with gaschromatography (GC)
- GC-separation and measuring their tritium content by Gasproportional counting

The GC-analysis of testsubstances of bacterial bio-mass which we have performed with our methods is shown in Fig.2. Work in the separation and measurement of lipids in algal fat and milk fat from tritium contaminated cows will be continued.

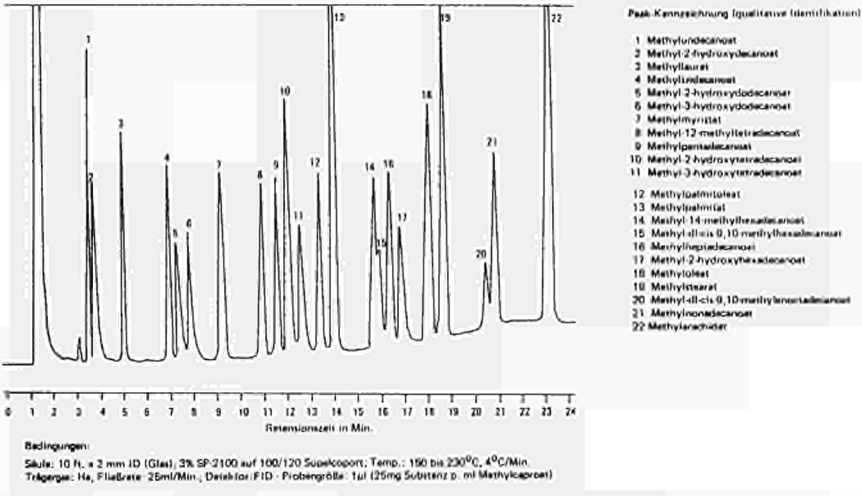

Fig. 2: GC-analysis of fatty acids from bacterial bio-mass

References: (I) Incorporation of tritium into proteins:

(1) Hill, R.L.: Advance in Protein Chemistry 20, 1965,p.37
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(3) Meloche, H.P., Monti, C.T., Cleland, W.W.: Biochim. & Biophys. Acta, 480, 1977, p. 517
(4) Hartshorn,S.R., Shiner,V.J.: J.Am.Chem.Soc.94, 1972, p.9002
(5) Merkenschlager, M., Schlossman, K., Kurz, W.: Biochem. Z. 329, 1957, 332
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(8) Nürnberger, E., Clausen, E., Kistner, G.: Annales . de l'Association Belge de Radioprotection vol.7,n° 3-4, 339-343, 1982

References: (II) Incorporation of tritium into lipids:

(1) Wood, B.J.B.: In: Stewart, W.D.P., Algal Physiology and Biochemistry,Botanical Monographs Vol 10 Oxford, London, 1974, S. 236-265
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(3) Leister, W., STH-Berichte 4/1981, Dietrich Reimer Verlag, Berlin
(4 Urbach, W., Rupp, W., Sturm, H.: Experimente zur Stoffwechselphysiologie der Pflanzen. Stuttgart, S. 208-210, 1976
(5) Hadorn, H., Zürcher, K.: Mitt. Lebensmittelunters. u. Hyg., 58, 326, (1967, 59, 78, 1968

List of Publications:

I. Publications in scientific journals, monographs, proceedings:

Leister,W.: Untersuchungen über die Biokinetik von Kohlenstoff 14 in Algenkulturen, STH-Bericht 4/1981, Dietrich Reimer Verlag, Berlin,1981

Kistner,G.: Zur Tritiumkontamination der Umwelt und des Menschen in: Kaul, A. (Herausg.) 20 Jahre Strahlenhygiene beim Bundesgesundheitsamt Wissenschaftliches Kolloquium, Neuherberg, 3.4.1981 STH-Bericht 12/1981, Dietrich Reimer Verlag,Berlin,

Kriegel,H., Schmahl,W., Kistner,G., Stieve,F.-E.: Developmental effect of prenatal irradiation Internationales Symposium, Neuherberg, 26.-28.11.1980, Gustav Fischer Verlag, Stuttgart - New York, 1982

Török, P., Kistner, G.: Reproductive performance of mice after injection of tritiated water at different embryonic developmental stages. In: Kriegel,H.,Schmahl,W.,Kistner,G.,Stieve,F.-E.,(ed.) Developmental effects of prenatal irradiation G.Fischer Verlag,Stuttgart-New York,S.91-96, 1982

Nürnberger, E., Clausen, E., Kistner, G. Investigation of the distribution of tritium and carbon 14 in the amino acids of labeled green algae (Sc. quadricauda ssp.) Annales de l'Association Belge de Radioprotection, vol. 7, n°. 3-4, 339-343, 1982

II. Short communications, theses, internal reports, patents, posters and presented papers:

Leister, W.: Untersuchungen über die Biokinetik von Kohlenstoff 14 in Algenkulturen Dissertation 1981, Freie Universität, Berlin

Török, P., Kistner, G., Kriegel, H.: Age dependent fertility of NMRI-mice after injection of tritiated water during organogenesis. Approximation of"dosis minimalis".Poster IX.Congreß of International Society of Developmental Biologists, Basel/Schweiz, 28.8.-1.9.1981

Török, P., Elsässer, U., Kistner, G.: Postnatal development of mice related to prenatal incorporation day of tritiated water (HTO) Poster, 9th Conference of the Europ. Teratology Society, 31.8.-3.9.1981, Basel/Switzerland

Kistner,G., Török,P., Schmahl,W., Meyer,I., Kriegel,H.: Radiobiological effects of tritium incorporation in mice. Paper presented at the Seminar "Environmental transfer and metabolism of tritium" of the Project-Group "Tritium" of the EG, Wageningen/Netherlands, 28.-30.6.1982

Kistner,G.: Behaviour of tritium in aquatic systems Paper presented at Europan Seminar on the Risks from Tritium exposure. Mol/Belgium, 22.-24.11.1982

Progress Report
1982

Contractor: Contract no.: BIO-B-484-82-D

Kernforschungszentrum Karlsruhe GmbH
Postfach 36 40
D-7500 Karlsruhe 1

Head(s) of research team(s):

Prof. Dr. H. Kiefer
Hauptabteilung Sicherheit
Kernforschungszentrum Karlsruhe GmbH
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General subject of the contract:

Microbiological aspects of the behaviour and control of radionuclides in terrestrial and aquatic ecosystems.

List of projects:

1. Microbiological influences on the mobility of radionuclides in soils and sediments.

Microbiological influences on the mobility and bioavailability of radionuclides in soils and sediments

Dr. S. Strack
Dipl.-Biol. A. Müller

1. General Aim

The aim of this project is to make a contribution to modeling the behavior of radioecologically relevant nuclides in terrestrial ecosystems after release from nuclear facilities. The work was initiated by the long-term changes observed in the vicinity of the Karlsruhe Nuclear Research Center (KfK) as regards the bioavailability in the soil of I-129 and Pu-nuclides. Microbiological processes in the humus horizons might account for it. Initially, these studies will be restricted to radionuclides such as H-3, C-14 and P-32, but it is planned to extend them very soon to include radioecologically relevant nuclides e. g. Sr-90, I-129, Cs-137 and Pu-139.

2. Particular Objectives

The migration behavior of radionuclides applied to soil columns as various compounds will be investigated. It is intended to determine the consequences of specific interferences in the composition and metabolic activity of the microflora. The fine distribution of the immobilized nuclides in the soil material will be studied.

3. Work Performed

Various methods of quantitative determination of the microflora and its metabolic activity were examined. Preliminary tests were performed with percolator apparatuses in order to find out suitable experimental conditions. Conditioned soil samples, incubated with H-3 and C-14 compounds, were examined using microautoradiography. Monthly determinations of the number of germs in the soil of a test field were performed since July this year.

4. Results Obtained

The data derived from the monthly determinations of the population density of soil bacteria and lower fungi have been summarized in Fig. 1. Fluctuations in the population density of the soil result from changes in the nutrient offer, soil temperature and, above all, moisture of the soil.

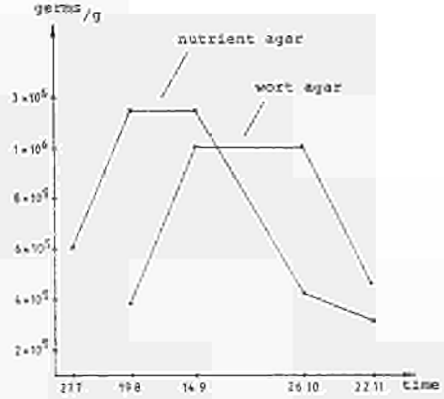

Fig. 1
Number of living germs of bacteria and fungi on nutrient and wort agar per gram of forest soil; humus horizon, duration of cultivation at 30°C: 48 h
(period of examination: July through November 1982)

Considerable dryup of the topmost soil layers and a small offer of degradable plant residues accounted for the low number of bacteria in July. Damp-warm weather conditions permit a fast growth of bacteria, but they cause a low density of germs of lower fungi because the latter, except for specific water species, prefer drier sites than most of the bacteria. The conspicuous multiplication of fungi in September and October is due to a long-term period of dryness beginning in early September and to degradable plant material produced in excess as a result of fall of leaves. The drop of the curves towards the end of the year was caused by low soil temperatures of approx. 10 °C and an excessive saturation of the soil with water leading to the accumulation of wetness.

The relations observed in the field test between the nutrient offer, soil aeration, macroclimate and microclimate, on the one hand, and species composition, metabolic activity and capability of division of the microorganisms, on the other hand, will be transferred at the laboratory to the percolation tests performed on soil columns treated with isotopes.

Microautoradiographic methods to determine the microdistribution of radionuclides in the soil matrix were tested. Soil samples, enriched with microorganisms, were examined by fluorescence microscopy and autoradiography (stripping film) after short-term incubation with tritiated and C-14 labeled substrates. Organisms with an active metabolism were identified by the corresponding black spots on the film.

I. Literature

1. S. Strack - "Metabolism of Tritium and Organic Binding in Micro-Organisms and Soil".
European Seminar on the Risks from ^{3}H Exposure, Mol, Belgium, November 22-24, 1982.

Progress Report
1982

Contractor: Contract no.: BIO-B-431-81-B

Centre d'Etude de l'Energie
Nucléaire, CEN/SCK
Avenue Plasky 144
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Head(s) of research team(s):

Dr. R. Kirchmann
Département de Radio-
biologie, CEN/SCK
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B-2400 Mol

General subject of the contract:

Simulation of tritium transfer in the environment : study of organic tritium transfer in the aquatic environment, the soil-plant and the plant-mammal system.

List of projects:

1. Behaviour of tritium in different chemical forms in the freshwater environment.
2. Behaviour of tritium in the soil-plant system.
3. Behaviour of tritium in mammals.

Title of project nr 1 : BEHAVIOUR OF TRITIUM, IN DIFFERENT CHEMICAL FORMS, IN THE AQUATIC ENVIRONMENT

Head of project and scientific staff : S.BONOTTO, G.ARAPIS(1), R.KIRCHMANN
Collaborators : G.GERBER(2), S.PUISEUX-DAO(3)

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1. Modelization of tritium transfer into the organic compartments of algae

Uptake of tritium oxide and its conversion into organic tritium was studied in four different types of algae with widely varying size and growth characteristics (*Acetabularia acetabulum*, *Boergesenia forbesii*, two strains of *Chlamydomonas* and *Dunaliella bioculata*). Water in the cell and the vacuoles equilibrates rapidly with external tritium water. Tritium is actively incorporated into organically bound form as the organisms grow. During the stationary phase, incorporation of tritium is slow. There exists a discrimination against the incorporation of tritium into organically bound form. A model has been elaborated taking in account these different factors. It appears that transfer of organic tritium by algae growing near the sites of release would be significantly only for actively growing algae. Algae growing slowly may, however, be useful as cumulative indicators of discontinuous tritium release.

2. Incorporation of ^{3}H into the proteins of algae

Analysis by electrophoresis on gels of polyacrylamide has shown that the main proteins of the algae (*Acetabularia* and *Chlamydomonas*)are labelled after one week treatment with tritiated water. The microalga *Chlamydomonas reinhardii* CW15, a mutant without cell wall, releases several labelled polypeptides into the external medium.

(1) Scientific and technical fellow of the Commission of the European Communities.
(2) Commission of the European Communities.
(3) University of Paris VII.

3. Factors affecting uptake and distribution of tritium into the algae

The main physical and chemical factors (light, temperature, culture medium composition, etc.) which may affect tritium uptake and incorporation into the algae are poorly investigated. In order to fill this gap, we have undertaken an experimental work of the incorporation of tritium (tritiated water and/or tritiated precursors) into the giant unicellular alga *Acetabularia*, kept under different experimental conditions. Since light was found to stimulate ^{3}H uptake and incorporation, tritium releases into the aquatic systems should be practisized during the night.

4. Publications

S. Bonotto, A. Bossus, G. Nuyts, P. Mathot, J. Colard, R. Kirchmann
Experimental study on the biological availability of selected radionuclides in several marine algae. Third International Ocean Disposal Symposium, Woods Hole, Massachusetts, U.S.A., October 12th-16th, 1981, Abstracts, p.40-41.

S. Bonotto, G. Nuyts, A. Bossus, R. Kirchmann
Apport de la biochimie à l'étude de la pollution radioactive du milieu marin : distribution du ^{3}H dans les différents constituants cellulaires des algues marines Acetabularia, Boergesenia et Dunaliella. Journées du Groupement pour l'Avancement de la Biochimie Marine (GABIM), "Indices Biochimiques et Milieu Marin", Brest, 18-20, novembre 1981. Publi. CNEXO (Actes Colloq.) n.14, pp.399-410 (1982).

D. Hoursiangou-Neubrun, A. Lüttke, G. Arapis, S. Puiseux-Dao, S. Bonotto
Apicobasal gradient of chloroplast DNA synthesis and distribution in Acetabularia. In : Cell function and differentiation. Part B : Biogenesis of energy transducing membranes and membrane and protein energetics. FEBS, Federation of European Biochemical Societies, Vol.65, p.333-345 (1982).

S. Bonotto, G.B. Gerber, G. Arapis, R. Kirchmann
Modelization of tritium transfer into the organic compartments of algae. Ann. Ass. Belge de Radioprotection, 7, 283-292 (1982).

G. Arapis, S. Puiseux-Dao, S. Bonotto
Factors affecting uptake and distribution of tritium in the giant unicellular marine alga Acetabularia. European seminar on the risks from tritium exposure, Mol, Belgium, 22-24 November 1982.

R. Kirchmann, J.C. Dupont, P. Fontaine-Delcambe
Evaluation de l'influence des tours de réfrigération sur le transfert à l'environnement terrrestre du tritium d'un cours d'eau récepteur. Annales de l'Association Belge de Radioprotection, vol.7,nr 3-4, 147-166 (1982).

H. Camus, J. Delmas, R. Kirchmann
Influence du climat sur la sorption et la désorption d'eau tritiée par des végétaux irrigués. Annales de l'Association Belge de Radioprotection, vol.7,nr3-4, 247-258 (1982).

R. Kirchmann, E. Fagniart
Bilan de recherches expérimentales sur le transfert de l'eau tritiée aux végétaux cultivés. Annales de l'Association Belge de Radioprotection, vol.7, nr3-4, 229-246 (1982).

Title of project nr 2 : BEHAVIOUR OF TRITIUM IN THE SOIL-PLANT SYSTEM
Head of project and scientific staff : R. KIRCHMANN

I. Production of tritiated feed

- Ray grass was grown under a plastic greenhouse (6m x 4,5m) located in the experimental fields of the C.E.N. farm at Mol.
- Potatoes were contaminated by the same conditions as ray grass but only covered during contamination.

a) Ray grass : two cuttings were produced with respectively 35,5 kg and and 11,5 kg fresh matter.
The content of the hay was about 0.7 nCi and 0.9 nCi/g dry matter.

b) Potatoes : The production of potatoes (tubers) at the final harvesting was 37,20 kg of fresh matter, with a content of incorporated tritium about 3,8 µCi/g dry matter.

For both plants the incorporated tritium was brought by tritiated spray water (20 mCi/l).
The potatoes shall be used in experiments on the transfer of tritium from feed to animal (project 4).

II. Transfer of C^{14} from feed to animal

Plants of maize have been exposed, under plastic greenhouses, to CO_2^{14} gas during 7 hours (conditions support 1981 project 3 : point 2).
The final harvest was made 16 days after contamination with a production of 120 kg fresh matter.
Analyses and experiments are in progress.

III. Transfer of C^{14} from Lupinus (as organic fertilizer) to plants

Lupinus has been contaminated by the same conditions as maize.
The production of about 8,17 kg/m² was ploughed into the soil, 58 days after contamination.

IV. Transfer of C^{14} from compost to maize, potatoes, lettuce and cabbage

A second culture is made on the same substratum of 1981 in order to follow the evolution in function of time.
Analyses are in progress.

Title of project nr 3 : BEHAVIOUR OF TRITIUM IN MAMMALS

(1982, work performed in Department of Radiobiology, SCK/CEN, Mol)

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1) Study of the transfer of inorganic and organically bound tritium, administered continously to milk during pregnancy and lactation, as well as on the turn-over of inorganic and organic tritium in different organs was performed in 1982.
Food mixed from equal amounts of organically labeled tritiated milk powder and normal food pellets was given to mice during pregnancy and lactation. At birth, some new-born were swapped with those from non-exposed mothers to compare separately accumulation and metabolism during pregnancy and lactation. Young mice were sacrificed at different times after birth, and tritium activity in different organs was determined. Tritium activity was also determined in maternal organs at various times during and after the 42 days feeding period. The activity per g in some tissues of the young particularly in fat, exceeded that of the food given, probably as a result of the high activity and low metabolic dilution of the fats in the food. Young mice contaminated during lactation and pregnancy contained still detectible activity at an age of 2 months. Activity was nearly the same in mice receiving tritium only during lactation as in those receiving it also during pregnancy. Dilution was more marked due to rapid growth when tritium application was discontinued at birth. Tritium water was replaced most rapidly, organic tritium in brain turned over most slowly with an additional metabolic component of a half life in the order of 1 month. Organic tritium in liver displayed an intermediate half life.
The analysis of the data concerning the continous administration of THO to mice during pregnancy and lactation are in progress.

2) Distribution of organically bound tritium in different fractions of organs after a continous administration of inorganic or organic tritium.

Activity of organic tritium after application of tritium oxide is higher and turnover is slower in developing brain than in other organs. This

had been demonstrated in an experiment where pregnant sows were given tritium oxide to drink, and the new-borns were either kept for additional exposure during lactation (43 days) or swapped with new-borns from uncontaminated mothers. Analysis of the cerebella of the piglets showed highest activities at birth in fatty acids and cholesterol whereas cerebroside activity increased during lactation. High levels of activity, particularly in fatty acids and cholesterol are still present at an age of 115 days. A large part of lipids and structural material in proteins etc. laid down in brain during development is thus retained for long periods of time. Brain represents therefore, together with the ovary, a critical organ for tritium exposure during development.

Adult non pregnant mice were given for three weeks milk powder whose organic constituents were labeled with tritium. Different tissue fractions, proteins, nucleic acids, residue, fatty acids, cholesterol, phospholipids, gangliosides and glycolipids, were isolated from organs, and their total and specific activity were determined. Highest specific activities were found in gangliosides and glycolipids and lowest ones in proteins and fatty acids. It is concluded that tritium labeled lipids, particularly, the more complex ones, undergo relatively little dilution when they are incorporated into body lipids whereas protein constituents, fatty acids and small molecular size metabolites are diluted to a greater extent. Uptake of tritium organically bound to lipids, particularly during development, may thus represent a critical pathway in the risk from organically bound tritium in food.

Publications

R. Van Bruwaene, G.B. Gerber, R. Kirchmann, J. Van den Hoek and J. Vankerkom
Tritium metabolism in young pigs after exposure of the mothers to tritium oxide during pregnancy.
Radiation Research 51 : 124-134 (1982).

M. Rochalska, R. Van Bruwaene, G.B. Gerber, R. Kirchmann
Organic tritium in brain of new-born pigs from mothers who had received tritium water during pregnancy.
Annales de l'Association Belge de Radioprotection, vol.7, nr 3-4, p.363-373 (1982).

M. Rochalska, R. Van Bruwaene, G.B. Gerber, R. Kirchmann
Organically bound tritium and its distribution in mice fed organically

labeled milk powder.
Annales de l'Association Belge de Radioprotection, vol.7, nr 3-4, p.345-351 (1982).

R. Van Bruwaene, G.B. Gerber, R. Kirchmann, J. Maes, E. Fagniart
Incorporation and metabolism of tritium in pregnant mice and their offspring after feeding organically labeled tritiated milk powder during pregnancy.
Annales de l'Association Belge de Radioprotection, vol.7, nr 3-4, p.353-362 (1982).

Progress Report
1982

Contractor:

Centre d'Etude de l'Energie
Nucléaire, CEN/SCK
Avenue E. Plasky 144
B-1040 Bruxelles

Contract no.: BIO-B-467-81-B

Head(s) of research team(s):

Ir. R. Kirchmann
Département de Radio-
biologie, CEN/SCK
Boeretang 200
B-2400 Mol

General subject of the contract:

Technetium transfer in the animal food chain.

List of projects:

1. Technetium transfer in the animal food chain.

Title of project nr 1 : TECHNETIUM TRANSFER IN THE ANIMAL FOODCHAIN

Head of project and scientific staff : R. KIRCHMANN, J.R. MAISIN,
R. VAN BRUWAENE

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1. Metabolism

Technetium metabolism was studied in sheep after an intravenous injection of Tc^{95m} as pertechnetate. Faecal and urinary excretion was followed for more than 100 days and the distribution in the organs was determined on days 1, 3, 7, 30 and 100. After an i.v. injection we observed a total faecal and urinary excretion of respectively 67.2% and 17.24%; about 99% and 71.5% respectively of the total faecal and urinary excretion were found within the first 5 days after administration.

The data were analyzed by non-linear regression using an exponential function $A_t = \sum_{i=1}^{n} A_i . C^{-t/T_i}$. The parameters A_i and T_i are summarized in table 1.

Table 1. Parameters of ^{95m}Tc excretion in faeces and urine of sheep after an intravenous injection.

Faecal		Urine	
A	T_o	A	T_o (days)
5650 ± 164	0.71 ± 0.006	∿26000	∿ 0.2
1.19 ± 0.20	8.66 ± 0.83	38.1 ±10	1.26 ± 0.21
0.0214 ± 0.0071	80 ± 44	5.30 ± 0.91	6.51 ± 1.25
		0.025 ± 0.013	55 ± 13

These data demonstrate that technetium in the body is principally excreted via the GI tract. Faecal excretion displays 3 components with half lives (T_o x 0,693) of 0.49 , 6.0 and 55 days. Urinary excretion is caracterized by 4 compartments with half lives of 0.14 , 0.87 , 4.5 and 38 days.

Table 2. Technetium (Tc^{95m}) distribution in organs of sheep after i.v. injection(in % of dose/organ).

day	total	thyroid	liver	lungs	kidneys	GI tract
1	3.11	0.054	0.063	0.194	0.150	2.42
3	0.535	0.016	0.026	0.083	0.098	0.227
7	0.187	0.011	0.012	0.025	0.040	0.016
30	0.087	0.015	0.006	0.030	0.011	0.011
100			in progress			

Distribution in organs (in % of dose/organ) is represented in table 2. Highest concentrations were observed in the thyroid; concentrations of technetium in lung tissue were also elevated.

2. Toxicity

An experiment concerning the chemical toxicity of technetium (Tc^{99}) was carried out in female rats (R-Cnb) of 3 months age. Technetium pertechnetate was added to commercial rat food at concentrations of 0, 0.5 , 1, 5, 10 and 15 µg Tc^{99}/g food and rats were sacrificed after 7 or 17 weeks of treatment; body weight, organ weight and blood formula were noted and did not show any major alterations. Determination of Tc concentrations, radio-immunoassays of thyroidal hormons, histological examinations and autoradiographies are in progress.

Publications

R.Kirchmann, J. Maisin, R. Van Bruwaene, G.B. Gerber and J. Colard
Metabolisme et transfert des métaux lourds chez l'animal.
Rapport scientifique final 1978-1981 du Programme National "Environnement-Air" nr 23, p.1-104 (1982).

R. Van Bruwaene, G.B. Gerber, R.Kirchmann and J. Colard
Transfer and distribution of radioactive cadmium in dairy cows.
Intern. J. Environmental Studies, Vol.19, p. 47-51 (1982).

R. Van Bruwaene, G.B. Gerber, R. Kirchmann and J. Colard
Metabolism of Antimony-124 in lactating dairy cows.
Health Physics, Vol.43, nr 5, 733-738 (1982).

R. Van Bruwaene, G.B. Gerber, R. Kirchmann, J. Colard and J. Vankerkom
Metabolism of Chromium-51, Manganese-54, Iron-59 and Cobalt-60 in lactating dairy cows.
sous presse.

Progress Report
1982

Contractor: **Contract no.:** BIO-B-314-81-D

Gesellschaft für Strahlen-
und Umweltforschung mbH.
GSF
Ingolstädter Landstr. 1
D-8042 Neuherberg

Head(s) of research team(s):

Prof. Dr. W. Kühn
Ökologische Physik
Inst. für Angewandte Physik
Herrenhäuser Strasse 2
D-3000 Hannover 21

General subject of the contract:

The influence of topography on the dispersion of nuclear pollutants, exchange processes of HTO vapor between soil and atmosphere and accumulation of I-129 in the human thyroid.

List of projects:

1. Investigations on the influence of topography and surface roughness on the dispersion of nuclear pollutants.
2. Study of exchange processes of HTO vapor in the soil-plant atmosphere system.
3. Investigations on the accumulations of iodine -129 in vegetation, milk, and human thyroid glands in the vicinity of nuclear facilities.

Title of project nr. 1
Investigations on the influence of topography and surface roughness on the dispersion of nuclear pollutants.
Head of project:
Dr. B. Georgi

The dispersion of aerosols in the lower atmosphere was investigated with help of a specially developed measuring equipment in the particle-size range between 0.06 and 10 µm. The equipment consists of a central measuring car for the registration of the meteorological data, for the preparation of the samples and for the control of the four independent aerosol measuring set-ups. The set-ups can be installed in inhabited as well as in uninhabited aeras at any distance from the source. The sources produce pyrotechnically generated model aerosols labeled with a tracer (dysprosium). The high reproducibilities of the generated aerosol distributions and the aerosol spectrometry (±2%) by means of low-pressure impactors were found by gravimetric and by neutron-activation-analytical evaluation. Therefore, it was possible to determine concentration inhomogeneities during dispersion in the lower atmosphere for all size classes. The measured ranges of variation were up to one order of magnitude.

The chemical composition and the connected shape alterations of the generated particles, shown already earlier, were analysed with help of an X-ray microprobe and by activation analysis. In the low-size range (nucleation or accumulation mode) the particles consist mainly of dysprosiumchloride $DyCl_3$, as the Dy-portion of the total mass is 54%. This portion increases to 75% in the large-size range (course mode), so that the main content is dysprosiumoxide Dy_2O_3. With the remaining chlorine portion follows a partitioning of 55% Dy_2O_3 and 45% $DyCl_3$ for those particles, where the latter form agglomerates of smaller particles (nucleation of accumulation mode). Variations of this distribution by meteorological influences in the presence of

other aerosol sources can be ascribed to the different chemical composition of the particles.

First measurements on the floating times of particles in the lower atmosphere yielded results contradicting to the existing model calculations. Even one hour after generation at a wind speed of 3m/sec and a source elevation of 3 m besides smaller particles also those $\geq$ 5 µm were detected. This shows that also the remobilization behaviour of aerosols can be studied with the pyrotechnically generated, polydisperse model aerosols and low-pressure impactors.

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

Georgi, B., Kühn, W.: Simulation of Aerosols from Industrial Processes by Pyrotechnically Generated Aerosols. J. Aerosol Sci. 13, (3), (1982)

Täschner, M., Georgi, B., Berner, A., Reischl, G.: Spectroscopy of Pyrotechnically Generated Aerosols. Proc. Aerosols in Science, Medicine and Technology, GAF, Bologna, 16.9. 1982

II. Short Communications, Theses, Internal Reports, Patents.

Georgi, B.: Coagulation of Aerosol Particles. EUROMECH Colloquium 161, Coalescence and Deposition of Aerosol Particles, Abstracts. University College London, London, 27. - 29.9. 1982

Georgi, B.: Vergleichsmessungen zwischen Impaktoren und Zentrifugen unter feldmäßigen Bedingungen. Staub- und Aerosolkolloquium, Institut für Experimentalphysik der Universität Wien, 20.1. 1982

Georgi, B.: Interaction among aerosol particles. First Annual Conference of the American Association for Aerosol Research, Santa Monica, 17.2. 1982

Georgi, B.: Elektronenmikroskopie in der Aerosolforschung. Elektronenmikroskopische Arbeitsgemeinschaft Tierärztliche Hochschule und Universität Hannover, 16.6. 1982

Georgi, B., Kreiner, H.-J.: Continous Aerosol Monitor for alpha emitting nuclides. Aerosols in Science, Medicine and Technology, GAF, Bologna, 16.9. 1982

Georgi, B.: Coagulation of Aerosol Particles. EUROMECH Colloquium 161, Coalescence and Deposition of Aerosol Particles, London, 27.-29.9. 1982

Täschner, M., Georgi, B., Berner, A., Reischl, G.: Spectroscopy of Pyrotechnically Generated Aerosols. 10. Konferenz der Gesellschaft für Aerosolforschung, Bologna, 16.9. 1982

Kreiner, H.-J., Georgi, B.: Monitor zur Messung von α-Aerosolen. Zusammenfassungen der Beiträge, 16. Jahrestagung des Fachverbandes für Strahlenschutz, München, 19.-22.10. 1982

Title of project nr. 2
Exchange processes of HTO-vapor in the atmosphere-soil-plant system.
Head of project:
Dr. C. Bunnenberg

In 1982, investigations on the exchange processes of HTO-vapor were concentrated on the laboratory simulation of the condensation of tritiated water vapor on the soil surface and the vapor diffusion into deeper layers. In a still running long-term experiment the model atmosphere above a semi-infinite soil column is supplied with HTO-labeled water vapor under controlled climatic conditions.
As expected according to the isotopic effect, preliminary results indicate a preferred condensation of HTO compared to H_2O on the soil surface, reducing the HTO concentration in the atmospheric humidity above the soil. This stresses the suggestion of an additional pathway of tritium released as tritiated vapor from nuclear facilities onto the soil surface. Theoretical considerations describing this new pathway as a kind of wet fallout or as a condensation contribution have shown that it may be as effective as washout processes. As due to isotopic effects the tritium contamination of soil and vegetation may considerably deviate from the estimations on the basis of the specific activity model. This pathway is of special interest after high short-term tritium emissions into the environment.
While the administered tritiated vapor diffuses into deeper layers of the soil column, further simultaneous measurements are taken of the changes of the moisture content and of the HTO-activities at different soil depths. The resulting HTO/H_2O-ratios yield informations on the extend of accumulations of HTO in certain soil layers due to isotopic effects.
The preliminary findings encouraged the modification of the

wind-tunnel set-up, in order to determine the influence of meteorological parameters on the HTO/H_2O condensation and evaporation ratios.
The possibilities to substitute HTO by the less harmful $H_2{}^{18}O$ for field measurements could not yet be tested due to measuring difficulties in the quantification of low concentrations.

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

Bunnenberg, C., Kühn, W., Ujeno, Y.: Vapor Exchange between Atmosphere and Soil with Respect to the Transfer of Tritium. Annales de l'Association Belge de Radioprotection 7 (3-4) (1982)

Glubrecht, H., Kühn, W., Handl, J., Bunnenberg, C.: Belastungspfade und ausgewählte Beispiele. Radioökologiesymposium Arbeitsgem. f. Umweltfragen e.V. und BMI, Universität Stuttgart, 15.-16. Oktober 1981 (ersch. 1982)

Kühn, W.: Ausbreitung radioaktiver Stoffe im Boden. Radioökologiesymposium Arbeitsgem. f. Umweltfragen e.V. und BMI, Universität Stuttgart, 15.-16. Oktober 1981 (ersch. 1982)

II. Short Communications, Theses, Internal Reports, Patents.

Bunnenberg, C., Kühn, W., Ujeno, Y.: Vapor Exchange between Atmosphere and Soil with Respect to the Transfer of Tritium. Seminar on International Assize of Radioecology of the Intern. Union of Radioecologists, June 28.-30., Wageningen, The Netherlands

Exhibition.

Bunnenberg, C., Feinhals, J.: Test der Wasserdurchlässigkeit von Verschlußmaterialien für Salzkavernen. "Neue Technologie" auf der Hannover-Messe 1982

Title of project nr. 3
Investigation on the accumulation of iodine-129 in vegetation, milk and human thyroid glands in the vicinity of nuclear facilities.

Head of project:
Dr. J. Handl

For the long-term tracing of iodine-129 in the soil and its uptake by plants a soil sample with an area of 2.5 m^2 and a depth of 1.5 m was established in the field. The surface of the sample was contaminated by 3.4 µCi of I-129 and grass seeds were sowed out. In the following 7 months 7 harvests were gathered in and the iodine concentrations were determined. There was a clear reduction of the I-129 concentration from 4.7 down to 0.2 pCi/g dry weight within 6 months.
In order to follow the iodine transfer and the pathway soil-grass-milk-man, the harvested grass was fed in equal quantities to a pasture animal within 9 days. During the application of the labeled grass and during the following 26 days milk samples were taken frequently to study the decrease of the I-129 concentration. Part of the daily milk yield was fed to a pig, which because of its similarity to human physiology represented the end of the investigated pathway. After slaughtering of the pig I-129 was extracted from its thyroid gland. As this series of experiments could be completed only shortly before the end of the year, the results concerning I-129 were not yet available at the time of report.
The inventory of iodine-129 in thyroid glands was continued with special consideration of human thyroids. In the investigations the separation method developed in the preceding year was applied, which provides an important reduction of the detection limit. It is 3 fCi for thyroid glands and other organic material (milk, vegetation) and about 1 fCi for inorganic substances (soil), so that very low concentrations can be determined now. A total of 14 human thyroid glands from Lower Saxony (Table 1) and 9 thyroid glands mainly from Dutch bovine (Table 2) could be investigated. The isotope-ratios of I-129/I-127 ranged between $<10^{-9}$ and 10^{-7}.

Table 1

Human Thyroid Glands

Date of taking	Origin	Fresh weight [g]	I-127 Content [mg/g f.w.]	I-129 Content [pCi]	I-129 Content [mg]	Isotope ratio I-129/I-127
May 1979	Lower Saxony (FRG)	29.0	0.36	$53.8 \cdot 10^{-3}$	$3.3 \cdot 10^{-7}$	$3.1 \cdot 10^{-8}$
May 1979	Lower Saxony (FRG)	12.5	0.22	$75.2 \cdot 10^{-3}$	$4.6 \cdot 10^{-7}$	$1.7 \cdot 10^{-7}$
June 1979	Lower Saxony (FRG)	24.6	0.46	$131.0 \cdot 10^{-3}$	$8.0 \cdot 10^{-7}$	$7.1 \cdot 10^{-8}$
June 1979	Lower Saxony (FRG)	15.2	0.24	$28.3 \cdot 10^{-3}$	$1.7 \cdot 10^{-7}$	$4.4 \cdot 10^{-8}$
July 1979	Lower Saxony (FRG)	9.6	0.46	$13.7 \cdot 10^{-3}$	$8.4 \cdot 10^{-8}$	$5.5 \cdot 10^{-9}$
October 1979	Lower Saxony (FRG)	14.7	0.60	$54.4 \cdot 10^{-3}$	$3.3 \cdot 10^{-7}$	$3.8 \cdot 10^{-8}$
October 1979	Lower Saxony (FRG)	13.3	0.42	$53.6 \cdot 10^{-3}$	$3.3 \cdot 10^{-7}$	$5.8 \cdot 10^{-8}$
November 1979	Lower Saxony (FRG)	15.9	0.52	$20.9 \cdot 10^{-3}$	$1.3 \cdot 10^{-7}$	$1.5 \cdot 10^{-8}$
November 1979	Lower Saxony (FRG)	18.8	0.63	$56.7 \cdot 10^{-3}$	$3.4 \cdot 10^{-7}$	$2.9 \cdot 10^{-8}$
June 1980	Lower Saxony (FRG)	26.3	0.34	$10.9 \cdot 10^{-3}$	$6.7 \cdot 10^{-8}$	$7.4 \cdot 10^{-9}$
March 1981	Lower Saxony (FRG)	26.5	0.38	$38.2 \cdot 10^{-3}$	$2.3 \cdot 10^{-7}$	$2.3 \cdot 10^{-8}$
May 1981	Lower Saxony (FRG)	26.0	0.31	$32.8 \cdot 10^{-3}$	$2.0 \cdot 10^{-7}$	$2.5 \cdot 10^{-8}$
July 1981	Lower Saxony (FRG)	41.3	0.18	$22.7 \cdot 10^{-3}$	$1.4 \cdot 10^{-7}$	$1.8 \cdot 10^{-8}$
September 1981	Lower Saxony (FRG)	46.4	0.07	$25.5 \cdot 10^{-3}$	$1.6 \cdot 10^{-7}$	$5.1 \cdot 10^{-8}$

Table 2

Bovine Thyroid Glands

Date of taking	Origin	Distance from nuclear facilities [km]	Fresh weight [g]	I-127 Content [mg/g f.w.]	I-129 Content [pCi]	I-129 Content [mg]	Isotope ratio I-129/I-127
March 1981	Mariensee (FRG)	> 200	18.4	0.59	$< 3.0 \cdot 10^{-3}$	$< 1.8 \cdot 10^{-8}$	$< 1.7 \cdot 10^{-9}$
March 1981	Mariensee (FRG)	> 200	13.3	0.73	$< 3.0 \cdot 10^{-3}$	$< 1.8 \cdot 10^{-8}$	$< 1.8 \cdot 10^{-9}$
September 1980	Kloetinge (NL)	12	19.0	0.34	$65.7 \cdot 10^{-3}$	$4.0 \cdot 10^{-7}$	$6.2 \cdot 10^{-8}$
September 1980	Ovezande (NL)	4	17.8	0.21	$24.8 \cdot 10^{-3}$	$1.5 \cdot 10^{-7}$	$4.1 \cdot 10^{-8}$
September 1980	Ovezande (NL)	4	18.0	0.53	$37.5 \cdot 10^{-3}$	$2.3 \cdot 10^{-7}$	$2.4 \cdot 10^{-8}$
May 1980	Hillegom (NL)	37	38.4	0.72	$236.5 \cdot 10^{-3}$	$1.45 \cdot 10^{-6}$	$5.2 \cdot 10^{-8}$
May 1980	Hillegom (NL)	37	48.7	0.25	$133.6 \cdot 10^{-3}$	$8.2 \cdot 10^{-7}$	$6.8 \cdot 10^{-8}$
July 1980	Mill (NL)	26	22.0	0.79	$191.7 \cdot 10^{-3}$	$1.17 \cdot 10^{-7}$	$6.8 \cdot 10^{-8}$
July 1980	Mill (NL)	26	24.5	1.70	$635.6 \cdot 10^{-3}$	$3.9 \cdot 10^{-6}$	$9.3 \cdot 10^{-8}$

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

Glubrecht, H., Kühn, W., Handl, J., Bunnenberg, C.: Belastungspfade und ausgewählte Beispiele. Radioökologiesymposium Arbeitsgem. f. Umweltfragen e.V. und BMI, Universität Stuttgart, 15.-16. Oktober 1981 (ersch. 1982)

Progress Report
1982

Contractor: Univ. Catholique de Louvain
Halles Universitaires
Place de l'Université 1
B-1348 Louvain-la-Neuve

Contract no.: BIO-B-328-81-B

Head(s) of research team(s):
Prof. H. Laudelout
Laboratoire de Pédologie
UCL
Place Croix du Sud 2
B-1348 Louvain-la-Neuve

General subject of the contract:
Hydrological and biogeochemical characteristics which condition the environmental behaviour of the mobile long-lived radionuclides. Application to the ultimate biological availability of Tc.

List of projects:
1. Hydrological and geochemical characteristics which condition the regional behaviour of the mobile long-lived radionuclides.
2. Study of the environmental behaviour of technecium (TcO_4^-).

Title of project nr 1 : HYDROLOGICAL AND GEOCHEMICAL CHARACTERISTICS WHICH CONDITION THE REGIONAL BEHAVIOUR OF THE MOBILE LONG-LIVED RADIONUCLIDES

Head of project and scientific staff : LAUDELOUT H., LAMBERT R., TANG VAN HAI, DE CUYPER X.

Numerous studies have been devoted to the interactions of technetium wi the soil-plant system. The primary aims being to calculate how ^{99}Tc wil be distributed in soil, migrate towards the water-table or be taken up by the plant.

One important aspect seems to have been neglected namely the extent of hydrodynamic dispersion which the pertechnetate ion may undergo on its way towards the water-table. The well known reactivity of pertechnetate with soils rich in organic matter and with an abundant biomass complica the problem the more so that the equilibrium between the ^{99}Tc compounds is not instantaneous.

Columns of 45 mm internal diameter and 20 cm length were packed with sa or soil and percolated with distilled water. From the wet and dry weigh of the columns, their volumetric water content was estimated. Water containing about 3 µC per liter of ammonium pertechnetate or $^{36}ClNa$ was percolated at a constant rate through the columns during a time corresponding to the displacement of 6 to 7 pore volumes from the column.

One experiment was made with a dry loamy sand through which the pertechn tate solution was eluted without any preliminary equilibration with wat

In all cases, the Darcy velocity of the solution entering the column wa 1.13 $mm.day^{-1}$.

After percolating the radioactive solution, distilled water or a 0.01 M solution of $CaCl_2$ was passed through the column. The activity of the percolates and ^{2} of the eluates was determined with a liquid scintillation spectrometer (Packard-Tri-Carb-Model 3380).

It was then calculated as relative concentration by referring it to the initial activity of the solution. The dimensionless set of experimental data could then be used for adjusting the four parameters present in th analytical solution using a programme described by M. Th. Van Genuchten

Using soil columns which had been moistened and had been brought at field capacity or higher did not allow the appearance of pertechnetate in the effluent even after passing about 10 pore volumes of solution through the column.

Since in columns of about 0.5 m length, using larger amounts of solutio at the flow rate chosen would result in a prohibitive amount of time, an attempt was made of passing the solution through a soil column initi ly dry with the result shown in fig. 1.

In that case, radioactivity appeared in the solution after passing one pore volume only and increased very rapidly and eluted from the column with the column with a pronounced tailing.

Using a sand medium which was not devoid of sorptive properties for pertechnetate gave the experimental results shown in fig. 2 on which a theoretical curve for the percolation and elution process has been supe

ɛters used were as follows (with their standard error) :
ıber : 67.5 ± 14.8, retention coefficient : 2.3 ± 0.02, mobile
ɜ : 0.87 ± 0.02, transfer coefficient ω : 0.51 ± 0.24.

:he theory allows a fairly good description of the movement of
:ate with parameters that are consistent with the material

ı with an ion such as chloride is illustrated in fig. 3.
ıs little adsorbed in sand and consequently half of the maximum
:he reduced concentration is reached after passing one pore
labelled solution.

ıificant that the retention factor for pertechnetate is about
sand which causes a pronounced displacement of the break-
ırve in comparison with that of chloride.

comparison of the behaviour of ^{99}Tc and ^{36}Cl has been made
ıd al. (1979).

ique used was quite different since initially dry soil samples
for observing the elution of a spot of $^{36}ClNa$. Nevertheless,
ısion was quite similar namely a definitely increased retention
ı terms of tailing and retardation factor.

ıilibrium between the solution and the solid surface past
pertechnetate ion are percolating is reached rapidly with
ɔ the residence time of the solute in the column, the composi-
ıe effluent may be calculated by making use of an appropriate
factor.

ɔeen done in fig. 4, where the relative concentration of
tate has been plotted for various values of the retention

ious that for RF values exceeding 15, no radioactivity will
ed in the effluent after passing 10 pore volumes through the
first seven of them being radioactive.

son between the retention factor as it is defined by

$\frac{\rho\ k}{\theta}$ and what has been called the transfer factor defined

$\frac{\text{activity in organism or sediment (wet weight)}}{\text{activity in water}}$ is fairly

both definitions. We must have : $\frac{(R - 1)\ .\ \theta}{\rho} = k.$

s dimensionless and k is not, taking the plausible values of
and 0.5 for θ, it is seen that k is about one third of the
This allows a comparison of the values found for the adsorption
which have been reported as either F or k.

rthcoming report on the factors determining the absorption of
oil rich in clay colloids at various levels of microflora
we have found values ranging from 0.1 to 0.7 g^{-1} ml for k

at 13°C. This is well within the range of values quoted by Wildung and al. (1979) who found coefficients ranging from 0.007 to 2.8 g^{-1} ml.

The retention factor should then range from about 1 to 10. These values are too low for accounting for the concentration of Tc in the upper cm of a soil column. As calculated above a retention factor of 10 is necessary for preventing pertechnetate of appearing in the effluent after passing 10 pore volumes through the soil column.

As shown in fig. 1, a break-through is observed if the soil is percolated with the pertechnetate solution while initially dry.

Gast and al. (1979) have found similar results of an asymetric pattern of elution together with the presence of measurable amounts of Tc in the first ml aliquot of the leaching solution. Their techniques of observation was quite different from the one used here but suffers equally from the fact that parameters obtained from it can hardly be applied to the description of Tc movement through moist soils with an active microflora. This is to be expected since the maximum elution time did not exceed twenty-five hours.

Similar results were observed by Saas et al. (1979) who stressed the fact that when very small amounts of technetium are deposited on top of the soil, its elution is practically nil and it remains at the surface.

A quantitative description of this process is feasible if information is available firstly about the parameters determining movement of water in an unsaturated soil and secondly the change of k, the distribution factors with time.

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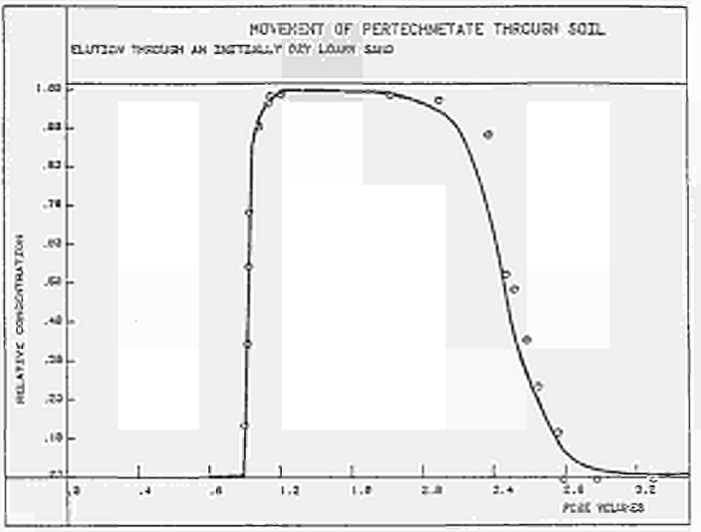
MOVEMENT OF PERTECHNETATE THROUGH SOIL
ELUTION THROUGH AN INITIALLY DRY LOAMY SAND
RELATIVE CONCENTRATION
PORE VOLUMES

Fig. 1

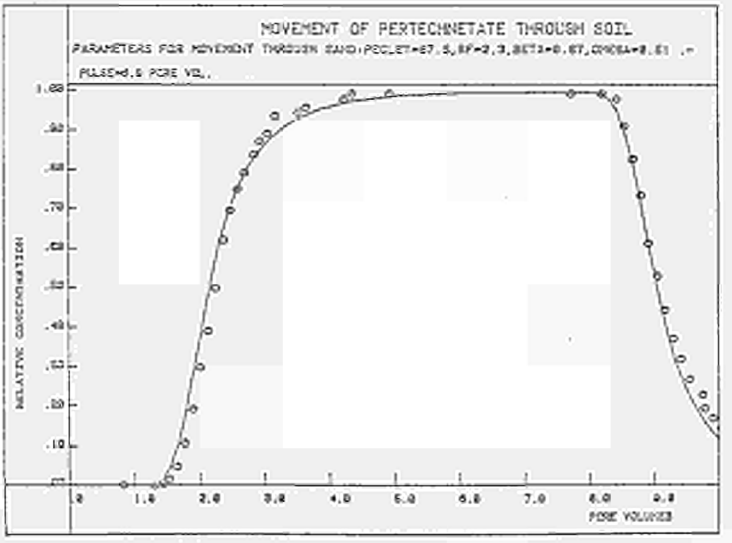
MOVEMENT OF PERTECHNETATE THROUGH SOIL
PARAMETERS FOR MOVEMENT THROUGH SAND:PECLET=67.5,RF=2.3,BETA=0.67,OMEGA=0.61 ,=
PULSE=4.5 PORE VOL.
RELATIVE CONCENTRATION
PORE VOLUMES

Fig. 2

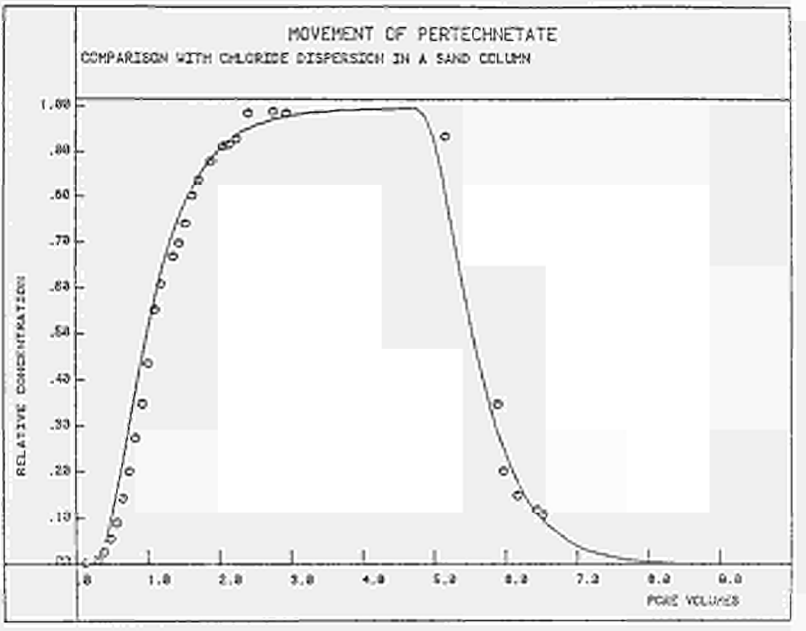
MOVEMENT OF PERTECHNETATE
COMPARISON WITH CHLORIDE DISPERSION IN A SAND COLUMN
RELATIVE CONCENTRATION
1.00
.80
.60
.50
.40
.30
.20
.10
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
PORE VOLUMES

Fig. 3

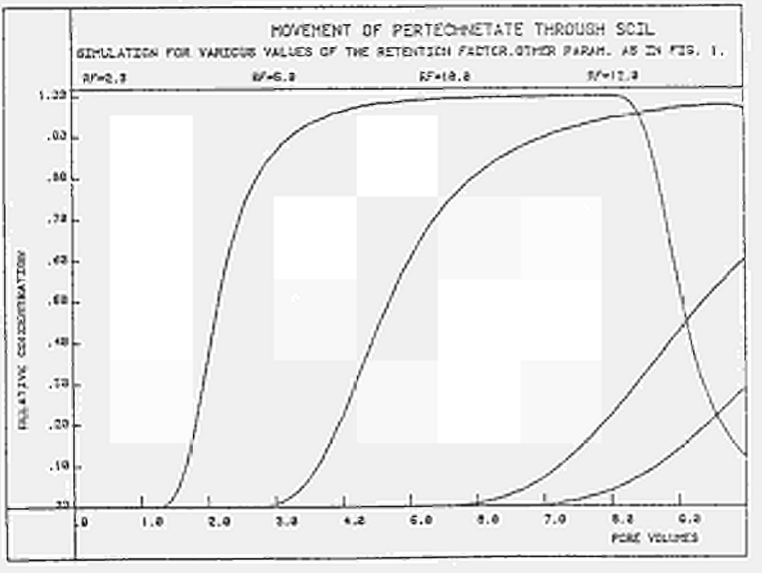
MOVEMENT OF PERTECHNETATE THROUGH SOIL
SIMULATION FOR VARIOUS VALUES OF THE RETENTION FACTOR.OTHER PARAM. AS IN FIG. 1.
RF=2.0
RF=6.0
RF=10.0
RF=12.0
RELATIVE CONCENTRATION
1.00
.80
.60
.40
.20
.10
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
PORE VOLUMES

Fig. 4

Title of project nr 2 : STUDY OF THE ENVIRONMENTAL BEHAVIOUR OF TECHNETIUM (TcO_4^-)

Head of project and scientific staff : MYTTENAERE C., MOUSNY J.M., RONNEAU C., TANG VAN HAI, VAN DE CASTEELE C., DE CUYPER X.

2.1. Estimation of the analog element approach

2.1.1. Study of the interaction Re-Tc

A preliminary experiment on the behaviour of Re (ReO_4^-) was performed using Phaseolus vulgaris. No toxicity symptoms were observed in the concentration range 10^{-7}M - 10^{-6}M Re. In a second experiment no interaction was observed between Tc (from 10^{-7}M to 10^{-6}M) and Re (5 10^{-7}M); such a result confirms the absence of analogy between both elements.

2.1.2. Study of the interaction Mo-Tc

Tc-99 absorption by P. vulgaris was studied in solutions containing different concentrations of Mo (MoO_4^{2-}); 10^{-7}M - 10^{-3}M). Mechanisms responsible of the inhibition are now under examination (Linewaever-Burk graphs) for non toxic concentrations of Mo (10^{-7}M - 10^{-5}M).

2.2. Estimation of the influence of time (long term behaviour of Tc)

Tc-95 m is now produced in Louvain-la-Neuve (Cyclotron - in collaboration with the Nuclear Chemistry Department). The behaviour of Tc present in very low concentrations in the medium (from 10^{-3}M to 10^{-6}M) has been studied thanks to the labelling of Tc-99. (P. vulgaris is water culture) with Tc-95m. In addition, a double-tracing experiment has confirmed that no Tc-99 was lost during the preparation of the samples (humid digestion) and that in our experimental conditions Tc-95m may be considered as a good tracer of the TcO_4^- (Tc-99). Long term experiments in soils will be considered in 83.

2.3. Study of the transfer of Tc in permanent flooded soils

Two models of flooded rice fields with continuous water flow were built in the greenhouse (five containers placed side by side at decreasing height so that the overflow of the first one runs into the next one, the first functionning as a reservoir, the last collecting the residual water.

In one of the model, the soil was uniformly traced (1,7 µCi/kg) meanwhile in the second, the irrigation water was contaminated (0,3 µCi/l). The Table 1 gives the concentration factors calculated between soil, irrigation water and plants.

2.4. Tc toxicity

2.4.1. Effects of Tc on the nutrient solution of higher plants

Dry matter and iron content of soybean plants are negatively correlated with the various levels of Tc (0 to 10^{-6}M) investigated. On the contrary, the iron transport coefficient was positively affected when the Tc is prensent in the medium. (Table 2). Moreover, the phytoferritin concentration decreases as the Tc-99 level increases.

2.4.2. Tc toxicity on N_2 fixation

The results formerly obtained (Report 81) have been confirmed using more accurate techniques (Interaction Tc-Mo-Mo/Fe nitrogenase - reduction of the N_2 fixation).

Table 1

Transfer factors (CPM G dry matter/CPM G dry soil or CPM ML irrigation water) obtained in models of rice field

Container / Plant organ	Plant - Water			Plant - Soil		
	B	C	D	B	C	D
Shoots basis	23,8	16,3	34,7	0,2	0,1	0,1
Leafy shoots	80,9	68,5	69,8	4,9	6,2	6,7
Empty panicles	11,2	3,1	3,2	0,04	0,02	0,02
Sterile grains	4,4	3,9	2,7	0,02	0,01	0,01
Hull	3,9	4,5	3,2	0,01	0,01	0,01
Caryopsis	0,2	0,3	0,2	0,004	0,003	0,004

Table 2

Coefficient of transport of Iron (leaf Iron/total Iron) and Tc-99

Element Tc	Fe 0.1 ppm Fe	Fe 1.0 ppm Fe	Tc 0.1 ppm Fe
0.0 M	0.161	0.178	0.0
10^{-8} M	0.170**	0.260**	0.72
10^{-7} M	0.181**	0.210	0.71
10^{-6} M	0.071	0.204	0.77

** Highly significative difference

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Progress Report
1982

Contractor:

Centre d'Etude de l'Energie
Nucléaire, CEN/SCK
Avenue Plasky 144
B-1040 Bruxelles

Contract no.: BIO-B-330-81-B

Head(s) of research team(s):

Dr. J. Maisin
Département de Radio-
biologie, CEN/SCK
Boeretang 200
B-2400 Mol

General subject of the contract:

Study of the impact of waste from PWR nuclear power stations on the fresh water ecosystem.

List of projects:

1. Study of the impact of waste from PWR nuclear power stations on the fresh water ecosystem.

Title of project no. 1 : STUDY OF THE IMPACT OF WASTE FROM PWR NUCLEAR POWER STATIONS ON THE FRESH WATER ECOSYSTEM

Head of project and scientific staff : R. KIRCHMANN

The main results gained during 1982 are presented below according to the various contributions received from the participating laboratories to this study.

1. Physico-chemistry of the river water : twenty four series of sampling were performed upstream and downstream of the NPP ; 10 parameters were analysed.
   The comparative behaviour of ^{60}Co and ^{54}Mn on one hand and of various heavy metals (Cd, Cs, Zn, Pb) on the other hand, regarding the suspended matter, is under investigation.
2. Primary producers : twenty four series of sampling of phytoplancton and zooplancton were performed at the same locations as under 1. The biomass was assessed (measurement of chlorophyll a) and the photosynthetic activity of phytoplancton was measured (O_2 production) as well as the respiration. These data will be used to make an oxygen balance during ten series of sampling performed from July to December and to compare the situation up- and downstream of NPP.
   The annual inventory of bryophitic populations in the sector Ben-Ahin to Argenteau has been achieved in September.
3. Primary and secondary consumers :
   Studies on the impact of thermal releases on macroinvertebrates and fishes were pursued in 1982 : the colonisation of artificial substratum by the macrofaunes (about one month of exposure) has been followed during seven different periods of time, furthermore the growth and fecondity of Asellus aquaticus L. were studied.
   The study of the fecondity of Rutilus rutilus L. and Alburnus alburnus L. caught up- and downstream of the NPS (14 fisheries campaigns) is in progress. The growth of both species and of Leuciscus cephalus L.

is also investigated.

Measurements of γ-emitters in various organs of the fishes caught were performed.

4. Laboratory experiments :

a. Kinetic studies of absorption and desorption have shown that the algae (Scenedesmus obliquus) present two compartments having different affinities for the radionuclides under study : Cs and Tc.
It was necessary to improve the equipment ("turbidostat") used for the continuous culture of algae ; three different culture mediums are compared.

b. Research on the mechanisms of organification of ^{3}H in the primary cooling water of a PWR was continued in 1982. Effect of irradiation (gamma rays from ^{60}Co) on resins used for the purification of the primary circuit containing tritiated water was investigated : algae grown on this medium do not indicate the presence of ^{3}H organic compounds biologically available.
Two samples of primary coolant having circulated through resins, irradiated and non-irradiated, were concentrated and exposed in the core of BR_2 (1.5×10^{13} $n.cm^{-2}.s^{-1}$) for 33 hours. The quartz vials exploded due to excessive radiolysis. Other samples were exposed in BR_1 (2.9×10^{11} $n.cm^{2}.s^{-1}$) and an integrated dose of 8.10^{16} n was applied : some vials were broken and the intacts were used as medium culture for algae (Scenedesmus obliquus), the work is still in progress.
Investigations on microorganisms present in the resins have been initiated.

I. Publications

De Clercq-Versele, H., Kirchmann R. (ed.) (1982). L'impact des rejets de la centrale nucléaire de Tihange (Belgique) sur l'écosystème Meuse : cinq années d'études in situ et d'approche expérimentale (1976-1980), BLG 555, 54 pp.

Descy J.P., Empain A. (1981). Inventaire de la qualité des eaux courantes en Wallonie (Bassin Wallon de la Meuse). Rapport de synthèse, Univ. de Liège, Vol. I (87 pp), Vol. II (194 pp), Vol. III (37 pp).

Descy J.P., Empain A., Lambinon J. (1981). La qualité des eaux courantes en Wallonie. Bassin de la Meuse. Secrétariat d'Etat à l'Environnement, à l'Aménagement du territoire et à l'eau pour la Wallonie, Bruxelles, 18 pp.

Descy J.P., Empain A. (in press). Hydrobiology of the Belgian basin of the Meuse. in : Ecology of European Rivers, ed. B.A. Whitton, Blackwell Publ., Oxford.

Kirchmann R., Dupont J.C. (1981). Rôle du tritium dans les rejets d'effluents radioactifs liquides provenant d'installations nucléaires. Bull. Rech. Agron. Gembloux, 16 (1), 111-136.

Mattheeuws A. et al. (1981). Etude de la reproduction du gardon Rutilus rutilus et des effets d'une élévation provoquée de la température en Meuse sur cette reproduction. Hydrologia, 85, 271-282.

II. Various reports

Etude de l'impact des rejets provenant des centrales nucléaires de type PWR sur la biocénose dulcicole. Volume VI (1981), rapport d'avancement.

Descy J.P., Lambinon J. (1982) L'écosystème Meuse. IAEA Coordinated Research Programme, 1st meeting : "Role of Sediments in the Accumulation and Transport of Radionuclides in Waterways", Mol, Belgium.

Mouvet C., Descy J.P. (1982). Le plancton et les matières en suspension de la Meuse : leur rôle dans la fixation des métaux lourds et des radionucléides. IAEA Coordinated Research Programme, 1st meeting : "Role of Sediments in the Accumulation and Transport of Radionuclides in Waterways", Mol, Belgium.

Progress Report
1982

Contractor:

University of Dublin
Trinity College
IRL-Dublin 2

Contract no.: BIO-B-338-81-EIR

Head(s) of research team(s):

Dr. I.R. McAulay
Dept. of Pure and Applied Physics
Trinity College
IRL-Dublin 2

General subject of the contract:

Measurement of levels of radioactivity in the marine life and waters of the Irish Sea and their contribution to radiation dosage of the population.

List of projects:

1. Measurement of levels of radioactivity in the marine life and waters of the Irish Sea and their contribution to radiation dosage of the population.

Title of project	Measurement of levels of radioactivity in the marine life and waters of the Irish Sea and their contribution to radiation dosage of the population.
Head of project and Technical Staff	Dr. I.R. McAulay Mr. T. Lane

The programme of work during 1982 included measurement of gamma emitting activities in fish and seaweed samples, determination of total beta activities in fish samples, examination of sediments from the Irish Sea coast for caesium deposition and an assessment of the population dose resulting from fish consumption.

Fish samples are now being obtained and measured on a more regular basis for a number of different species from different ports of landing. Measurements indicate differing activites between species and port of landing and these factors can now be taken into account in estimating the population dose. It is not always possible to establish the location of the fish catch, though this is not considered of importance so long as the samples measured are representative of the fish consumed.

Measurement of total beta activities of fish samples was commenced during the year and is now working on a routine basis and dealing with the backlog of samples which have been preserved.

Measurement of seaweed samples is continuing with routine sampling at a number of sampling points. The results indicate a gradient in Cs-137 and Cs-134 decreasing from North to South. Some indications have been found which suggest a variation in the concentration factors between different parts of the plant and this is under investigation.

A number of sediment cores have been taken at points on the Irish coast where high sedimentation rates exist. These show a variation in caesium condentration with depth that accords with the published levels of discharge from the Windscale nuclear fuel reprocessing plant. More work will be carried out in this area to try to overcome difficulties associated with possible reworking of the sediments

following the initial deposition.

The population dose to the Irish population resulting from fish consumpiton is now assessed at approximately 2 man sievert per year as a consequence of previous discharges from the United Kingdom nuclear industry. As much of the Irish fish catch is exported directly or in processed form, there is a re-exportation of population dose of approximately 8 man sievert per year.

A research paper on some of the work has been prepared and will be submitted for publication shortly.

Progress Report
1982

Contractor:

Ministry of Agriculture,
Fisheries and Food
Fisheries Laboratory
Pakefield Road
Lowestoft
GB-Suffolk NR33 OHT

Contract no.: BIO-B-331-81-UK

Head(s) of research team(s):

Dr. N.T. Mitchell
Fisheries Radiobiol. Lab.
Pakefield Road
Lowestoft
GB-Suffolk NR33 OHT

General subject of the contract:

Behaviour of radionuclides in the marine environment in support of the disposal of wastes arising from the utilization of nuclear energy.

List of projects:

1. Behaviour of transuranic and long-lived fission product nuclides in coastal waters.
2. Deep sea radioecology.

Title of Project No 1. Behaviour of transuranic and long-lived fission product nuclides in coastal waters.

Head of project and scientific staff: Dr R J Pentreath, Mr B R Harvey, Dr D S Swift, Mr M B Lovett, Mr R Ibbett.

Studies on the behaviour of transuranium and long-lived fission product nuclides in UK coastal waters were continued during 1982 and one extended research vessel cruise was made specifically for this purpose; many of the samples taken on the cruise are still being analysed. Sea water samples were collected in the Irish Sea, off the North Scottish coast, and off the Channel Islands, for comparative purposes. All of the evidence obtained on radionuclide speciation so far suggests that, away from the immediate discharge areas, ^{237}Np is present primarily as Np(V) and ^{99}Tc as the pertechretate. There is evidence that ^{241}Am, however, may be present in more than one chemical form becuse only $\simeq$ 50% of Am in filtered sea water could be removed by co-precipitation with $Fe(OH)_3$, the remainder requiring reduction with Na_2SO_3. Speciation studies have been made using ^{243}Am and ^{243}Cm as yield tracers in known chemical forms.

A variety of box cores and gravity cores were obtained at 41 stations within the Irish Sea during 1982, some of which (those >2m in length) are being used to interpret the long-term processes of sediment accumulation and erosion in this area. The clay mineralogy of surface sediment samples is also being studies using XRD techniques, and the presence of small 'hot' particles is being investigated using CR-39 as an alpha-track detector.

The incorporation of radionuclides into settled sediments is affected by, amongst other things, bioturbating processes. Bioturbation to depths of up to 10cm can be brought about, in coastal waters, by a large variety of infauna but evidence has been obtained that one particular organism in the Irish Sea can affect the distribution

of Pu amd Am down to depths in excess of 35cm. The organism is an echiuroid (Maxmulleria lankasteri) which, by constructing extensive burrows into which faecal material is deposited, substantially alters the Pu isotope ratio profiles in certain areas. The long-term consequences of such bioturbatory effects are being incorporated into a mathematical model to describe the role of sediment/nuclide interactions in shallow sea areas.

The accumulation of these long-lived nuclides by marine organisms has also been studied further, with samples being collected from various parts of the UK. A variety of zooplankton samples has also been taken; there consisted largely of calanoid copepods and ctenophora. Analyses of algae and crustaceans from the Sellafield area has also continued and it appears that the distribution of ^{99}Tc in crabs and lobsters sampled so far indicate highest concentrations in the green gland, gonad and muscle tissues. These results differ somewhat from those obtained in laboratory experiments using ^{95m}Tc in which, apart from the green gland, the hepatopancreas (digestive gland) consistently attained the highest concentrations when the isotope was introduced in either the food or in the water. Laboratory experiments have also been made to study the absorption of transuranium nuclides by crustaceans and fish across the gut. The results indicate that ^{241}Am is slightly better absorbed than either ^{237}Pu or ^{235}Np by both crabs and lobsters. Fish also absorbed ^{241}Am better from food than ^{237}Pu, and thornback rays absorbed more per unit than plaice. These studies are continuing.

Title of Project No 2. The behaviour of radionuclides in the deep sea.

Head of project and scientific staff: Dr R J Pentreath, Dr P Kershaw, Dr D S Woodhead, Mr C W Baker, Mr B R Harvey.

A number of deep sea water, sediment and biological materials have been obtained during 1982 from the North Atlantic. Water samples are being analysed for ^{226}Ra, ^{228}Ra and ^{14}C and a multi-depth grid study made of excess ^{222}Rn indicated that it was not found above avout 50m off the sea bed.

Modifioations to a Reineck box oorer trigger system has allowed high quality undisturbed cores to be obtained at depths in excess of 4000m. Sections from these cores were x-rayed on board ship and provided clear evidence of extensive biological activity and sediment mixing. The sedimentation rates and depth and rate of bioturbation have been modelled using ^{14}C and ^{210}Pb data from 2 cores. Bioturbation mixing rates were found to be similar to those calculated for the Western Atlantic and Pacific, in spite of the differing water depths and sediments composition.

Laboratory experiments have also been made to study the sorption of Pu (IV) by deep sea sediments. It has been shown that Pu absorbs and desorbs more readily to sediments with a high carbonat content. The relationship of the Pu K_d with particle size has also been determined for some deep sea sediments.

Further analyses of deep sea fish for ^{210}Po have also been made and some of the preliminary findings published (1). This paper also includes the development of ideas for modelling the possible return of radionuclides to man along critical pathways by using a transfer coefficient approach, the necessity of further data for collective dose commitment calculations, and of modelling the role of biological processes which serve to retard the return of radionuclides from the deep sea to man. The analyses of deep sea biological material is continuing.

(1) PENTREATH, R.J. (1983). Biological Studies. In interim oceanographic description of the NEA Dumpsite for the disposal of low-level radioactive waste. Ed. by P.A. GURBUTT and R.R. DICKSON, NEA/OECD, Paris.

Progress Report
1982

Contractor:

United Kingdom Atomic Energy
Authority, UKAEA
Charles II Street 11
GB-London SW1Y 4QP

Contract no.: BIO-B-334-81-UK

Head(s) of research team(s):

Dr. A. Morgan
Env.& Med.Sciences Div.
AERE
Harwell, Didcot
GB-Oxon OX11 ORA

General subject of the contract:

The remobilisation of actinides from contaminated intertidal sediments.

List of projects:

1. Remobilisation of actinides from contaminated intertidal sediments.

Title of project nr 1: The remobilisation of actinides from contaminated intertidal sediments

Head of project and scientific staff: A Morgan, J D Eakins, A E Lally D G Humphreys, P J Burton

Laboratory Studies

A study of the distribution coefficients (Kds) for $^{239+240}Pu$ and ^{241}Am between Ravenglass sediment and water has been undertaken. Equilibrium is established within 15 min between contaminated sediment stirred with inactive North Sea water, the Kds for $^{239+240}Pu$ and ^{241}Am being 1.2×10^6 and 2.0×10^6 respectively. In an experiment in which a sample of sediment was leached successively with 5 fresh samples of seawater, the Kd values for $^{239+240}Pu$ were constant but those for ^{241}Am increased by a factor of 2. Dialysis of actinides in the aqueous phase showed that they were in true solution.

When actinides leached from contaminated sediment were equilibrated with inactive North Sea sediment, they were resorbed by the inactive sediment, the Kd values for both $^{239+240}Pu$ and ^{241}Am being 2.2×10^4. Further measurements with filtered contaminated Irish Sea water and North Sea sediment gave Kd values of 7.8×10^3 and 2.1×10^4 for $^{239+240}Pu$ and ^{241}Am respectively. Speciation measurements during this experiment showed that >95% of the Pu in the filtered seawater was Pu V or VI, but when adsorbed by sediment it was reduced to Pu III or IV.

The distribution of actinides between Ravenglass sediment and North Sea water was investigated while the suspension was being sparged with different gases. Kd values for $^{239+240}Pu$ were increased by a factor of 1.7 with nitrogen sparging, reduced by 2 with air sparging, by 5 with carbon dioxide and by 10 with oxygen. Distribution coefficients for ^{241}Am were not affected by any gas except carbon dioxide, which reduced the Kd by a factor of 40. The results suggest an oxidative process for the leaching of $^{239+240}Pu$ which does not apply to ^{241}Am. Both nuclides pass more readily into solution in the presence of CO_2, suggesting the formation of carbonate complexes, although the associated pH change from 7.8 to 5.5 during the experiment may have been a contributing factor.

In an experiment in which Ravenglass sediment was dried and exposed to air for 3 days prior to equilibration, the Kds for $^{239+240}Pu$ and ^{241}Am were reduced by factors of 2 and 1.5 respectively.

Distribution measurements have also been made at different salinity and pH values but the analytical work is not yet complete. A full evaluation of the results will be made when all the data are available.

Field Work

An experiment has been carried out to compare the amount of plutonium and americium entering the Ravenglass estuary with that leaving it during a tidal cycle. The experiment was carried out over a spring tide during the 5 September 1982. Measurements were made of water flow, width, depth, salinity, pH and actinide content on the seaward side of the estuary from a boat anchored in the mouth. In Fig.1 the $^{239+240}Pu$ passing the sampling point is plotted against time during the flowing and ebbing tide. The difference between the plutonium entering and leaving the estuary in solution was probably within the experimental limits but there was significantly more plutonium leaving the estuary in suspension than entering on this occasion. The results for ^{241}Am are still being evaluated but appear to follow a similar trend.

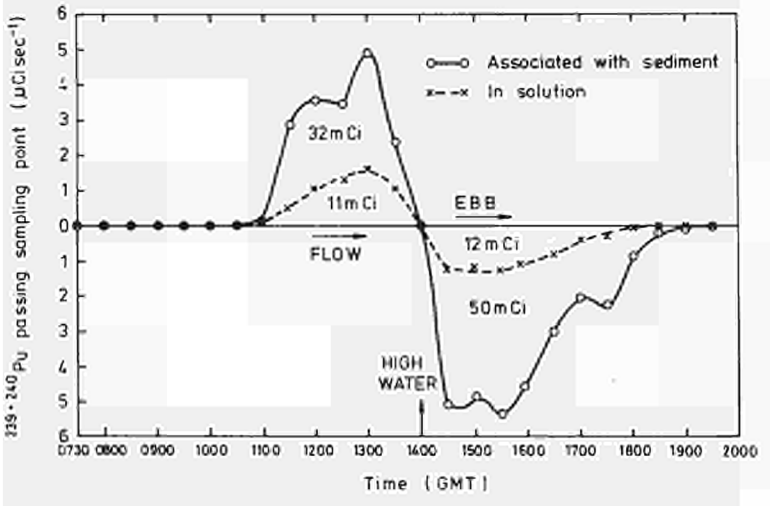

FIG.1. $^{239+240}Pu$ ENTERING AND LEAVING THE RAVENGLASS ESTUARY DURING A TIDAL CYCLE.

This is a slightly unexpected result as, overall, the estuary is an area of net sediment accumulation. However, annual discharges of plutonium from BNFL, Sellafield have decreased by a factor of 3 since 1978 and it is possible that the equilibrium between the estuary and the sea may have shifted from it being a net accumulator of radioactivity to being a net source. Two possible mechanisms whereby this could occur whilst the estuary remained a net accumulator of sediment are as follows:

(1) Plutonium passing into solution from sediment deposits is being resorbed by suspended sediment and being transported on the ebb tide.

(2) Sediment containing relatively low concentrations of plutonium is being transported into the estuary on the flood tide whilst bottom sediment, rich in plutonium, is being suspended by current and wave action and transported on the ebb.

In either case the specific activity of sediment leaving the estuary should be greater than that entering, but unfortunately this parameter was not measured. The second mechanism could well apply on this occasion as, because of the spring tide, current velocities were high and, although there was little wind on the flood, it increased later and there was considerable wave action on the ebb. However the laboratory studies show that desorbed plutonium is readily resorbed and the first mechanism is also possible. Further studies are required during neap as well as spring tides, including specific activity measurements of the suspended sediment, to determine whether the results are typical of the situation in the estuary.

Progress Report
1982

Contractor:

The Royal Veterinary and
Agricultural University
Bülowsvej 13
DK-1870 Copenhagen V

Contract no.: BIO-B-486-82-DK

Head(s) of research team(s):

Dr. N.E. Nielsen
The Royal Veterinary and
Agricultural University
Thorvaldsensvej 40, Entr. 8
DK-1871 Copenhagen V

General subject of the contract:

Soil-plant transfer of iodine with special reference to iodine-129 in the environment.

List of projects:

1. Test of a soil-plant transfer function of iodine in field experiments.

Title of project no. 1: Test of a soil-plant transfer function of iodine in field experiments.

Project leader and scientific staff: Henning Kaufholz

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1. Introduction

Following escape of radioisotopes of iodine to the environment from the nuclear industry these radioisotopes may accumulate in the food-chain and may ultimately cause radiation damage to the human thyroid. The long-lived I-129 will not be eliminated by radioactive decay before uptake by plants from soil may take place. There is therefore a demand for a model, which can predict the quantity of I-129 uptake by plants from soil. Uptake of I by plants could be described by a transport kinetic model of nutrient uptake by plants[1]. The aim of the present work is to test if an extended form of this transport kinetic model is reliable in predicting transfer of I from soil to plants under field conditions. The quantity of I transferred from soil to plants per unit area of field in the time interval Δt will depend on: a) the kinetic parameters for mean net inflow of I^- into roots at the considered pH in the soil solution, b) the mean root length per unit area of field, c) the concentration of iodide in the soil solution at the root surface, and d) the pH of the soil solution.

2. Experimental

In the field trial division of The Royal Veterinary and Agricultural University, 17 km west of Copenhagen, a field experiment (2 by 3 factorial with 3 replications) was laid out with the treatments: no lime and 10 ton lime ha^{-1} in combination with 0, 12.5 and 25.0 kg I ha^{-1} in KI.

P- and K-fertilizer, lime, and I were applied in September 1981. N-fertilizer was applied four times during 1982. The figure in parenthesis following the date denotes kg N ha^{-1} applied in calcium nitrate: 26/4 (100), 21/7(100), 10/8(75), 13/9(50).

Perennial ryegrass was sown in October 1981 but due to unfavourable weather conditions the germination was poor. The ryegrass was therefore resown at the date 21/4-1982. The field was not irrigated.

Four main cuts were taken in 1982. Except for the first main cut sub-cuts were taken within the growth period of the main cuts in order to determine changes in growth rate (Figure 1) and changes in the rate of iodine uptake within the growth period of the main cuts. Except for the first cut the area cut per sample was 2 m^2. Soil cores for root length determinations were taken out to 1 m of depth at the dates 26/7 and 1/9. At the former date more than 80% of the total root length to 1 m of depth was present in the surface layer of the soil profile to 10 cm of depth (Table 1). Soil samples for chemical analyses were taken out to 1 m of depth at the dates 29/5 and 30/8. The I^- concentration in suspensions of u dried soil in 0.01 M $CaSO_4$, an approximation to the I concentration in soil solution, was determined in soil from the former da-

te of sampling (Table 2). Increases in iodide concentration in soil suspensions due to KI application were notable only in the surface soil layers to 10 cm and to 20 cm of depth at the intermediate and highest rates of KI application respectively.

The field experiment is under execution and the validity of the soil-plant transfer function of iodine cannot be tested with the limited number of data presently available.

3. References

1. Kaufholz, Henning and Nielsen, Niels Erik, 1981: Iodine uptake by plant roots with special reference to I-129 in the environment. Report EUR 7169 DE/EN/FR, 319-326 (ISBN 3-7186-0062-5).

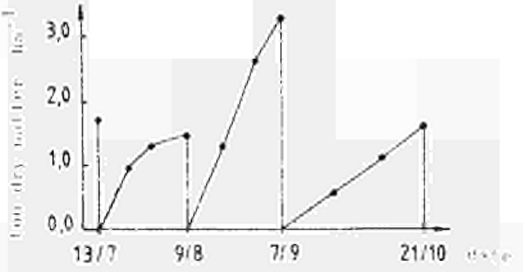

Figure 1. Yield of top dry matter in four successive cuts of perennial ryegrass in the field experiment in 1982. Results from the treatment: no lime and 25 kg iodine ha^{-1} in KI.

Table 1. Mean root length of perennial ryegrass per unit area of field ($km\ m^{-2}$) in various depth intervals at the date 26/7-1982 in plots without and with lime application respectively.

Depth interval cm	0 ton $CaCO_3$ ha^{-1}		10 ton $CaCO_3$ ha^{-1}	
0 - 10	27	(9)*	23	(9)
10 - 20	3.3	(8)	2.8	(9)
20 - 40	0.9	(9)	1.6	(6)
40 - 60	0.3	(4)	0.6	(3)
60 - 80	0.2	(1)	0.1	(1)
80 - 100	-	(0)	-	(0)

* The figure in parenthesis indicates number of root samples.

Table 2. Iodide concentration (μM) in suspensions of undried soil in 0.01 M $CaSO_4$. Soil samples were taken from the field 29/5-1982 to 5/6-1982 (preliminary results) *

Depth-interval cm	ton $CaCO_3$ ha^{-1}					
	0			10		
	kg I ha^{-1}					
	0	12.5	25	0	12.5	25
	μM I^-					
0 - 10	0.038	0.300	0.902	0.047	0.242	0.542
10 - 20	0.021	0.027	0.107	0.022	0.038	0.170
20 - 40	0.021	0.018	0.046	0.019	0.031	0.061
40 - 60	0.038	ND	0.051	0.024	0.031	0.094
60 - 80	0.042	ND	0.055	0.045	0.048	0.071
80 - 100	0.100	0.114	0.121	0.051	0.056	0.079

* The values are means of 1 to 3 determinations; ND denotes not yet determined.

List of publications in 1982

II. Abstracts.

Kaufholz, H. and Nielsen, N.E. 1982: The kinetics of iodide uptake by intact plants (Hordeum vulgare (L.) cv. Nordal and Lolium perenne (L.) cv. Vigor) in: SPPS XIIIth Nordic Congres for Plant Physiology, Aarhus 1.-6. August 1982, Abstracts p. 49.

Progress Report
1982

Contractor:

United Kingdom Atomic Energy
Authority, UKAEA
Charles II Street 11
GB-London SW1Y 4QP

Contract no.: BIO-B-332-81-UK

Head(s) of research team(s):

Dr. D.H. Peirson
Env.& Med.Sciences Div.
AERE
Harwell,Didcot
GB-Oxon OX11 ORA

General subject of the contract:

Measurement of particulate airborne radioactive materials.

List of projects:

1. Measurement of particulate airborne radioactive materials.

Title of the project nr. 1

THE MEASUREMENT OF PARTICULATE AIRBORNE RADIOACTIVE MATERIALS

Head of Project and scientific staff : M Marshall
D C Stevens
J A B Gibson

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1. Introduction

There is now a greater awareness of the need to minimise the committed dose equivalent from inhaled aerosols and to determine aerosol concentration reliably. This requires a greater knowledge of the variations in concentration, particularly between the sampler and the breathing zone, the efficiency of sampling inlets and the losses in sampling systems. Variations in concentration depend upon the sources of release, which include resuspension, ventilation patterns and the aerodynamic properties of the aerosol.

2. Techniques

A vibrating orifice generator has been commissioned to complement a spinning top generator for the production of monodisperse aerosols. Difficulties have been experienced in producing particles suitable for automatic counting and sizing on an image analyer as suitable mounting media tend to dissolve the particles. This is being investigated further.

3. Deposition in Sampling Pipes.

Previously this was measured by taking samples upstream and downstream of the pipe being investigated. However the concentration profile downstream was found to be non-uniform and so a total sampling system was introduced. The input airstream was split into two by a symmetrical Y-junction and the flow rates were made equal. Experiments continue.

Measurements have been made of losses in sampling systems where the airflow turns through a right angle so that a detector can continuously monitor the front face of the filter. A low flow rate system (2 l/min) gave >90% collection on the filter up to 12 μm whereas a similar system with a flow rate of 37 l/min gave only 80% collection at 5 μm although this did

not decrease further up to 12 μm.

4. Removal of Large Particles from Sampling Lines.

Where sampling lines lead to continuous alarm monitors it is important that insignificant amounts of aerosol are deposited since they may become re-entrained and cause false alarms. The methods of removing large particles reported previously were presented at the 3rd International SRP Symposium along with some information on deposition in sampling pipes. It was not found possible to construct a small cyclone with a cut-off of 10 μm at a flow rate of 90 l/min and the final version had a cut-off of 8 μm. The increase in wall losses when the centripeter design was scaled from 30 to 90 l/min as described previously and in the higher flow-rate right-angle sampler described above both show the problems of scaling and the necessity for experimental studies in this field.

5. Resuspension

The relationship between surface contamination levels and airborne concentrations has been reviewed and areas which require further work identified. The possibility of modelling the effects in large areas using small rooms is being studied.

6. Blunt samplers

In blunt samplers the dimensions of the entry orifice are small in relation to those of the sampler. Most practical samplers are in this category. Data on the efficiency of sampling inlets has been reviewed and calculations have been made on the properties of blunt samplers using data in the literature. Discussions have taken place with the Institute of Occupational Medicine, Edinburgh who are very active in this field.

Progress Report
1982

Contractor:

United Kingdom Atomic Energy
Authority, UKAEA
Charles II Street 11
GB-London SW1Y 4QP

Contract no.: BIO-B-333-81-UK

Head(s) of research team(s):

Dr. D.H. Peirson
Env.& Med.Sciences Div.
AERE
Harwell, Didcot
GB-Oxon OX11 ORA

General subject of the contract:

Environmental studies of artificial radioactivity in soil, plants and the sea-air interface.

List of projects:

1. Radionuclides in arable soils and crops.
2. Radionuclide enrichment of the sea - surface microlayer and transfer to the atmospheric aerosol.

Title of project nr 1 Radionuclides in arable soils and crops

Head of project and scientific staff: P A Cawse

Establishment of current levels of actinides

Collection of rainwater and dry deposition has been made over the period Jan-Dec 1982 at eleven locations in Gt Britain (Fig. 1). Samples are analysed each quarter year. From analysis completed so far for the first quarter, deposition of plutonium 239+240 from nuclear weapons fallout amounts to 7.2 x 10^{-4} mCi/km^2 at locations on the west side of Gt Britain, with mean quarterly rainfall of 440 mm, compared to 3.5 x 10^{-4} mCi/km^2 at easterly stations having 153 mm rainfall for the January to March quarter year.

At the same locations (Fig. 1), hay, wheat, barley, oats and potatoes have been collected in 1982. In addition, soil samples were taken from the 49 fields that produced these crops, to derive transfer factors for Pu and Am-241. Results so far obtained from hay fields show concentrations of Pu-239+240 in the range 0.0027 to 0.013 pCi/g, being highest at the sites with highest rainfall. The average ratio of Am-241/Pu-239+240 is 0.29. Other crop samples and soils are being prepared for analysis.

Uptake of radionuclides by ryegrass and clover

To complement studies with ryegrass started in 1981, clover was sown in April 1982 in pots, each containing 3 kg dry soil, with application of 14 separate fertiliser treatments. This study was made on two soils subjected to contamination by Pu. Foliage has been cut regularly and further samples will be collected in 1983 before analysis of actinides is made to derive soil/plant transfer factors.

Depth profiles of radioactivity

Plutonium and Am-241 has been analysed in 10 cm sections of a peat profile from Dartmoor (SW England). The accumulation of Pu-239+240 to 20 cm depth amounts to 98.3% of the total amount recovered to a depth of 50 cm. The ratio Am-241/Pu-239+240 is 0.27, similar to that found in arable soils and in other peat profiles sampled at Garvault and Stroupster in NE Scotland. At all three peat sampling sites, retention of Pu by the top 20 cm is greater than found for Cs-137.

To study the depth distribution of actinides in mineral soils under different ecosystems, grassland, coniferous and deciduous woodlands near Penrith (Cumbria) were sampled in Autumn 1982, to a depth of 50 cm.

Biological effects on soils

Nitrification rates have been measured for six soils with concentrations of Pu-239+240 that are 2-3 orders of magnitude in excess of levels from nuclear weapons fallout. Modified Audus soil perfusion units were used to allow continuous percolation of ammonium substrate through the soils over a period of 7 weeks. Nitrate and nitrite were analysed in the perfusate every 2 to 3 days.

Only two of the soils achieved nitrification rates of 21 and 54 μg NO_3-N/g /day following a growth phase of nitrifying organisms and a build up of nitrite that was subsequently oxidised to nitrate. In the other soils nitrifying organisms failed to proliferate, little nitrite was detected and nitrate production was poor, in the range 6-15 μg NO_3-N/g/day. In these soils, inoculation and other amendments are being applied to resolve the reasons for poor biological oxidation of ammonium to nitrate.

Collaborative discussions

Discussions on soil/plant transfer of radionulcides were held at KFA Julich in March 1982, organised by the IUR. Recommendations were made for the standardisation of experimental conditions to permit valid comparison of transfer factors. Subsequently, an IUR Workshop to evaluate the status of current research in western Europe on transfer factors was attended at the ITAL Laboratory, Wageningen, in December 1982. Valuable contacts were made with EEC colleagues.

Publications

A literature survey has been published in the Harwell report series, entitled "The Uptake of Radionuclides by Plants: a Review of Recent Literature" by P.A. Cawse & G.S. Turner, AERE R9887, February 1982, H.M. Stationery Office, London.

Key to Locations

Site No.	Location	Grid Reference
1	South Brent, Devon	SX 695620
2	Glynllifon, Gwynedd	SH 455554
3	Dumfries	NX 982747
4	Fort William, Inverness-shire	NN 288017
5	Strathkanaird, Ross and Cromarty	NC 153018
6	Wellesbourne, Warwicks	SP 271565
7	Hadlow, Kent	TQ 628497
8	Bratoft, Lincs	TF 868640
9	Ponteland, Northumberland	NZ 159798
10	Lawrencekirk, Kincardineshire	NO 732770
11	Wick, Caithness	ND 364527

Figure 1. LOCATION OF TOTAL DEPOSITION COLLECTORS AND CROP SAMPLING AREAS

Title of project nr 2. Radionuclide enrichment of the sea-surface microlayer and transfer to the atmospheric aerosol

Head of project and scientific staff: N J Pattenden
R S Cambray
M I Walker

Development of field sampling equipment

A motor generator and a high volume air sampler, suitable for operation above the sea, have been acquired. A series of tests have been performed with these in the laboratory, together with a tank to hold seawater and a source of bubbles. The tests have demonstrated that the collection of water in droplet form by the air sampler, is sufficient to enable useful samples of seawater drops to be obtained under field conditions. As an example of the results, with an airflow input to the bubbling source of about 3 l min^{-1}, water droplets were collected on a filter 30 cm above the water surface at a rate of about 1 ml water per minute.

Delays in the delivery of the equipment, followed by bad weather, have meant that testing of the complete equipment in the field has not been possible in 1982. This is now planned for the Spring of 1983.

Collaborative discussions

A study group meeting on the transfer of Am and Cm in the environment, was held at Monaco on 12-13 October 1982, at which we were represented and delivered a contribution. Following this, discussions were held at the Laboratoire de radioécologie marine, Cherbourg, with our colleagues from France, on 10 December 1982. These were valuable, and may result in some collaborative research in the future.

Progress Report

1982

Contractor:

Université de Nantes
Laboratoire de Biochimie
Chemin de la Houssinière 2
F-44072 Nantes Cédex

Contract no.: BIO-B-435-81-F

Head(s) of research team(s):

Prof. J. Pieri
Lab. de Biochimie
Université de Nantes
Chemin de la Houssinière 2
F-44072 Nantes Cédex

General subject of the contract:

Chelation of radioelements (plutonium-239 and -237) in the marine environment - Roles of microorganisms and various natural and bioorganic degradation compounds.

List of projects:

1. Chelation of plutonium-239 and -237 in the marine environment - Roles of microorganisms and various natural and bioorganic degradation compounds.

Contractant de la Commission : Université de Nantes
Laboratoire de Biochimie
2 Chemin de la Houssinière
F - 44072 NANTES Cédex

N° du Contrat : BIO-B-435-81-F

Chef du Groupe de Recherche : Professeur J. PIERI

Thème général du contrat : "Complexion de radioéléments (^{239}Pu ^{237}Pu) en milieu marin (Rôles des microorganismes et de divers composés naturels et de dégradation bioorganiques)".

Titre du projet : Distribution subcellulaire de ^{241}Am, ^{252}Cf chez des Invertébrés d'eau de mer.

Chef du projet et collaborateurs scientifiques : Pr. J. PIERI J. Galey F. Goudard.

Sachant que l'Américium a très souvent un taux de concentration particulièrement élevé dans l'hépatopancréas des Invertébrés marins, organe impliqué dans de nombreuses activités métaboliques d'absorption, de synthèse de stockage et d'excrétion, nous avons poursuivi l'expérimentation, commencée en 1981, sur des animaux marins (Mollusques et Echinodermes).

"UPTAKE" ET DISTRIBUTION DE Am (III) CHEZ E. COLI

Après des séparations biochimiques que nous ne mentionnons pas ici, nous avons fait un essai préliminaire de contamination de E. Coli, bactérie commune dans l'intestin de l'Homme, par ^{241}Am et nous avons constaté :

1 - E. Coli concentre l'Américium (Fc = 220) à la fin de la phase de croissance :
2 - L'Américium est presque entièrement fixé sur la paroi, seulement 5 % sont répartis entre le cytosol et la membrane plasmique.

Les résultats sont pratiquement identiques que le mode de cassage de la Bactérie soit la lyse hypotonique ou la sonication.

"UPTAKE" ET DISTRIBUTION DE Am (III) CHEZ MUREX

Cette expérience a été effectuée avec la collaboration du Laboratoir de Chimie C.C.R. EURATOM, ISPRA, ITALIE.

Comportement de l'Américium dans l'eau : L'Américium-241 (III) est solubilisé dans l'eau de mer (pH = 7) avant d'être fourni à 2 espèces de Murex. Ces animaux accumulent le radionucléide très rapidement; après 3 jours de contact, près de 65 % de la radioactivité initiale a disparu de l'eau de contamination.

La distribution des formes chimiques de l'eau témoin et de l'eau de contamination fait apparaître essentiellement une chute de la fraction particulaire $> 0,01 \mu m$ et une augmentation de la fraction cationique.

Répartition dans les tissus : Les branchies (FC = 150) et la coquille (FC≃95) concentrent particulièrement l'Américium. On ne peut cependant pas conclure que les concentrations les plus élevées se trouvent dans les tissus en contact direct avec la solution contaminante puisque le rein a un facteur de concentration ≃100. L'analyse statistique des facteurs de concentration des 2 espèces fait apparaître une différence significative de l'accumulation de ^{241}Am ($P<0,05$) pour l'hépatopancréas, le rein et les tissus mous restants (contiennent notamment le tractus intestinal).

Distribution intracellulaire : Après 3 jours de contamination, l'Américium est incorporé dans les cellules de l'hépatopancréas et la séparation biochimique met clairement en évidence son association avec la fraction lysosomes-mitochondries. Compte tenu de leur hétérogénéité, l'isolement total des lysosomes est très difficile à réaliser, mais, la concentration de ^{241}Am dans le rein est très élevée, et l'hypothèse d'une élimination par l'intermédiaire des lysosomes peut être formulée.

CONCENTRATION DE ^{241}Am ET ^{252}Cf PAR L'ETOILE DE MER MARTHASTERIAS GLACIALIS.

Lors de cette expérience, nous avons comparé le comportement du Californium et de l'Américium chez l'Echinoderme Marthasterias glacialis contaminé par la nourriture au Laboratoire International de Radioactivité Marine, IAEA MONACO.

Distribution dans les tissus : Ces 2 radioéléments sont concentrés et stockés dans les caecums pyloriques (90 et 99 % du Cf total, 82 et 87 % du Am total). Un faible pourcentage d'Américium (2,5 et 4 %) est fixé sur l'exosquelette, ce qui n'est pas vrai pour le Californium.

Distribution subcellulaire : La séparation des organites cellulaires par ultracentrifugation confirme l'association de l'Américium avec la fraction lysosomes-mitochondries. Près de 45 % de Californium est lié à cette fraction et 20 % est sous forme soluble.

En séparant le culot lysosomes-mitochondries sur gradient isopycnique la courbe de radioactivité de ^{241}Am, bimodale, présente un pic d'activité lié aux fractions les plus denses et un pic de moindre amplitude superposé au pic d'activité spécifique de la phosphatase acide et indique une contamination à la fois des lysosomes et des mitochondries. Par contre, la courbe de radioactivité du Californium présente un seul pic d'activité lié aux fractions les plus lourdes du gradient (mitochondries).

PUBLICATIONS :

. Répartition tissulaire et subcellulaire de ^{252}Cf et de ^{241}Am dans l'étoile de mer M. Glacialis (en collaboration avec S. FOWLER).

. Répartition subtissulaire et subcellulaire de ^{241}Am chez Murex. (En collaboration avec N. MURRAY).

. Métabolisme de ^{241}Am chez Cardium et Littorinea et du Technetiun 95 chez Homarus. (En collaboration avec P. MIRAMAND. M. MASSON et P. GERMAI La Hague C.E.A.)

Progress Report
1982

Contractor:

Contract no.: BIO-B-336-81-UK

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

Head(s) of research team(s):

Dr. H. Smith
Biology Department
NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

General subject of the contract:

Experimental studies of environmental factors influencing the uptake of radionuclides by man.

List of projects:

1. Uptake of radionuclides into foodstuffs grown in contaminated soil.

Title of project nr 1

Uptake of radionuclides into foodstuffs grown in contaminated soil

Head of project and scientific staff :

Dr. D. S. Popplewell

Mr. G. J. Ham

Crops of potatoes have been grown outdoors at Chilton in tubs containing a constant sample of radioactively-contaminated soil for four consecutive seasons. The objectives of the work have been to measure season-by-season the soil-to-plant transfer factors for various radionuclides and to observe how these factors are influenced by the use of fertilizers.

Sediments from an estuary near Ravenglass, Cumbria were used in the experiments. These sediments had been contaminated as a result of discharges of radioactive waste into the Irish Sea from the nuclear fuel reprocessing plant at Sellafield. Although the sediments contain high levels of radioactivity relative to the adjacent farmland, they do not readily support plant growth. On the other hand, the farm soils support plant growth but have low levels of artificial radioactivity which would make it difficult to measure the radionuclide uptake into plants. Moreover the chemical forms of the artificial radionuclides deposited on the soil from nuclear weapons fallout may be different from the chemical forms of the radionuclide present in the sediments, and different with respect to their uptake into plants. Therefore a blend of approximately equal volumes of soil and sediment was prepared, giving a medium which would support plant growth and have an acceptable specific activity. The resulting blend was watered during the winter of 1978-79 so as to reduce its salinity. This same batch of material was used to grow potatoes in the four seasons up to 1982.

The 1981 potatoes were pooled for analysis because the reproducibility of the results from the individual tubs in 1979 and 1980 showed that it was unnecessar to analyse separately the crop from each tub. For the 1982 growing season different fertilizer regimes were used between these pots, but the small scale of the experiment made it impossible to assess the significance of the small differences between the observed transfer factors. Therefore the table quotes mean of the results, irrespective of the fertilizer treatment.

The uptake factors for plutonium and americium are similar in magnitude to each other, and showed marked reductions for the third and fourth seasons. The uptake factors for ^{90}Sr and ^{137}Cs showed no dramatic changes over the period of experiment.

The season by season transfer factors of radionuclides from soil (dry weight) to potato (fresh weight) x 10^4

	^{239}Pu + ^{240}Pu	^{241}Am	^{90}Sr	^{137}Cs
1979 Peel	12	11.7	82	100
Flesh	0.086	0.21	16	62
1980 Peel	8.4	7.3	125	79
Flesh	0.11	0.12	24	74
1981 Peel	0.70	0.81	106	170
Flesh	0.015	0.05	28	65
1982 Peel	2.4	3.4	(150)*	90
Flesh	0.025	0.029	(41)*	100

*Calculated using soil ^{90}Sr concentration from previous seasons' results

The radiological consequences of eating potatoes of the specific activity of the 1980 crop are of interest. If peel, representing 17% of the fresh weight of the potato, is regarded as unpalatable and discarded, 95% of the plutonium and americium in the tuber would be discarded with it. Caesium was distributed uniformly throughout the potato therefore discarding the peel would do little to reduce the specific activity of ^{137}Cs in the ensuing meal. Strontium-90 was enriched in the peel relative to the flesh and removing the peel would remove approximately 54% of the total radiostrontium. However, if whole potatoes were eaten the radionuclides ingested for ^{238}Pu + ^{239}Pu + ^{240}Pu, ^{241}Am, ^{90}Sr and ^{137}Cs would represent respectively 0.9%, 0.5%, 0.5% and 4.4% of the Generalised Derived Limits for these radionuclides. Whether the potatoes were to be eaten peeled or unpeeled, it is apparent that the dose to man would be small.

It should be emphasised that the transfer factors have been derived for a particular blend of soil and silt, the latter having been contaminated with radionuclides in a particularly natural process. Details of the soil type, on which the transfer factors depend, have been described.(1)

Publication

(1) POPPLEWELL, D. S., HAM, G. J. and STATHER, J. W. "The uptake of ^{239}Pu + ^{240}Pu, ^{241}Am, ^{90}Sr and ^{137}Cs into potatoes", paper presented to Workshop Comparing Soil-Plant Transfer Factors of Radionuclides, International Union of Radioecologists, Wageningen, 7-10th December 1982.

Progress Report
1982

Contractor:

The National Radiological
Protection Board, NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

Contract no.: BIO-B-521-82-UK

Head(s) of research team(s):
Dr. H. Smith
Biology Department
NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

General subject of the contract:
The speciation of radionuclides in plants and foodstuffs and the influence of this on their gastrointestinal uptake.

List of projects:

1. The speciation of radionuclides in plants and foodstuffs and the influence of this on their gastrointestinal uptake.

Title of project nr 1

The speciation of radionuclides in plants and foodstuffs and the influence of this on their gastrointestinal uptake

Head of project and scientific staff :

Dr. J. R. Cooper

The plutonium-binding ligands in a variety of crops have been identified by expressing juice from the edible material, adding plutonium-239 nitrate and then separating the complexes by gel permeation chromatography. The following binding ligands have been identified so far:- citrate, isocitrate, phytate (myo-inositol hexakis-phosphate) and probably malate (Table 1).

The identification of phytate as a plutonium-binding agent is of interest because it occurs in several important foodstuffs including peas, beans and grain. In order to find out how much plutonium was absorbed across the gut, plutonium-labelled phytate was administered to rats and rabbits through a stomach tube. The rats were kept thereafter for one week and the rabbits for three weeks before the animals were killed and the tissues were analysed. 0.13% and 0.01% of the plutonium was absorbed in rat and rabbit respectively. This compared with 0.04% and 0.02% respectively, for the amount of plutonium absorbed after administration of the plutonium-citrate complex. The marked difference in the absorption of plutonium administered as the phytate complex between rat and rabbit was investigated further. It was proposed that it may be due to different concentrations of phytate-digesting enzyme phytase. Experimental data confirmed this view. The enzyme was shown to be localised in the small intestinal brush border, the membrane lining the small intestine and through which foodstuffs are absorbed and its concentration was 3-4 times higher in the rat than in the rabbit. It is suggested that plutonium-phytate is broken down by phytase at the brush border, momentarily releasing monomeric plutonium in the immediate vicinity of the absorptive surface. Thus some absorption of plutonium could occur before the competing reaction of hydrolysis and polymerisation. The higher concentration of phytase in rat intestine would allow a higher absorption than in the rabbit.

Measurement of phytase concentrations in human gut mucosa are underway.

Table 1 - Plutonium binding species in plant juices

Species	Plutonium-binding ligands
Potato	Phytate and citrate
Soya bean	Phytate and citrate
Carrot	None detected
Beetroot	None detected
Turnip	Malate*
Apple	Malate*
Tomato	Citrate
Onion	Isocitrate

The complexes were identified by gel permeation chromatography using Sephadex gels

*It is difficult to separate plutonium-malate from the plutonium citrate on Sephadex gels and this designation is based on the relative amounts of the two acids in the juices and the stability of the complexes

Progress Report
1982

Contractor:

Landbouwhogeschool Wageningen
Laboratorium Dierfysiologie
Haarweg 10
NL-Wageningen

Contract no.: BIO-B-432-81-NL

Head(s) of research team(s):

Dr. J. van den Hoek
Lab. Dierfysiologie
Landbouwhogeschool
Haarweg 10
NL-Wageningen

General subject of the contract:

Simulation of tritium transfer in the environment; study of organic tritium transfer in the aquatic environment, the soil-plant and the plant-mammal system.

List of projects:

1. Behaviour of tritium in mammals.

Title of project nr. BIO-B-432-81-NL: Behaviour of Tritium in Mammals

Head of project and scientific staff: Dr. J. van den Hoek

As was outlined in the working programme for 1982, six miniature goats were equipped with rumen fistules and fed tritiated hay for the entire gestation period of 150 days. Some baby goats were removed from their mothers after birth, fed non-tritiated milk and killed at 0, 5 and 20 days. Tritium content was determined in over 20 different tissues and organs. Tritium is lost rapidly in organs such as liver, small and large intestine, stomach and thymus, which probably indicates a high metabolic turnover rate in these organs. Rather surprisingly, tritium levels decrease relatively rapidly also in mesentery, omentum, the fatty tissue around the kidney and in the subcutaneous tissue. At birth, these tissues are found in very small quantity only, and the rapid decrease in tritium activity may merely indicate the growth of these tissues from non-tritiated precursors after birth. The lowest turnover so far was found in hair, at least during the first month of life, followed by the neck ligament (ligamentum nuchae), brain, bone and muscular tissue, including that of the heart. More animals will have to be sacrificed, particularly at longer periods after birth, in order to be able to calculate accurate turnover times of the various components which are already apparent in most organs and tissues. At the present time, differences in tritium metabolism in various organs and tissues can be presented schematically in the following order of decreasing turnover rates: *liver* < *small intestine* < *thymus* < *large intestine* < *kidney* < *stomach* < *omenta* < *lung* < *fatty tissue* < *spleen* < *heart* < *testis* < *skin and hair* < *neck ligament* < *brain* < *bone* < *muscle*.

A second group of baby goats was allowed to suckle their mothers for about 2 months while these mothers continued to receive tritiated hay during their lactation period. Analysis of their tissues and organs is in progress.

Tritium content of organic milk constituents of miniature goats was determined during the administration of tritiated hay and after this was stopped. Interesting differences were noted with the results observed in the cow. For example, tritium content of the milk protein (casein) was higher than that in milk fat at steady state (Figure 1). In the lactating cow, tritium levels in casein were less than 40% of those in milk fat. After stopping the administration of tritiated hay, casein continues to show higher tritium activity than milk fat. A regression analysis will be carried out as soon as sufficient samples have been analysed.

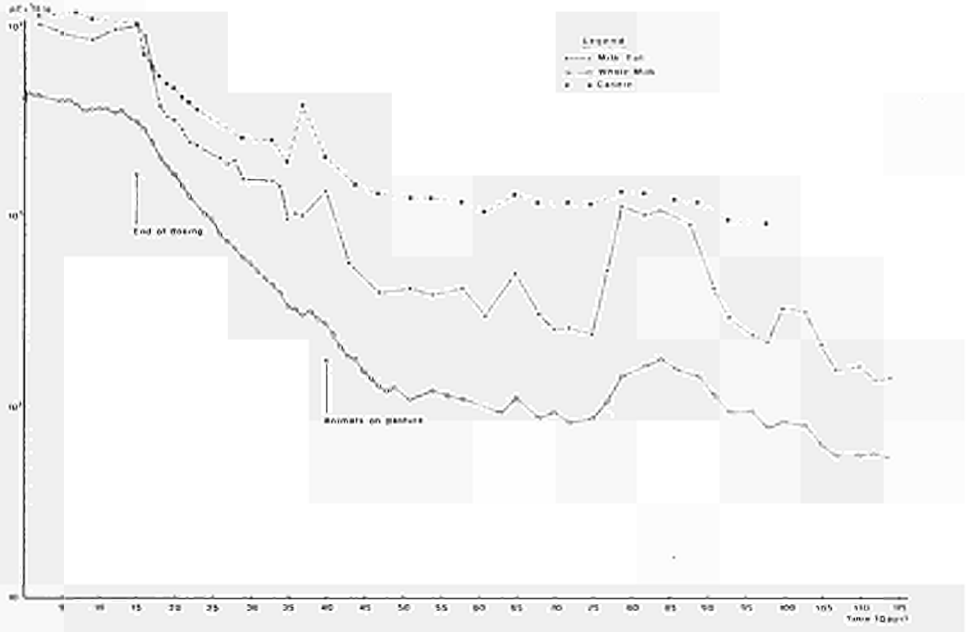

Figure
Tritium activity in milk fat, casein and whole milk during and following ingestion of OBT for 160 days by a miniature goat.

List of publications in 1982

I Publications in Scientific Journals, Monographs, Proceedings.

- Tritium metabolism in cow's milk after administration of tritiated water and of organically bound tritium.
  J. van den Hoek, G.B. Gerber, R. Kirchmann.
  Annales de l'Association Belge de Radioprotection, 7 (1982), nr. 3-4, 317-330.
- Incorporation of tritium in milk lipids after feeding organically bound tritium to cows.
  M. Rochalska, J. van den Hoek, R. Kirchmann, G.B. Gerber, R. van Bruwaene.
  Annales de l'Association Belge de Radioprotection, 7 (1982), nr 3-4, 331-338.
- Conversion to organic tritium in ruminants and the implication for radiation protection.
  J. van den Hoek, G.B. Gerber.
  Invited paper presented at the "European Seminar on the Risks from Tritium Exposure", November 22-24, Mol, Belgium. To be published by the Commission of the European Communities.
- Tritium metabolism in young Pigs after Exposure of the Mothers to tritium oxide during Pregnancy.
  R. Van Bruwaene, G.B. Gerber, R. Kirchmann, J. van den Hoek and J. Van Kerkom.
  Radiation Research, 91 (1982), 124-134.

II Short Communications

- Tritium in cow's milk after ingestion of inorganic and organic tritium.
  J. van den Hoek, G.B. Gerber.
  Paper presented at the 17th Annual Meeting of the European Society for Radiation Biology, 26-30 July, 1982, Bordeaux, France.

Progress Report
1982

Contractor: Contract no.: BIO-B-485-82-B

Institut Royal des Sciences
Naturelles de Belgique (IRSNB)
Rue Vautier 29
B-1040 Bruxelles

Head(s) of research team(s):

Dr. D. Van der Ben
Section Océanographie
IRSNB
Rue Vautier, 29
B-1040 Bruxelles

General subject of the contract:

Study of the impact of technetium on the environment and organisms of the Belgian coast and risk of its transfer to man.

List of projects:

1. Kinetics of accumulation and transfer of technetium by certain organisms and sediments and its influence on the primary productivity.

Title of project nr BIO-B-485-82-B:

Kinetics of accumulation and transfer of technetium by certain organisms and sediments and its influence on the primary productivity.

Scientific staff: S. BONOTTO, J.M. BOUQUEGNEAU, M. COGNEAU, R. KIRCHMANN, B. MANIA, M.N. MAQUET, Z. MOUREAU, L. PIGNOLET, O. VAN DER BEN, C. VERTHE, S. WARTEL.

Progress report 1982

I. Bacteriology: Effect of 99Tc on some marine bacteria

1. Bacteria were enumerated in samples of intertidal sediments of the North Sea (Belgium). Some representative strains were isolated in order to determine their biochemical characters.

2. Bacteria from similar samples were counted after addition of 99Tc in the culture (1.10 and 100 ppm). No significant difference was observed between the number in contaminated and non contaminated cultures. However, the contaminated cultures were pigmented.

3. Marine infusoria (Uronema marinum) were cultivated successfully. We intend to nourish these organisms with bacteria contaminated by 99Tc.

II. Tc accumulation in North Sea sediments and in phytoplancton

The coefficients of Tc-99 distribution (with Kd expressed in ml/g) in two types of North Sea sediments, have been determined at a radiopollutant concentration level of 0.1 ppm. In spite of very different characteristics, the values obtained for each type of sediment as well as their evolution in the process of time appeared to be fairly similar (reaching rapidly the maximum: Kd = 8, and then decreasing slowly during ± 30 days towards an equilibrium value: Kd = 2). These first results suggest a possible influence of microflora in the second phase.

As a first species representative of phytoplankton, Dunaliella bioculata has been cultivated in a turbidostat - a device created in our laboratories and allowing the control of the population levels. The growth of the alga and the Tc-99 transfer factor have been measured at a radiopollutant concentration level of 0.1 ppm. If referred to dry weight of algae, the transfer factor = ± 1.

III. Tc accumulation in marine algae

Technetium uptake and distribution was investigated in three species:two

unicellular green algae (Acetabularia acetabulum and Boergesenia forbesii) and a pluricellular brown alga (Ascophyllum nodosum). ^{95m}Tc has been added to the sea water as $^{95m}TcO_4^-$ (pertechnetate). Vegetative Acetabularia cells extracted ^{95m}Tc from the surrounding medium, the concentration factor being 25 already after a few hours. Moreover,after a 30 or 120 min incubation,the apical region of the cells (which is that mostly grazed by fishes in nature) was more labelled than the basal one. ^{95m}Tc seems thus to be distributed in the cells according to an apico-basal gradient. Vegetative Boergesenia cells cannot concentrate ^{95m}Tc: even after ten days incubation, the transfer factors remain lower than 1. However, ^{95m}Tc is able to cross the cell wall and the cytoplasmic layer, since after 120 min incubation, a measurable amount (2.6 nCi/ml) was found in the vacuole, by using the puncturing technique. The brown macro-alga Ascophyllum shows a rapid uptake of ^{95m}Tc, the concentration factor being about 50 already after a 4 days incubation. All regions of the plant were found to accumulate ^{95m}Tc; in some cases the young apical region was labelled more strongly than the other parts of the plants. Our results suggest that marine algae may play a role in the transfer of technetium through the food chain.

IV. Tc accumulation in two marine mollusks

The accumulation by a common periwinkle, Littorina littorea, of ^{99}Tc (ammonium pertechnetate) at the concentration level of 2.6 ppb in seawater, has been measured over a period of 60 days. Six specimens have been selected every other day and the content of Tc measured both in the viscera and in the foot. It appears that considerable quantities of Tc are concentrated, resulting mainly from the accumulation in the viscera. The concentration factor (C.F.) for the whole animal is of about 53.8.

On the other hand, preliminary experiments have been carried out by contaminating mussels (Mytilus edulis) with Tc at the concentration level of 10 ppb in water. This species seems to accumulate much smaller quantities of Tc (the C.F. here ranging from 1 to 2 in the viscera of the animal). Traces of metallothionein have been sought in both species. These proteins which contain a high proportion of cysteine and are well-known for their affinity for metals, do not seem to have any particulate affinity for Tc. As far as the soluble fraction of the organism is concerned, Tc seems to appear mainly in the form of a molecular weight inferior to 500 Daltons. Further investigation is on the way in order to identify more closely the compound to which Tc might mainly be bound.

Progress Report
1982

Contractor:

Studiecentrum voor
Kernenergie, SCK/CEN
Plaskylaan 144
B-1040 Brussel

Contract no.: BIO-B-329-81-B

Head(s) of research team(s):

Prof. O. Vanderborght
Département Radio-
biologie, SCK/CEN
Boeretang 200
B-2400 Mol

General subject of the contract:

Bioavailability of actinides in selected freshwater, estuarine and seawater species and the related effects of environmental factors on the modeling of their behaviour.

List of projects:

1. Biological availability of transuranics in freshwater ecosystems.
2. Biological availability of transuranics in estuarine and coastal systems.
3. Chemical speciation of transuranics and their bioaccumulation.
4. Mathematical models on the mechanisms affecting bioavailability of transuranics.

Title of project nr 1 : BIOLOGICAL AVAILABILITY OF TRANSURANICS IN FRESHWATER ECOSYSTEMS

Head of Project : O. VANDERBORGHT
Scientific staff : J. VANGENECHTEN, J.BIERKENS, S.VAN PUYMBROECK

Under the current and future practice of radioactive alpha-waste storage in deepsea sediments and in underground geologically stable formations, americium radioisotopes can become a major hasard source from the nuclear fuel cycle as their relative importance grows during the ageing of the nuclear waste.

The present project aims to study the biological availability of ^{241}Am in freshwater ecosystems for different animal species whereby special attention has been focused on the role of organic compounds in the water on bioaccumulation. Next to this, experiments were performed on the uptake of ^{241}Am by marine benthic organisms from contaminated deep-sea sediments.

1. Influence of organic compounds in the water on bioaccumulation of ^{241}Am by freshwater biota.

During the past year, biological uptake of ^{241}Am has been studied in detail in the crustacean crayfish Astacus leptodactylus, in the waterbug Corixa punctata and in following mollusc species : Lymnaea peregra, Planorbis corneus, Anisus planorbis.
The influence of organic matter on bioaccumulation in the crayfish was investigated and revealed higher concentration factors (C.F.) in surface water with high levels of organic matter (C.F. = 40-150 in lake water with high content of organic matter from biotic origin and C.F. = 20-70 in a stream water polluted with organic material from a papermill) in contrast with C.F. values of 0.5-20 in organically poor stream water.
The biological half-life of ^{241}Am in A. leptodactylus, measured during a retention experiment, was calculated to be about 55 days.
In another experiment bioaccumulation of ^{241}Am in waterbugs C. punctata

was compared in freshwaters which contained organic compounds (with Molecular Weight above 10 000) and the same waters which did not contain these organic compounds. It was clear that the organic compounds had an important impact on the physico-chemical behavior of the transuranic in the water and thus possibly on the uptake by the animals. Other techniques to measure the extend of binding of ^{241}Am with organic compounds were tested out and will become operational during the next year.

2. Bioaccumulation of ^{241}Am by marine benthic organisms from sediments.

The uptake of ^{241}Am from artificially contaminated deep-sea sediments by marine benthic organisms (the bivalve mollusc : Tapes decussatus, the polychaete Hermione hystrix and the isopod : Cirolana borealis) has been studied during a 3 month working visit at the IAEA laboratories in Monaco.

Accumulation of the radionuclide by the animals, measured over a period of 40 to 50 days, yielded transfer factors lower than unity. Uptake was up to 2 to 5 times higher for animals from Pacific sediment (taken at a seabed disposal feasibility investigation site in the Pacific Ocean) than from Atlantic sediment (from near the NEA = North East Atlantic dumpside). Nevertheless the affinity of the transuranic (measured as the K_d factor) was comparable in both sediments and was of the order of 1×10^5.

Conclusions

Organic compounds present in the water may influence bioavailability of the transuranic ^{241}Am for freshwater animals. Clearly more information is needed on the association of the transuranic with organic compounds in the different watertypes if one wants to elucidate their role on bioavailability.

The bioaccumulation of ^{241}Am from the sediments seems not to be directly related to the measured affinity of the element to these sediments (K_d-value).

These results emphasise the need to investigate thouroughly the impact of organic compounds in the water for bioavailability and further to study more closely the transfer of the ^{241}Am from sediments (also freshwater sediments) to biota.

Title of project nr 2 : BIOLOGICAL AVAILABILITY OF TRANSURANICS IN ESTUARINE AND COASTAL SYSTEMS

Head of project : M. HOPPENHEIT (Biologische Anstalt Helgoland)

The project aims to study accumulation and retention in marine invertebrates in relation to environmental factors, furthermore it aims to estimate the absorbed dose to species from alpha radiation.
Lack of personnel during this year has retarded the research (this project does not receive any support from the C.E.C.). Breeding of the experimental animals has been maintained. Furthermore important statistical aid has been given to project number 1 and 3.

Title of project nr 3 : CHEMICAL SPECIATION OF TRANSURANICS AND THEIR BIOACCUMULATION

Head of project : G.M.R. THIELS, N.C. MURRAY

An attempt was made to characterize the bioavailability of ^{237}Pu and ^{241}Am for the pond snail Lymnaea stagnalis L. in selected surface waters. The uptake, distribution and retention patterns were studied through the contamination route water-snail. The effects of a number of parameters, such as seasonal variation, water type (pH, conductivity, ionic composition) and presence of food, on the bioavailability of the transuranics were evaluated. Finally, an assessment was made concerning the chemical speciation of plutonium and americium in the selected freshwaters.

It was shown that L. stagnalis quickly accumulates both transuranics from the water, yielding whole body concentration factors between 40 and 900 after several days. The concentration factors in the organs generally display the same decreasing pattern : shell margin > shell > gastrointestinal (G.I.) tract > hepatopancreas > foot muscle ≃ soft tissues > blood. Two major processes are responsible for the accumulation of both elements in the pond snail. The first, which accounts for more than 60% of the total amount taken up, occurs by external fixation on the shell, while the second appears to be the result of oral uptake via the drinking water. This is followed by true assimilation within the organs as reflected by the association of the transuranics with subcellular components.

The elimination of ^{241}Am by L. stagnalis is essentially described by two loss components with biological half-lifes of 3 days and 40 to 60 days. The rate of americium removal from the shell and soft parts depends on the organ concerned.

The concentration factors of ^{241}Am in L. stagnalis, unaffected by the initial americium concentration in the water (between 3×10^{-10} and 3×10^{-9} M), undergo cyclic seasonal changes and are inversely related to wet weight. The presence of uncontaminated food results in a lower

assimilation as compared to starving animals. A limited amount of radioactive food, however, enhances both external and internal fixation. The water type has the most pronounced effect on the concentration factors of ^{241}Am, inducing differences of an order of magnitude. These differences are mainly related to the specific conductivity and the pH of the water, but not to the measured americium species (i.e. particulate, cationic, anionic or neutral) in the water. On the other hand, ageing of americium solutions significantly reduces its bioavailability this phenomenon is pH dependent. Compared to ^{241}Am, ^{237}Pu is less available for L. stagnalis. Moreover, different oxidation states of this element seem to have dissimilar fixation patterns.

Finally, an insight was obtained concerning the chemical speciation of both transuranics in some freshwaters. It was found that the particle formation of (> 0.01 µm) ^{241}Am is determined by the chemical composition of the water, pH, ageing and to a lesser extent by turbulence. The chemical species of this transuranic are thought to be the result of hydrolysis or a combination of hydrolysis and complexation reactions. Hydrolysis, as reflected by the particulate fraction (> 0.01 µm) decreases with acidity and with the anionic and organic carbon content of the water. Similar chemical processes govern the speciation of Pu(III), while Pu(IV) and (VI) are mainly subject to hydrolysis. The presence of L. stagnalis in the contaminated solutions results in a further dissolution of both transuranics apparently through the formation of negatively charged organic complexes. The relative importance of these anionic species is related to the amount of faeces and to the water type. L. stagnalis also exerts a strong reducing effect on the plutonium oxidation states in solution.

The present findings, although based on a limited number of variables and pertaining to a relatively simple microcosm, might aid in identifying the more important processes which govern the bioavailability of transuranics.

Title of project nr 4 : MATHEMATICAL MODELS ON THE MECHANISMS AFFECTING BIOAVAILABILITY OF TRANSURANICS

Head of project : C.N.MURRAY (CCR-Ispra)

The study on the requirements to put up a general model for the behavior of ^{241}Am in marine estuarine and freshwater systems showed that high priority must be given to the study of the chemical forms and their bioavailability in these systems.

Publications

Bierkens,J., J.H.D. Vangenechten, S. Van Puymbroeck and O.L.J. Vanderborght (1982). Biological availability of the transuranic Americium-241 in different types of freshwaters by four freshwater animals. Presented at the Joint Radiation Protection Meeting of the Soc. Française de Radioprotection & Fachverband für Strahlenschutz (CH). Lausanne, 30 Sept. 1981, Proceedings of the Meeting.

Thiels,G.M. (1982). The bioavailability of the transuranic elements ^{237}Pu and ^{241}Am for the pond snail, Lymnaea stagnalis L. and their behaviour in selected natural surface waters. Ph.D. study University of Antwerp (UIA-Universitaire Instelling Antwerpen), Wilrijk (Belgium).

Vangenechten,J.H.D., S.R. Aston and S.W. Fowler. Uptake of Americium from two experimentally labelled deep-sea sediments by three benthic species : a bivalve mollusc, a polychaete and an isopod. Submitted for publication in Marine Ecology - Progress Series.

Communications

Bierkens,J., J.H.D. Vangenechten, S. Van Puymbroeck and O.L.J. Vanderborght (1982). Effect of organic pollution on the bioavailability of Americium-241 for the crayfish Astacus leptodactylus Eschscholtz. Presented at the Meeting on the Transfer of Am and Cm in the Environment. Jointly orgnised by the CEC and the IAEA. Monaco 12-13, October 1982.

Thiels,G.M. (1982). Bioavailability of ^{241}Am for the pond snail Lymnaea stagnalis L. in six surface waters at the same pH. Presented at the Meeting on the Transfer of Am and Cm in the Environment. Jointly organised by the CEC and the IAEA. Monaco 12-13 October 1982.

Vangenechten,J.H.D., S.R. Aston and S.W. Fowler (1982). Bioavailability of the transuranic Americium-241 in two marine sediments by sediment-dwelling invertebrates : the Mollusc Tapes decussatus, the Polychaete Hermione hystrix and the Isopod Cirolana borealis. Presented at the meeting on the Transfer of Am and Cm in the Environment. Jointly organised by the CEC and the IAEA. Monaco 12-13 October 1982.

Abstracts

Bierkens,J. (1982). Biological availability of the transuranic ^{241}Am in different types of freshwater for Astacus leptodactylus Eschscholtz (Turkish Crayfish). Presented at the Colloquium on the Toxicity of Radionuclides. Liège, 19-20 November 1982. Abstract to be published in the Journal Belge de Radiologie.

Vanderborght,O.L.J., J.H.D. Vangenechten, and J. Bierkens (1982). Biological Problems concerning Transuranics in the Aquatic Environment. Presented at the Colloquium on the Toxicity of Radionuclides. Liège, 19-20 November 1982. Abstract to be published in the Journal Belge de Radiologie.

Vangenechten,J.H.D. (1982). Bioaccumulation of ^{241}Am in different freshwater types by tadpoles (Rana esculenta) and waterbugs (Corixa punctata). Presented at the Colloquium on the Toxicity of Radionuclides. Liège, 19-20 November 1982. Abstract to be published in the Journal Belge de Radiologie.

Progress Report
1982

Contractor:

International Union
of Radioecologist IUR
Rue Card. Cardyn 5
B-4480 Oupeye

Contract no.: BIO-B-468-81-F

Head(s) of research team(s):

Ir. P. Bovard
Président de l'I.U.R.
Rue Card. Cardyn 5
B-4480 Oupeye

General subject of the contract:

Promotion of research and exchange of information in radioecology.

List of projects:

1. Promotion of research and exchange of information in radioecology.

Title of project nr BIO-B-468-81-F

Promotion of research and exchange of information in radioecology

Head of project and scientific staff :

Ir.P.Bovard.

P.O.Agnedal, S.I.Auerbach, F.Führ, R.Kirchmann, G.Kistner,- J.Pieri,J.Van den Hoek.

Progress Report for the period 1 January to 31 December 1982.

1. Intercomparison and harmonization of methodologies.

a) Marine Environment.

A workshop convened by I.U.R. on " The transport of radionuclides from the Nordic Sea into the Baltic Sea and their behaviour" task place in Vienna 9 - 10 of July 1982,with the project leader P.O.Agnedal as chairman. The meeting was attended by 11 Scientists (see list of participants in Annex).

Data were presented respectively by D.F.Jefferies, P.Guegueniat ; data from research work in Nordic Countries and in Germany were also reported. It was found that generally a good agreement existed between the results from different laboratories.

The points in connection to what could be achieved during the "Gauss" expedition in the Baltic Sea (May-June 1983) were discussed; there was agreement on the sampling location , on the sample preparation and on the radionuclides to be measured (all samples : Sr-90, Cs-137.In water also H-3. In selected samples : Pu-239+240, Pu 238, Am-241 and if possible Tc-99,Np-237 and Cs-134).

Reporting of data was also discussed as the main aim is to calculate the inventory of radionuclides within the different main compartments of interest, in order to make a realistic budget for the Baltic Sea ecosystem.

From the Monaco Lab.samples have been distributed for in-

tercalibration of sea water and bottom sediments. Methods for Tc and Np analysis ara available from different laboratories and the analytical procedures will be distributed to the participants.

As the behaviour of most radionuclides is salinity dependent, it is therefore of great interest to establish the behaviour or radionuclides in brackish water.

The collected data should be used for modelling purposes and will provide a great opportunity for model validation. The models developed at NRPB and STUDSVIK should be connected at the transition zone in the Skagerac area.

The models and the analytical results will be used to predict the impact of radionuclides entering the Baltic Sea from Windscale and La Hague.

b) Terrestrial environment.

-Report IUR project Soil-Plant Transfer factors.

The leader of the project,M.J.Frissel,organized a workshop at ITAL,Wageningen, 7 - 10 december 1982. The workshop was attended by 30 participants from Sweden, the Federal Republic of Germany, the Netherlands, Belgium, France, Switzerland,Italy and the United Kingdom.(See list of participants in Annex).
It was chaired by Dr.Cawse.

The main goal was to discuss the results of TF (Transfer Factors of radionuclides)determinations,which were set up according to the recommendations of the IUR meeting, 9,10 April 1982, at Jülich. Of course also other TF values were reported and discussed during the first 2 days of the workshop.

The 3rd day the participants divided themselves into three subgroups, each of the groups dealing with a specific topic. The first group prepared a kind of "Status of the art" report on the determination of TF values. The second subgroup was asked to evaluate the Tf values which were

reported. Due to the success of the meeting the number of TF values, however, is large (about 800), so that this appeared to be impossible. Therefore a method was developed to process the data with a computer. A code was developed which can include the most important information of the TF values determined. Already 500 TF values, together with their particular details, were coded at the workshop. Another 300 will be coded as home work. The preparation of the data file will be done at ITAL. A method is developed which allows particular combinations of data to be listed so that e.g. all data on specified radionuclides, crops and soils can be combined. These listings will be available early 1983.

The third group discussed details of how to determine the availability of radionuclides for plant roots and worked out recommendations for the determination of additionnal data for transfer experiments.

A next meeting of the IUR Soil-Plant transfer project group is scheduled for March 1984. It is expected that at that time all 1982 and 1983 uptake data will be available.

2. Inventory of present means and ongoing research as well as of future objectives of research in Radioecology.

A workshop organized by IUR was held in Brussels on March 30 1982, 21 participants from twelve countries attented this meeting. (see list of participants in Annex).

The reviewers presented the reports prepared on the basis of the questionnaire sent to the leaders of the radioecological laboratories in the world. The authors and the title of the reports are given in the list of publications herewith.

A session was devoted to a general discussion (Chairman : S.I.Auerbach) on the following topics :

- the radioecology within the field of radiological protection;

- the state and role of the radioecologist;
- the preparation of a booklet on the "state of the art" in radioecology".

It is expected that the booklet will be issued early in 1983

3.Exchange of information.

Organization, during the Second International Assizes of Radioecology held in Wageningen (june 28 - 30,1982) of a Seminar on "Environmental transfer and metabolism of Tritium The title of the papers presented are given in the list of publications herewith.

List of publications in 1982.

I.Publication in Proceedings.

D.Papadopoulos, L.A.König, K.G.Langguth.
Tritium contamination of rain water due to tritium release to the atmosphere.
Annales de l'Association Belge de Radioprotection,Vol.7,N°3-4 129 - 146,1982.

R.Kirchmann, J.C.Dupont, P.Fontaine-Delcambe.
Evaluation de l'influence des tours de réfrigération sur le transfert à l'environnement terrestre du tritium d'un cours d'eau récepteur.
Annales de l'Association Belge de Radioprotection,Vol.7,N°3-4 147 - 166,1982.

C.Bunnenberg, W.Kuhn, U.Ujeno.
Vapor exchange between atmosphere and soil with respect to the transfer of tritium.
Annales de l'Association Belge de Radioprotection,Vol.7,N°3-4 167 - 182, 1982.

Y.Belot, J.Delforge.
Modélisation du transfer de HTO entre l'atmosphère et le sol. Application au transfert de la vapeur d'eau tritiée.
Annales de l'Association Belge de Radioprotection,Vol.7,N°3-4 183 - 198, 1982.

L.A.König, K.G.Langguth, D.Papadopoulos.
Tranfer of tritium discharged with the liquid effluent in the environment of the Karlsrühe Nuclear Research Center.
Annales de l'Association Belge de Radioprotection,Vol.7,N°3-4 199 - 212,1982.

S.Strack.
Behaviour of tritium in the water pool and organic pool of the leaves of a beech tree. First results of a long term investigation.
Annales de l'Association Belge de Radioprotection,Vol.7,N°3-4 213 - 228,1982.

R.Kirchmann, E.Fagniart.
Bilan de recherches expérimentales sur le transfert de l'eau tritiée aux végétaux cultivés.
Annales de l'Association Belge de Radioprotection,Vol.7,N°3-4 229 - 246,1982.

H.Camus, J.Delmas, R.Kirchmann.
Influence du climat sur la sorption et la désorption d'eau tritiée par des végétaux irrigués.
Annales de l'Association Belge de Radioprotection,Vol.7,N°3-4 247 - 258,1982.

L.Foulquier, M.Pally.
Données sur la teneur en tritium lié de poissons des grands fleuves français.
Annales de l'Association Belge de Radioprotection,Vol.7,N°3-4 259 - 282,1982.

S.Bonotto, G.B.Gerber, G.Arapis, R.Kirchmann.
Modelization of tritium transfer into the organic compartments of algae.
Annales de l'Association Belge de Radioprotection,Vol.7,N°3-4 283 - 292,1982.

J.Guenot, C.Caput, Y.Belot.
Rôle des réactions de photosynthèse et d'échange dans l'incorporation du tritium dans la matière organique des végétaux.
Annales de l'Association Belge de Radioprotection,Vol.7,N°3-4 293 - 306,1982.

S.L.Commerford.
The relative amount of organic and inorganic tritium present in the tissues of animals exposed to tritium through their drinking water or through their diet.
Annales de l'Association belge de Radioprotection,Vol.7,N°3-4 307 - 316,1982.

J.Van den Hoek, G.B.Gerber, R.Kirchmann.
Tritium metabolism in cow's milk after administration of tritiated water and of organically bound tritium.
Annales de l'Association Belge de Radioprotection,Vol.7,N°3-4 317 - 330,1982.

M.Rochalska, J.Van den Hoek, R.Kirchmann, G.B.Gerber, R.Van Bruwaene.
Incorporation of tritium in milk lipids after feeding organically bound tritium to cows.
Annales de l'Association Belge de Radioprotection,Vol.7,N°3-4 331 - 338,1982.

E.Nurnberger, E.Clausen, G.Kistner.
Investigation on the distribution of tritium and Carbon-14 in the animo acids of labeled green algae (scenedesmus quadricauda ssp.).
Annales de l'Association Belge de Radioprotection,Vol.7,N°3-4 339 - 344,1982.

M.Rochalska, R.Van Bruwaene, G.B.Gerber, R.Kirchmann.
Organically bound tritium and its distribution in mice fed organically labeled milk powder.
Annales de l'Association Belge de Radioprotection,Vol.7,N°3-4 345 - 352,1982.

R.Van Bruwaene, G.B.Gerber, R.Kirchmann, J.Maes, E.Fagniart.
Incorporation and metabolism of tritium in pregnant mice and their offspring after feeding organically labeled tritiated milk powder during pregnancy.
Annales de l'Association Belge de Radioprotection,Vol.7,N°3-4 353 - 362,1982.

M.Rochalska, R.Van Bruwaene, G.B.Gerber, R.Kirchmann.
Organic tritium in brain of new-born pigs from mothers who had received tritium water during pregnancy.
Annales de l'Association Belge de Radioprotection,Vol.7,N°3-4 363 - 374,1982.

II. Papers presented at the U.I.R. workshops.

A) Workshop "Present and Future Objectives in Radioecology", Brussels 30 March,1982.

- P.Bovard. - Rapport National (France).
- A.Grauby. - Un avenir pour la Radioécologie?
- S.I.Auerbach. - Overview of North.South American Radioecology,Research programs.
- A.Polykarpov. - Etude des problèmes actuels en radio-écologie à la lumière des solutions apportées aux problèmes d'énergie atomique.
- P.O.Agnedal. - Review of Radioecology in the European Nordic Countries.
- N.S.Fisher. - Review of radioecological research activities in the Mediterranean region (including Portugal,Switzerland and Austria).
- Nishiwaki . - Radioecology and radioactivity studies in Japan and other Far-East countries.

B) Workshop "Comparing Soil-Plant Transfer factors of Radionuclides".Wageningen, 7 - 11 December 1982.

- W.Steffens, W.Mittelstaedt, F.Führ.
  Soil-plant radionuclide transfer of 90-Sr,137-Cs,60-Co and results from lysimeter experiments 1979/80.

- E.Haak.
  Long term transfer of 137-Cs and 90-Sr from soil to barley and grass under Swedish field conditions.

- M.Pimpl, H.Schüttelkopf.
  Investigation of the soil-plant transfer of Pu, Am, Cm,and Np in a greenhouse under controlled climatic conditions.

- D.S.Popplewell, G.J.Ham and J.W.Stather.
  The uptake of 239-Pu + 240-Pu, 241-Am, 90-Sr and 137-Cs into potatoes.

- A.Eriksson.
  Long term open field lysimeter study on the transfer of transuranics from soil to crop plants.

- C.Grouzelle, D.Riff, C.Colle, H.Camus, R.Ducousson, A.Saas.
  Facteurs de transfert du Cesium-137 entre certains sols polynésiens et une herbe à fourrage.

- A.Saas.
  Transfert sol-plante pour un sol brun calcaire.

- P.A.Cawse.
  Data on transfer factors for Cs-137 and Pu-239 + 240.

- A.Saas.
  Valeurs expérimentales de transfert sol-plante des isotopes obtenues par le laboratoire de Radioécologie terrestre du CEN Cadarache (France).

- A.Wiechen and K.Heine.
  Some remarks on the Significance of concentration factors (Transfer factors).

- K.Heine and A.Wiechen.
  Field studies of the transfer factors soil plant of 137-Cs and Sr-90.

- W.Kühn, J.Handl, P.Schuller.
  The influence of soil parameters on 137-Cs^{+}uptake by plants from long-term fallout on forest clearings and grass land.

- A.Haisch, P.Capriel, H.Stärk.
  Report on the preliminary results from the first two years of a three years lasting investigation.The experiment served for the assessment of transferfactors soil-plant for caesium and strontium at different soils.

- J.F.Stoutjesdijk, J.Sinnaeve, J.H.van Ginkel, R.M.J.Pennders, R. Sibbel and G.M.Desmet.
  Determination of soil-plant transfer factors at ITAL, Wageningen,The Netherlands.

- U.Boikat, A.Fink.
  Cesium transfer on permanent pastures from soil to vegetation : comparison of methods to determine the concentration ratio.

- R.Kirchmann, E.Fagniart.
  First year results of field experiments of soil-plant transfer of Co-60, Sr-85 and Cs-134.

III C

SOMATISCHE SOFORTWIRKUNGEN IONISIERENDER STRAHLUNG

SHORT-TERM SOMATIC EFFECTS OF IONIZING RADIATION

EFFETS SOMATIQUES A COURT TERME DES RAYONNEMENTS IONISANTS

Weitere Forschungsarbeiten zu diesen Themen werden auch in folgenden Tätigkeitsberichten beschrieben :

Further research work on these subjects is also described in the following progress reports :

D'autres travaux sur ces thèmes de recherche sont également décrits dans les rapports suivants :

III A. Muth, H./Grillmaier, R.E.	Univ. Homburg	BIO 289 D
III B. Kühn, W.	GSF Hannover	BIO 314 D
III D. Duplan, J.F.	Fond. Bergonie Bordeaux	BIO 371 F
III D. Maisin, J.R.	CEN, SCK Mol	BIO 378 B
III D. Maisin, J.R.	CEN, SCK Mol	BIO 379 B
III D. EULEP	Bordeaux	BIO 390 F
III E. Bootsma, D.	Univ. Rotterdam	BIO 404 NL
III E. Bridges, B.A.	MRC Brighton	BIO 414 UK
III E. Lohman, P.H.M.	TNO Rijswijk	BIO 403 NL
III E. Radman, M.	Univ. Bruxelles	BIO 420 B
III E. Rörsch, A./Van de Putte, P.	Univ. Leiden	BIO 408 NL
III E. Simons, J.W.I.H.	Univ. Leiden	BIO 407 NL
III E. van der Eb, A.J.	Univ. Leiden	BIO 405 NL
III E. van der Eb, A.J.	Univ. Leiden	BIO 476 NL

Progress Report
1982

:ractor:

Contract no.: BIO-C-363-81-UK

stitute of Cancer Research
, Summer Place
-London SW7

ı(s) of research team(s):

of. G. E. Adams
rector,
C Radiobiology Unit
rwell; Didcot
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Dr. E.M. Fielden
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Harwell, Didcot
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ɐral subject of the contract:

lecular processes involved in radiation damage and protection at
e cellular and sub-cellular level.

ɔf projects:

Investigation of the properties of radiation induced radicals derived from DNA and membranes.
Time scale of DNA damage and repair and its modification by radioprotective agents.
Application of rapid mixing techniques in the study of mechanisms of radiation action and protection.

Project Nr.1. Investigation of the properties of radiation induced radicals derived from DNA and membranes.

E.M. Fielden, G.E. Adams, P. O'Neill and A.B. Robins

The interaction of ·OH-radical adducts of dGMP(dG) with thiols and ascorbate

Using the technique of pulse radiolysis, the interactions of ·OH-radicals with 2'-deoxyguanosine (dG) and dGMP and the subsequent interactions of the resulting ·OH-radical adducts with a series of oxidants and reductants have been studied in aqueous solution in the pH range 6-11. It has been demonstrated using tetranitromethane (TNM) and N,N,N',N'-tetramethyl-p-phenylene-diamine (TMPD) that two types of ·OH-radical adducts of dGMP (dG) are produced with respect to their redox properties. The yields of the oxidising and reducing type ·OH-radical adducts of dGMP (dG) are 51% and 56% of the yield of ·OH radicals, respectively, and are independent of pH. Typical radioprotective agents such as the thiols, cysteine, glutathione and mercaptoacetic acid, and ascorbate have been shown to interact preferentially with those ·OH-adducts of dGMP (dG) with oxidising properties in an electron transfer process ($k \sim 3 \times 10^7 - 1.4 \times 10^9$ $dm^3mol^{-1}s^{-1}$) as implied from the pH dependence of the rate constants. It is inferred from experiments performed in the presence of oxygen (30-70 μmol dm^{-3}) and TNM that the ·OH-radical adduct of dGMP (dG) with oxidising properties does not interact with O_2 or other oxidants ($k < 10^6$ $dm^3mol^{-1}s^{-1}$).

The ·OH radical is suggested to add preferentially to dGMP (dG) at C_4 to yield the adduct with oxidising properties and at C_5 and C_8 to yield the adduct with reducing properties. In fact, it is not necessary to invoke the formation of a radical cation of dGMP to interpret the experimental results.

Repair of radical adducts of dGMP (dG) by antioxidants such as thiols and ascorbate only occurs with those adducts with oxidising properties and, furthermore, a competition for these radicals by O_2 or other oxidants should not be important. On the other hand, radicals produced by H-atom abstraction are repaired by thiols, via H-atom donation, but not by ascorbate. However, since RS·(RSSR·⁻) radicals interact with ascorbate ($k \sim 6.5 \times 10^8 dm^3mol^{-1}s^{-1}$ for cysteine), ascorbate may indirectly repair H-atom abstraction radicals,

via an electron cascade whereby RS· is converted to RS^- (H^+) in its reaction with ascorbate.

Since the ·OH radical adducts of dGMP (dG) with reducing properties interact with oxidants (TNM) it is envisaged that "electron-affinic" type radiosensitisers may interact with these radicals in a kinetically-controlled redox process. Structure-activity relationships of radiosensitisers based on electron affinity may, in part, be related to such processes.

Insofar as guanosine is concerned, radioprotective and radiosensitising effects appear to occur via different radical pathways.

Project Nr.2. Time scale of DNA damage and repair and its modification by radioprotective agents.

E.M. Fielden, G.E. Adams, M.J. Tilby and A.B. Robins

We have selected and developed two methods of cell inactivation for our work (see previous report). The first is a chloroform saturated solution of detergent and edta (CSE). After inactivation the DNA remains in a compact configuration within the remains of the cell walls. Consequently it is resistant to shearing and can be easily handled. The second method of inactivation is by adding ethanol.

These techniques have been used to study, in E. coli, the formation of double strand breaks (dsb) and also double strand damage that is not expressed as a dsb. Equipment has been designed and constructed for forming isokinetic exponential type neutral sucrose gradients for centrifugational measurement of DNA molecular weights. The DNA was released from the inactivated cells by digestion with the enzyme pronase, and was shown to be in a form suitable for centrifugational analysis.

Using CSE solution to inactivate cells, we have determined the yields of dsb after irradiation in the presence and absence of oxygen (1.8×10^{-2} and 0.4×10^{-2} dsb/genome/Gy respectively). The yields and OER are in excellent agreement with other results obtained at high dose rates, but are different to those reported by other workers who used X and γ irradiation. We are presently investigating whether or not a dose rate effect is involved in this discrepancy.

When ethanol was used to inactivate cells, the oxic dsb yield was nearly twice the yield observed after CSE inactivation (3.2 dsb/genome/Gy). This could result from the denaturing effect of ethanol causing a destabilisation of the DNA double helix between staggered trans-oriented single strand breaks (ssb). By delaying the ethanol lysis the possibility of rapid dsb repair occurring before CSE solution had inactivated intracellular enzymes was ruled out.

We have also investigated the effect on dsb yield of exposing the DNA to a moderately elevated pH (pH 9.6) as is widely used in the so-called "neutral elution technique". Exploiting the advantages of our techniques, the DNA from irradiated and CSE inactivated cells was exposed to buffers at pH 9.6 and 7.0 for 4 hr at 37°C. The yield of dsb induced by oxic irradiation was doubled by the exposure to pH 9.6. After anoxic irradiation the effect was slightly less marked (70% increase). The increase could have been due to alkaline hydrolysis of damaged sites in the DNA, comparable to the well known alkali-labile sites that give rise to an increase in ssb yields. Another explanation is that the denaturing action of elevated pH caused destabilization of the double helix between staggered trans-oriented ssb, as proposed for the effect of ethanol. When cells were irradiated oxically and inactivated with ethanol the pH effect was almost (75%) as great as when CSE inactivation was used, suggesting that pH and ethanol act largely by different mechanisms.

Thus we have evidence for 3 classes of double strand damage - initial dsb, sites that can be converted to dsb by ethanol and sites that can be converted to dsb by elevated pH.

Project Nr.3. Application of rapid mixing techniques in the study of mechanisms of radiation action and protection.

G.E. Adams, E.M. Fielden and A.B. Robins.

Using the modified rapid-mix apparatus described in the last report, the inactivation dose (D_{37}) for V79-753B cells was found to be 360 $\pm$ 36 rads in air, and 1010 rads in nitrogen, giving an enhancement ratio of 2.80. The addition of a radioprotective agent, 1 M DMSO, to aerobic cells was studied as a function of contact time before irradiation, from 120 to 600 msec. The maximum dose-modifying protective factor for 1 M DMSO was 1.74 when contact was allowed for 30 minutes, and a half maximum value of 1.47 was achieved after 450 msec pre-exposure. The protective effect of DMSO was evidenced by an increase in D_o as has been reported previously, using γ irradiation.

The effect of 1 mM cysteine as a typical sulphydryl protective agent, was also studied under the same conditions. This compound has been reported to increase the shoulder of inactivation curves under both aerobic and anoxic conditions. However, under rapid-mix conditions this could not be confirmed, and both aerobic and anoxic inactivation curves were unaffected by 1 mM cysteine for contact times of less than 600 ms. Increasing the cysteine concentration, up to 5 mM had no effect upon cell viability when incubated in buffer solution for 30' in culture vessels. However, under rapid mix conditions, cell viability declined sharply at concentrations of cysteine of 2 mM and greater, being very concentration-dependent. Pre-irradiation of the cysteine did not enhance this toxic effect so it is postulated that the shear stress produced in cell suspensions during flow through a capillary, and which normally leads to a loss in viability of less than 15%, may be potentiated in the presence of sulphydryl compounds. The mechanism by which this occurs is unclear at the present time. It is evident that radioprotective agents require much longer cell contact times than do radiosensitising agents.

Progress Report
1982

Contractor: **Contract no.:** BIO-C-349-81-F

Institut Curie
Section de Physique et de Chimie
Rue Pierre et Marie Curie 11
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Head(s) of research team(s):

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General subject of the contract:

Lethal effects induced by the transmutation of P-32, P-33, Cu-64 and I-125 incorporated in DNA molecules.

List of projects:

1. Comparison between the fragility of a single - or a double - stranded DNA : study with P-32 and P-33.

Title of project nr1 : Comparison between the fragility of a single -or a double-stranded DNA : study with ^{32}P and ^{33}P.

Head of project and scientific staff : S. APELGOT, P. COLS and E. GUILLE.

Our previous in situ and in vitro studies on the single-stranded DNA of phage S13 labelled with ^{32}P have shown that the strand break probability per transmutation (ϵ_{SSB}) is 1. This event accounts for the lethal effect occurring via the ^{32}P transmutation, the lethal efficiency of which (α) is also 1 (1).

The results are different in the case of ^{33}P transmutation. As the β particles emitted by this isotope have an energy which is lower (248 KeV) than that of ^{32}P (1,71 MeV), their path lengths are also much shorter. This means that, for ^{33}P, the irradiation was more intense than in the experiments with ^{32}P. We defined experimental conditions where the irradiation damage was as small as in the experiments with ^{32}P.

For ^{33}P, the strand break probability per transmutation (ϵ_{SSB}) is less than O.5. This event could not account for the lethal effect occurring via the ^{33}P transmutation, the lethal efficiency of which (α) is O.72 (2). From these results we conclude that ^{33}P transmutation induced at least 2 lethal modifications, one of which is a strand break. Moreover, for ^{33}P-DNA stored in vitro, we observed the appearance, with time, of heavy fractions which can only be a consequence of the hybridation of DNA fragments. Such heavy fractions were never observed for ^{32}P, although they were after irradiated. As irradiation works via radical processes, we suggest that the radical phenomenon of the neutralization of the positively charged sulfur atom formed in the decay could be at the origin of the hybridation observed.

With ^{32}P, this neutralization also exists, but its consequences are masked by the brutality of the strand break due to the high recoil energy available in this decay (77 eV, against 5 eV for ^{33}P).

We planed to obtain more information on this hybridation and to extend this work for a double-stranded DNA.

(1). Apelgot S. (1981) Programme Radiation Protection, Progress Report, 349.

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Progress Report
1982

Contractor:
Medical Research Council
MRC
Park Crescent 20
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Contract no.: BIO-C-361-81-UK

Head(s) of research team(s):
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Biology section
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General subject of the contract:

Radiation damage to cellular membranes at dose levels encountered in industrial and clinical use of radiation.

List of projects:

1. Effects of membrane perturbation on radiation response.
2. Directly induced membrane changes by radiation.

Title of project:

"Effects of membrane perturbation on radiation response"

W.A. Cramp
J.C. Edwards
A.M. George
J. Lunec

D. Chapman (Royal Free Hospital, School of Medicine)

Acholeplasma laidlawii, a mycoplasma, is unable to synthesise unsaturated fatty acids but it will incorporate them into its plasma membrane if they are supplied exogeneously. Thus the fatty acid composition of the cell membrane can be modified by growing the organism in media of defined fatty acid composition. We obtained cells containing one type of unsaturated fatty acid (oleic, linoleic or linolenic acid) and cells containing saturated fatty acid. The radiation sensitivities of these cell lines were determined in air or nitrogen conditions, at ice temperatures or at 37°C and at two different dose rates.

When the cells were irradiated at ice temperature at either dose rate those grown in saturated fatty acids were more radiosensitive than those grown in unsaturated fatty acids. When the temperature of irradiation was 37°C there was little difference in the sensitivities.

The cells were more effectively killed when irradiated in air at 5 Gy/min than when irradiated at 200 Gy/min. Irradiation in air at a dose-rate of 5 Gy/min was more effective at 37°C than at ice temperature. We concluded that cells with unsaturated (fluid) membranes were less radiosensitive than cells with saturated membranes and that lipid peroxidation did not seem to be involved in the cell killing process.

The last conclusion is at variance with the implicit conclusions of earlier workers although the non-involvement of lipid peroxidation has been observed in a similar series of experiments using the E.coli auxotroph K1060.

We have tested the possible involvement of radiation induced lipid peroxidation by directly measuring peroxide products from irradiated pure unsaturated phospholipids and lipid extracts from A. Laidlawii. It was found that pure unsaturated phospholipid dispersions are readily peroxidised by ionizing radiation. Irradiation in nitrogen produces peroxides but also causes breakdown of pre-formed peroxides. Peroxides produced in air are stable to further irradiation in air.

Only a small proportion (<5%) of the total fatty acid available for oxidation is attac. ed. More lipid peroxidation is seen if the dispersion is irradiated at the lower dose-rate. Significant amounts of peroxidation occur for some time after the irradiation period.

No peroxidation was observed in the unsaturated lipids extracted from A. Laidlawii grown on either linolenic or linoleic acid after irradiation with doses which caused peroxidation of the pure phospholipid dispersion and very extensive killing of the intact cell. We deduce that polyunsaturated acids when incorporated in the cell lipids of A. Laidlawii are protected from ionizing radiation by other ingredients not present in the pure unsaturated phospholipid.

In further work with human LDV cells it was confirmed that the clonogenic capacity of these cells after substantial alteration of their overall membrane composition was unaltered. This lack of effect on radiation sensitivity, in contrast to work reported for the bacteria E.coli K1060, was consistent with the fact that little or no change was brought about in the nuclear membrane composition of the radiation sensitive site. The ability of the cells to maintain a low level of unsaturated phospholipids in its nuclear membrane may be an important general defence mechanism against free radical damage.

List of publications

"Radiation studies on Acholeplasma Laidlawii"

J.C. Edwards, D. Chapman and W.A. Cramp.

European Society for Radiation Biology, 17th Annual Meeting, Bordeaux, 1982.

"Effect of membrane fatty acid changes on the radiation sensitivity of human lymphoid cells"

A.M. George, J. Lunec and W.A. Cramp.

Int. J. Radiat. Biol. 1983 in press.

"Radiation studies of Acholeplasma Laidlawii: the role of membrane composition"

J.C. Edwards, D. Chapman and W.A. Cramp.

Int. J. Radiat. Biol. 1983 in press.

Progress Report
1982

Contractor: Contract no.: BIO-C-359-81-B

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General subject of the contract:

Effects of irradiation on the stability and expression of genetic information.

List of projects:

1. Structures and reaction pathways of free radicals induced in DNA-protein complexes.
2. Genetics and biochemistry of DNA repair, mutagenesis and recombination in procaryotes and eucaryotes.
3. Genetic versus epigenetic effects of ionizing radiations and the mechanisms of chromosomal rearrangements.
4. Radiosensitivity of mammalian cells (mouse and human).
5. Low dose irradiation, autoimmunity and immuno competent radiation chimaeras.

Title of project nr 1.

Structures and reaction pathways of free radicals induced in DNA-protein complexes.

Head of project and scientific staff: A. Bertinchamps, S. Gregoli and M. Olast.

Research activity in 1982 has concentrated on the establishment of cause-effect relationships between the transient radical species induced by the radiation in biological substrates and the final degradation products, sources of biological disorder. This is a typical interdisciplinar work, and the clear-cut results already obtained are essentially due to the tight collaboration between our ESR unit in Brussels and the radiation-chemical unit of Dr. Cadet in Grenoble. A coherent approach and a standardized experimental protocol are, in fact, imperative conditions for the obtention of reliable results.

Experimental results are summarized below.

- The model system chosen was the frozen state (to study radiation effects in conditions of prominent direct effects).
- The irradiation dose was 20 Mrads (as great as to permit the formation of workable amounts of degradation products).
- The substances investigated were: a) 5-bromo-6-hydroxy-5,6-dihydrothymidine; b) 5-bromo-6-hydroxy-5,6-dihydrothymine; c) 5-bromo-5,6-dihydrothymine.
- In all of the above substances, the radical structures formed at low temperature were identified as due to the loss of bromine atoms from C5 (5-yl radicals).
- In the presence of molecular O_2, all 5-yl radicals are converted into C5-located peroxyl radicals.
- Single radical kinetics of both, the 5-yl radicals and the peroxyl radicals were determined by the application of the computer analysis of composite ESR spectra developed in our laboratory.
- The final product arising from the chemical conversion of radical 5,6-dihydrothymid-5-yl, was unambiguously characterized as 5,6-dihydrothymine. This result was substantiated by experiments in which thymidine is irradiated in the presence of additives capable to modify its radiation chemistry. Thus, when N-iodoacetamide (a very efficient electron acceptor) was added to the thymidine solution prior to irradiation at 77 K, it has been obser-

ved: (i) an almost complete prevention of the 5-yl radical (due to strong inhibition of its anionic precursor) and a significant reduction of the yield of 5,6-dihydrothymidine. Conversely, when thymidine is irradiated in the presence of equimolar amounts of 2'deoxyadenosine (electron donor with regard to thymidine) it has been observed: (ii) a strong increase in the yield of the 5-yl radical (due to complete electron transfer from A^- to T at the anionic stage of the radiolytic paths) and a significant increase of the yield of 5,6-dihydrothymidine.

List of publications in 1982

1. S. Gregoli, M. Olast and A. Bertinchamps, Mécanismes radiolytiques dans l'ADN hydraté. "Journé d'étude sur la chimie des radiations", Louvain-la Neuve. 111-115 (1982).
2 R. Mathur -DeVré, R. Grimée-Declerck, P. Lejeune and A. Bertinchamps, Hydration of DNA by tritiated water and isotope distribution: a study by ^{1}H, ^{2}H and ^{3}H NMR spectroscopy. Radiat. Res. 90, 441-454 (1982).

Title of project nr 2

Genetics and biochemistry of DNA repair, mutagenesis and recombination in prokaryotes and eukaryotes.

Head of project and scientific staff :

A) M. Radman, F. Bourguignon-Van Horen, A. Brandenburger, P. Caillet-Fauquet, M. Jones, G. Maenhaut-Michel, C. Piarrat*, R. Wagner*, C. Dohet*(research assistant);

B) J. Rommelaere, J.J. Cornelis, C. Dinsart, Z.Z. Su, J.M. Vos.

A) The role of DNA methylation and base pair mismatch repair (MR)in the maintenance of genetic stability. : studies on spontaneous and UV and γ-rays induced mutagenesis.

1. Genetic conservation and diversification by mismatch repair : studies with heteroduplex DNA.

DNA containing mismatched base pairs mimicking replication errors or recombinational heteroduplex were constructed with λ phage DNA strands with controlled DNA methylation. Newly developped methodologies involve in vitro packaging of heteroduplex DNA and infection of relevant E.coli mutants (see previous report). Furthermore selected outside markers in both DNA strands allow a quantitative study of mismatch repair, DNA recombination and DNA strand loss. All possible kinds of mismatches have been introduced into this system in order to determine their reparability, sensitivity to methylation and the specificity of various mismatch correction deficient mutants. Mismatch repair in replicational heteroduplex conserves nucleotide sequence of the parental (methylated) DNA strand ; mismatch repair in recombinational heteroduplex diversifies nucleotide sequence by random localised mismatch repair.(ref. 2, 3)

2. Signal sequences in mismatch repair.

Full methylation of GATC sequences in a DNA strand prevents MR in that strand in E.coli. We are using GATC-free ØX174 phage DNA with and without inserted GATC sequences to construct mismatched heteroduplex molecules in order to test whether GATC sequences are required for mismatch repair. Preliminary results show efficient residual mismatch repair in GATC free ØX heteroduplexes DNA, but indicates a role of GATC sequences in the efficiency of correction.

3. The role of mismatch repair in spontaneous and induced mutagenesis. (see also Euratom contract BIO-E-420-81-B).

We have studied the recA dependent SOS mutator effect induced by UV irradiation of E.coli using unirradiated λ phage as the mutational probe. The finding that some mut$^-$ mutants (deficient in MR) yield a higher UV-induced increase in λ mutagenesis (as observed on two different mutational targets ($c^+ \rightarrow c^-$; $R \rightarrow R^+$)), than the wild type strain favors the hypothesis

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*Present address : C. Piarrat (2 months visitor), R. Wagner (1 month visitor) and C. Dohet : Institut Jacques MONOD, Université Paris VII, France.

that i) SOS induction increases error rate during DNA synthesis, and that ii) such errors reside in the newly synthesized strands. The recA$^+$ dependence of all UV induced mutagenesis observed in mut$^-$ mutants argues against a direct competition for mismatch repair enzymes by the irradiated host DNA. On the basis of the pattern of specific effects of the different mut mutations (mutH, mutL, mutS and uvrE) on the two mutational targets (c$^+ \rightarrow$ c and Ram221 $\rightarrow$ R+), it can be argued that the mutational specificity of the SOS mutator effect is similar to that of spontaneous mutagenesis.(ref.6,7)

4. The isolation of mutH, mutL and mutS proteins by a combined proteine fusion immunological technique.

We are isolating E.coli clones with Mu:dlacAp (Casadaban) insertions in mutH, mutL and mutS genes in order to obtain synthesis of mut/βgal hybrid proteins. Our selection method is based on the fact that inactivation of mut genes renders viable a dam$^-$ recA ts double mutant at 42°C. For this purpose we have constructed a Δ(pro lac) dam$^-$recA ts strain which allows us to measure the expression of the β gal gene in the Mu lysogen. A Mu X derived from the Mu:dlacAp has been isolated that does not kill lysogens at 42°C (Mu:dlacAp has a thermosensitive repressor).

5. Cloning of mut$^+$ genes in cosmid DNA.

We are screening a library of E.coli mut$^+$ DNA cloned in cosmid vector (received from D. Schwartz) in order to isolate cloned mut$^+$ gene copies. Markers mapping very closely to the mut genes are used for selection. Since mutH, L, S and U mutator mutants are recessive, introduction of the appropriate cloned mut$^+$ gene copy should suppress the mutator phenotype in mut$^-$ mutants.

B) Mammalian cells

1. Analysis and early detection of human radiosensitivity syndromes

Several hereditary radiosensitivity syndromes have been identified in humans and are often associated with cancer-proneness (e.g. Ataxia telangiectasia,AT) or tissue degeneration (e.g. Huntington's Disease, HD). The eradication of these diseases would require the early detection of heterozygous or homozygous carriers. We are challenging whether PARVOVIRUSES can be used to that purpose. A number of parvoviruses can be propagated in human cells. The replication of parvoviruses depends on the enzymatic machinery of the host cells. We are testing whether AT and HD cells are impaired in their ability to reactivate γ-irradiated parvovirus H-1 and whether this property can be used for the early identification of afflicted individuals. Cellular defects in the repair and/or tolerance of DNA lesions causing strand breaks or blocking replication, are likely to affect dramatically the survival of parvoviruses, owing to the single-strandedness of the viral genome. In order to test this possibility we first had to transform AT, HD and normal human fibroblasts with the tumor virus SV40. Indeed, we showed previously that human cells are not permissive to parvoviruses unless they are transformed. A series of transformants were isolated from normal and mutant cell cultures inoculated either with SV40 virus or with a fragment of SV40 DNA comprising the transforming region. Transformed cells were shown to support H-1 replication and to display the X-ray sensitivity characteristic of untransformed parent cells. We are now using these transformed cultures to compare the survivals of γ-irradiated parvovirus H-1 in cells from normal donors and from AT and HD patients.

II. Analysis of radio-induced cell transformation

We showed recently that many proliferating mammalian cells resist the lytic action of parvoviruses because they pose an intracellular barrier to parvovirus replication. Moreover this barrier was found to be lifted by cell transformation with tumor viruses, such as SV40. For example, mouse cells selected for their resistance to parvovirus MVM become sensitive to the killing action of this virus after SV40 transformation. It follows that MVM inhibits selectively the *in vitro* transformation of these cells by SV40. Permissivity to parvoviruses can then be considered as a marker of SV40 transformation. In order to test whether this property is specific of SV40 or a general feature of cell transformation, we isolated a series of transformants after γ-irradiation of MVM-resistant mouse cells. Contrary to normal cells, these transformants are able to form colonies in semi-solid medium. We are now testing whether transformation induced by γ-radiation sensitizes cells to the lethal action of MVM, similarly to what was found with SV40. The effect of γ-ray transformation is also studied at the molecular level by comparing the ongoing parvoviral cycles in normal and transformed cultures.

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Minute-Virus-of-Mice inhibits cell transformation by Simian Virus 40.
Nature 300, 537-539.

Title of project nr 3 : Genetic vs epigenetic effects of ionizing radiations and the mechanisms of chromosomal rearrangements.

Head of project and scientific staff :
R. THOMAS, J. RICHELLE.
C. DAMBLY, L. TENENBAUM.
A. TOUSSAINT, M. FAELEN, A. RESIBOIS, M. COLET, F. VAN GIJSEGEM, L. DESMET
J. ROSCAM-SZPIRER, C. SZPIRER, A. POLIARD, D. SAGGIORO.

1. Quantitative analysis of mutational, epigenetic and recombinational effects of radiations and mutagens.

We have constructed a serie of isogenic bacterial strains suitable for the detection of three consequences of DNA damage caused by carcinogens : the mutagenic effect (M), the including effect (I) (i.e. the induction of a λ prophage) and the recombinational effect (R).

This year the test system has been developed both qualitatively and quantitatively :

1. First, we introduced in the tester strain a recB⁻ mutation which decreases the spontaneous level of the recombinational effect by a factor of 10 (from 10^{-2} to 10^{-3}). In this way, we have considerably improved the sensitivity of the recombinational test.
2. Second, we developed a mathematical method that allows us to formalize the experimental curves and calculate the "three potencies" (Mu, I, R).

On the basis of the theoretical curves obtained by fitting the experimental data with a computer program, we have evaluated and compared the capacities of UV, γ rays, MNNG, EMS and mitomycin C to produce mutations (M), induction (I) and recombination (R).

2. Theoretical analysis of epigenetic processes.

A class of epigenetic differences is now well analyzed from the theoretical viewpoint. They result from the occurence in the genetic network of positive feedback loops, i.e., loops which comprise an even number of negative interactions. These systems can be decribed by sets of logical or of differential equations ; the two descriptions fit (and complement) nicely.

3. Isolation of bacterial mutations affecting transposition and replication of the phage Mu.

The viral determinants involved in transposition and replication of the temperate bacteriophage Mu-1 have been identified previously (Toussaint et Résibois, in press); they include the ends of the Mu chromosome and a segment comprising genes A, B and arm.

There was, however evidence that host functions also take part in transposition and replication of this phage. We decided a method to detect such functions. The method relies on the assumption that killing of an induced Mu lysogen is due to either the expression the kil gene of Mu or transposition/replication of the prophage. If this view is correct, a lysogen for a thermoinducible (c_{ts}) Mu prophage carrying a kil mutation should survive heat induction if it lacks a function essential for the transposition and replication of Mu. Moreover, those mutants in which the deficiency corresponds to a host function should survive induction even if the strain carries more than one copy on the Mu prophage.

Using this selection procedure, we succeded in deriving several bacterial mutants. These strains fail to support the growth of Mu (either kil^- or kil^+) but also of the related heteroimmune phage D108. in some of them, the mutation affects not only the expression of the Mu transposition/ replication machinery, but also other early viral functions. Integration and transposition were found to be reduced to different extents by different mutations. Preliminary results suggest that some of these mutations also modulate the transposition frequency of Tn elements.

4. Study by cell fusion of the dominance vs recessivity of cell transformation.

We have previously isolated and characterized several transformed lines of rat fibroblasts ; these lines were obtained either after X ray irradiation or from untreated cultures (spontaneously transformed cells). Somatic cell hybrids have been generated by fusion of these different transformed lines with normal rat fibroblasts. Our results show that in both cases (x-induced, spontaneous) transformation behaves a recessive trait. However, suppressed hybrids may give rise to subclones reexpressing that transformed phenotype. Analysis of the chromosome constitution of the suppressed hybrids and their transformed derivatives showed that the relative dosage of chromosomes derived from the transformed and normal parental cells determines the phenotype (normal vs transformed) of the hybrid cells ; in other words, the two genomes seem to affect cell growth properties in an antagonistic manner.

Publications

A. Toussaint and A. Résibois
Phage Mu : Transposition as a life style (1983) Mobile genetic elements.
J.A. Shapiro ed. Academic press, in press.

D. Saggioro, J. Szpirer & C. Szpirer.
The effect of ploidy and colcemid on the frequency of spontaneous transf-formation of cultured cells .
Cell Biology International Reports 6, 29-38 (1982).

J. Papaconstantinou, E. Wong, H. Ratrie, C. Szpirer & J. Szpirer.
The molecular mechanism of extinction of liver specific functions in mouse hepatoma x rat fibroblast hybrids.
Extinction of the albumin gene.
Somatic Cell Genetics, 8, 363-376. (1982)

J. Szpirer
Le contrôle de l'expression de fonctions différenciées dans les hybrides cellulaires.
Mémoire de la Classe des Sciences de l'Académie Royale de Belgique, ed. Duculot 1982) sous presse.

J. Leclercq et R. Thomas
Analyses booléenne et continue de systèmes comportant des boucles de rétroaction.
II. Système à deux attracteurs formé d'une boucle positive et d'une boucle négative conjuguées.
Acad. Royale de Belgique - Bull. de la Classe des Sciences - 5ème série - Tome LXVII - 1981-3 67, pp. 190-225.

R. Thomas.
Logical description , analysis and synthesis of biological and other networks comprising feedback loops (Adv. Chem. Phys. 1983 - sous presse).

R. Thomas
Fully asynchronous logical description of networks comprising feedback loops. (1982a) - Lecture Notes in Biomathematics, in press.

R. Thomas
Du bacteriophage λ aux organismes supérieurs : la logique de la régulation (In : Biologie Mol. : acquis et perspectives , Ed. Univ. libre de Bruxelles, 1982b - sous presse).

J. Richelle
Analyses booléenne et continue de système comportant des boucles de rétroaction.
I. Analyse continue des boucles de rétroaction simples.
Acad. Royale de Belgique - Buul. de la Classe des Sciences - 5ème série Tome LXVI 1980-11.

D. Saggioro, J. Szpirer and C. Szpirer
The effect of ploidy and colcemid on the frequency of spontaneous transformation of cultures cells.
Cell. Biol. Int. Rep. 6, 29-38. (1982)

A. Résibois, A. Toussaint et M. Collet
DNA structures induced by Mini-Mu replication.
Virology 117, 329-340 (1982)

M. Colet, A. Résibois et A. Toussaint.
Identification of a mini-Mu by computer analysis of partially denaturated DNA.
The EMBO Journal Vol.1., n°8, pp.959-963. (1982)

A. Résibois, M. Colet et A. Toussaint
Localisation of mini-Mu in its replication intermediates.
The EMBO Journal, Vol.1., n°8, pp.965-969 (1982).

Title of project nr 4. Radiosensitivity of mammalian cells (mouse and man).

Head of project and scientific staff :
P. Van Gansen and J. Brachet
F. Zampetti-Bosseler and H. Alexandre.

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1. Chromosome damage and mitotic delay in normal human and ataxia-telangiectasia fibroblasts treated with X-rays or bleomycin (F. Zampetti-Bosseler and D. Scott, Paterson Laboratories, Manchester, U.K.).

X-ray-sensitive cells from patients with ataxia-telangiectasia (A-T) have been shown to be sensitive to bleomycin (Taylor et al., 1979, Cancer Res., 39, 1046). We have shown that this is also the case for X-ray-sensitive mouse lymphoma cells. These observations suggest a common lethal mechanism for X-rays and bleomycin. Since the induction of chromosome aberrations is believed to be the predominant mechanism of cell killing after ionising radiation, we have compared chromosome aberration frequencies in normal and A-T fibroblasts exposed to equitoxic doses of X-rays and bleomycin.

At similar levels of cell killing, bleomycin produces very much less chromosome damage than X-rays. The two agents may have a common mechanism of action which involves cell-lethal chromosome damage, but we must postulate an additional and quantitatively more important mechanism for bleomycin, which does not involve such damage.

We have previously postulated a common DNA lesion involved in mitotic delay and chromosome aberration (F.Zampetti-Bosseler and D. Scott, 1981, Int. J. Radiat. Biol., 39, 547). The observation of less mitotic delay induced by bleomycin than by X-rays at equitoxic doses supports this hypothesis.

2. Radiosensitivity of early mouse embryos (H. Alexandre).

We proposed previously that the lack of early teratogenic risk but the very high level of preimplantation and early postimplantation abortions result of a cell number insufficient to form an inner cell mass. We therefore analysed the relationships between the cell cycles progress and the morphogenesis in the mouse. The use of aphidicolin, a specific inhibitor of DNA polymerase α , enabled us to conclude that the 4^{th} replication cycle is "quantal" for the cavitation process. Aphidicolin has now been applied on activating mouse blastocysts : it exerts a clearcut differential effect on trophectoderm, which undergoes normally its outgrowth and giant cells

formation, and inner cell mass which fails to develop : this may suggest that DNA endoreduplication could be less dependent on DNA polymerase α than embryonic cells do.

On the other hand, we showed that inhibition of DNA polymerase β and γ by d_2TTP exerts no deleterious effects on preimplantation development ; this demonstrates that both enzymes are not required for this developmental stages and confirmed indirectly the absence of any mitochondrial DNA replication during this period. We now try to estimate the effects of this drug on UDS induced by UV and/or X-rays in cleaving mouse embryos.

Finally, in collaboration with Dr M. Geuskens, we analysed, at the ultrastructural level, the ontogenesis of the fibrillar centres of the nucleoli in relation to the onset of rDNA transcription: it is obvious that the centres are already present at the periphery of the primary nucleoli at the late 2- and early 4- cell stage and that they are actually the sites of rDNA transcription.

List of publications in 1982

I. Publications in Scientific journals, Monographs, Proceedings.

F. Zampetti-Bosseler

- D. SCOTT et F. ZAMPETTI-BOSSELER.
  Comparative studies on ataxia telangiectasia and radiation-sensitive mouse lymphoma cells.
  "Ataxia telangiectasia - A Cellular and Molecular Link Between Cancer, Neuropathology, and Immune Deficiency."
  Eds. B.A. Bridges and D.G. Harnden. 1982 John Wiley and Sons Ltd. P. 227-234.
- D. SCOTT et F. ZAMPETTI-BOSSELER
  Cell cycle dependence of mitotic delay in X-irradiated normal and A-T fibroblasts.
  A-T Newsletter 2, 6-7, 1982.
- D. SCOTT et F. ZAMPETTI-BOSSELER.
  Mitotic delay, chromosome damage and cell death in ataxia-telangiectasia cells.
  Brit. J. Radiol., 55, 471, 1982.
- D. SCOTT et F. ZAMPETTI-BOSSELER.
  Cell cycle dependence of mitotic delay in X-irradiated normal and ataxia-telangiectasia fibroblasts.
  Int. J. Radiat. Biol., 42, 679-683, 1982.

H. Alexandre

- H. ALEXANDRE
  Effect of Aphidicolin on the blastocyst formation in the mouse.
  Abstracts book : XVth EDBO International Embrylogical Conference. Abstract Nr 101, P. 48, 1982.
- H. ALEXANDRE, B. DE PETROCELLIS and J. BRACHET
  Studies on Differentiation Without Cleavage in Chaetopterus. Requirement for a Definite Number of DNA Replication Cycles Shown by Aphidicolin Puses. Differentiation 22, 132-135, 1982.
- H. ALEXANDRE
  Effet de l'inhibition spécifique de la réplication de l'ADN par l'aphidicoline sur la différenciation primaire de l'oeuf de Souris en préimplantation. C.R. Acad. Sc. Paris 294, 1001-1006, 1982.
- M. GEUSKENS and H. ALEXANDRE
  Effects of an Inhibitor of Spermidine and Spermine Synthesis on the ultrastructure of early mouse embryos.
  Biol. of the Cell 56, 10 p., 1982 (In Press)

II. Short Communications, Theses, Internat Reports, Patents..

- H. ALEXANDRE :
  Etude du déterminisme du premier événement morphogénétique chez un Mammifère : la formation du blastocyste de la souris. In : "Biologie Moléculaire 1976-1981", U.L.B., Faculté des Sciences, vol. III, 415-430, 1982.
- H. ALEXANDRE :
  Biologie de la reproduction et du développement embryonnaire des Mammifères. Sommaire. - Cours libre de la Faculté des Sciences. Presses Universitaires de Bruxelles, première édition 1982-1983.

Title of project nr 5. "LOW DOSE IRRADIATION, AUTOIMMUNITY and RADIATION CHIMAERAS".

Head of project and scientific staff : J.Urbain, M.Slaoui, J-D Franssen, G.Vansanten, C.Demeur, A.Van Acker et P.Meyers.

Broadly speaking, regulation in the immune system operates at two levels :

a) The Selection of Available Repertoires. During any immune response, only a small part of the potential repertoire is used. A large part of the repertoire is silent because of natural tolerance and the development of active suppressive mechanisms. Lymphocytes are in a state of dynamic equilibrium. The normal life span of B lymphocytes is only a few days. Furthermore, the repertoire available to antigens is not set up once and forever. There is a constant release of newly differentiating lymphocytes from bone marrow to periphery.

b) The regulation of an ongoing immune response. Triggering of an immune response leads to activation of a small subset of helper T cells and to the concomitant induction of suppressor T lymphocytes.

The effects of irradiation are much more complex, than previously thought. Suppressor T lymphocytes are extremely radiosensitive in certain phases of their development and some subsets of B lymphocytes are extremely radioresistant.

Particular attention must be given to the induction of autoimmune processes after low dose irradiation (5 to 100 rads). Several methodologies have been developed : the measurement of B cell frequencies under conditions of polyclonal activation, the

study of in vitro primary responses of peripheral blood lymphocytes (there is a high enhancement after low dose irradiation), the method of splenic focus assay in radiation chimaeras. In this last method, limiting numbers of primed or unprimed T lymphocytes (irradiated or not) are mixed with a constant number of B lymphocytes. The mixture is then used to repopulate irradiated recipients. After allowing homing of grafted cells during 48 hours, the recipient spleen is diced into fragments, containing 0, 1, 2 or 3 T cells. These fragments are then stimulated with antigen.
This kind of methodology allows to study the effects of irradiation on a few clones of lymphocytes specific for self or non self antigens.

PUBLICATIONS

- "Reduced tumor growth after low-dose irradiation or immunization against blastic suppressor T cells". A.F.Tilkin, N.Schaaf-Lafontaine, A.Van Acker, M.Boccadoro,J.Urbain. 1981. Proc.Natl.Acad.Sci.USA, 78, 3, 1809-1812.
- "A study of in vitro primary responses from rabbit peripheral blood lymphocytes". C. Demeur, G.Urbain-Vansanten, M.Vaeck, C.Bruyns and J. Urbain.1981. Immunology Letters,2, 297-304.
- "Idiotypic lymphocytes in human monoclonal gammopathies". M. Boccadoro, A.Van Acker, A.Pileri and J. Urbain. 1981. Ann. Immunol. (Inst.Pasteur), 132C, 9-19.
- "Idiotypic regulation in immune networks".J.Urbain and C. Wuilmart. 1981. Contemporary Topics in Molecular Immunology, 8, 113-148.
- "Le réseau immunitaire". J. Urbain. 1981. dans La Recherche, mensuel, n° 126, vol.12, octobre 1056-1066.
- "Monoclonal antibodies against urokinase". P.Hérion, C.Glineur, J-D Franssen,J.Urbain, A.Bollen. 1981. Bioscience Reports, 1, 885-892.

- "Selection of myeloma lines suitable for hybridoma. 1981. J-D Franssen, P. Hérion and J. Urbain. Protides or the Biological Fluids.
- "A study of hybridoma soft agar cloning". P. Hérion, J-D Franssen, J.Urbain. 1981. Protides of the Biological Fluids.
- "Some thoughts on idiotypic networks and immunoregulation". J.Urbain and C.Wuilmart. Immunology Today, 1982. vol.3, 4, 88-92.
- ""Antigens in the inside". 1982. Edit. I. Schnurr Hoffman La Roche.
- "Idiotypes, recurrent idiotypes and internal images. 1982. Urbain, J., Slaoui, M., Leo, O. Ann. Immunol.(Inst.Pasteur, 133 D, 179-189.
- "Idiotypic manipulation in mice : Balb/c mice can express the crossreactive idiotype of A/J mice. 1983. M. Moser, O. Leo, J. Hiernaux, J. Urbain. Proc.Natl.Acad. Sci. USA, in press.
- "From clonal selection to immune networks : induction of silent idiotypes. 1981. Ann. N.Y. Acad. of Sciences, in press. J. Urbain et al.
- "Induction of antiarsonate CRI positive antibodies in Balb/c mice. 1982. O. Leo, M. Moser, Hiernaux, J., J. Urbain. Ann. N.Y. Acad. of Science, in press.
- "Studies of the arsonate system using monoclonal antibodies. 1982. J.Hiernaux et al. Ann. N.Y. Acad. of Science, in press.
- "Study of helper and suppressor T cells in cystic fibrosis. 1982. R.Van Geffel, E.Hubert and J.Urbain. Immunology Letters, 5, 155-169./

Progress Report
1982

Contractor:

Gesellschaft für Strahlen-
und Umweltforschung mbH
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

Contract no.: BIO-C-343-81-D

Head(s) of research team(s):

Prof. Dr. U. Hagen
Inst. für Biologie
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

General subject of the contract:

Radiation induced degradation of nucleobases in DNA : primary effects and structural changes.

List of projects:

1. Radiation induced degradation of nucleobases in DNA : primary effects and structural changes.

Radiation induced degradation of nucleobases in DNA: primary effects and structural changes. Bio-C-343-81-D

Prof. Dr. Ulrich Hagen
Dr. Wolf Bors
Dr. Heidi Martin-Bertram
Dipl. Phys. Manfred Saran
Dr. David Tait

I. Competition kinetic experiments have been carried out to determine the reactivity of one-electron reduced fatty acid hydroperoxides with components of DNA. These studies indicated a rather inefficient reaction of the 9- and 13-hydroperoxides of linoleic acid and the 13-hydroperoxide of linolenic acid with the DNA components uracil, thymine, cytosine, adenine and 2-deoxy-D-ribose. Thus, only an upper limit of 2×10^5 $M^{-1}s^{-1}$ for the 2nd order rate constant K could be calculated for these reactions. These studies are being continued to include the corresponding nucleosides and nucleotides.
In the above experiments, the hydroperoxides were reduced using $FeSO_4$. The discrepancy between these results and those obtained from experiments in which the hydroperoxides were homolytically decomposed with u.v. radiation may imply a difference in the reactivity of the initially formed alkoxy radicals produced by these two methods.
The optimal experimental conditions have been ascertained for measuring the melting characteristics and kinetics of re-annealling of a synthetic, double stranded polynucleotide* following irradiation in the absence and presence of lipid components (e.g. unsaturated fatty acids and their hydroperoxide derivatives). Preliminary work shows that peroxidising arachidonic acid substantially alters the melting behaviour of the polynucleotide, while incubation with $FeSo_4$/13-hydroperoxy linoleic acid mixture had no measurable effect on the melting behaviour of the polynucleotide. Commercial samples of unsaturated fatty acids are not pure enough for these experiments. High performance liquid chromatography has therefore been used to isolate the pure acids.
Studies on the reactions of peroxy radicals, peroxy radical/O_2^- mixtures and hydroxyl radical/O_2^- mixtures with DNA components have been postponed until the pulse radiolysis detection system has been fully commissioned.

*
Poly[deoxy(adenylic-cytidylic)acid]:poly[deoxy(thymidylic-guanylic)acid]

II. In th second year of th contract, we continued to probe λ-DNA for radiation- induced "bulky lesions". For 1982 we obtained the following results:
1. After γ-irradiation of bacteriophage λ particles in situ (anoxic), each bulky lesion - as analyzed by S1 nuclease cleavage - was associated with a radiation- induced nick. Thus, the number of bulky lesions per dose can now easily be counted (f.e. 2500 Gy produced 2-3

Sl- sensitive sites). Sl nuclease incised unmatchable "loops" facing each radiation- induced nick and leaving the DNA molecule only with double strand breaks. In contrast to complete Sl nuclease properties, no hydrolysis of the incised single strand occured - probabely due to muliple helix distortions and base modifications.
2. γ-irradiation of λ-DNA solutions ("in vitro") exhibited no extra Sl- sensitivity, but pronounced changes in melting properties as a result of misparings. However, these mispairings were obtained after complete separation of both DNA strands (denaturation) and reannealing under conditions where otherwise intact DNA, as well as in situ γ- irradiated DNA, matched complementary. This implies strongly, that the distribution of multiple lesions was different after γ- irradiation in situ or in vitro, resulting in clustered damages (in situ) or minor random distributed lesions (in vitro). It might be speculated that clustered DNA damages, introduced by direct absorption of energy in DNA, might be prereqisites for production of double strand breaks.
3. UV- irradiation (254 nm) on λ-DNA induced also sensitivity against Sl nuclease. As a preliminary result we got with each dose of UV- light about three times more single strand breaks after Sl enzyme administration. The DNA molecule was all times free from double strand breaks. Melting temperatures were dose- dependently low, also from enzyme treatment and indicated temperature- labile mispairings in the extended area of the bulky lesion. It became abvious that complete Sl nuclease activity (incision and hydrolysis) was inhibited by structure and size of the bulky lesions, cleaving the DNA strands facing the main lesions.
4. Studies with various restriction endonucleases and UV- irradiated DNA resulted in shifting DNA fragment patterns up from 500 J/m^2 (Eco R I, Hind III). The extend of shifting was dependent from the size and pyrimidine composition of the recognition sequence (Hind III > Eco R I > Hha I, Hae III). We will continue this work with respect to bulky lesion analysis in defined DNA fragments to get some knowledge about localization and size (see programme 1983).

List of publications in 1982

Bors, W., Saran, M., Michel, C.: Radical intermediates involved in the bleaching of the carotenoid crocin. Hydroxyl radical, superoxide anions and hydrated electrons. Inter. J. Radiat. Biol. 41, 493-501 (1982)
Bors, W., Michel, C., Saran, M.: Antioxdidants: their function and mechanism as radical scavenger. Proceedings III. Int. Conference on SOD, Nevele, New York (in press)
Bors, W., Saran, M., Michel, C.: Assays of oxygen radicals: methods and mechenisms. In: Superoxide Dismutase (Ed.: L.H. Oberley) Boca Raton, Fla.: CRC Press (in press).
Martin-Bertram, H.: Heteroduplex DNA in bacteriophage Tl after ionizing radiation. In: Chromosome Damage and Repair (Eds.: E. Seeberg, K. Kleppe). New York, London: Plenum Press, 35-40 (1982)
Saran, M., Tait, D., Bors, W., Michel, C.: Formation and reactivities of alkoxy radicals. Proceedings III Inter. Conference on SOD, Nevele, New York (in press).

Progress Report
1982

Contractor:

Universität Regensburg
Postfach 397
D-8400 Regensburg

Contract no.: BIO-C-342-81-D

Head(s) of research team(s):

Prof. Dr. J. Hüttermann
Universität Regensburg
Postfach 397
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Prof. Dr. A. Müller-Broich
Universität Regensburg
Postfach 397
D-8400 Regensburg

General subject of the contract:

Studies of the effects of ionizing radiation on DNA and its constituents.

List of projects:

1. Free-radical mediated DNA-strand-break induction.

Title of project nr. 1:

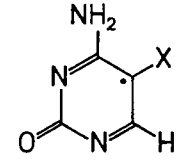
Free-radical mediated strandbreak-induction in DNA

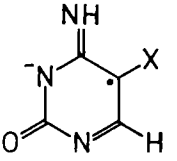
Head of project and scientific staff:

Prof. Dr. J. Hüttermann

Dr. H. Riederer (1.1.-30.9.82)
J. Krieger

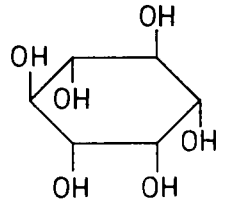
Two aspects of previous work which dealt with the possible free radical mechanisms involved in DNA radio-sensitization by 5-halouracil incorporation had to be followed up. Having elucidated in detail the base oxidation mechanisms by $SO_4^{-\cdot}$ and (excited) solvent holes in acidic glasses[1], a cooperation was started with the group of M.D. Sevilla (Rochester, Michigan, USA) to probe the corresponding oxidations by Cl_2^- in neutral and basic LiCl glasses for both the halouracils and the group of 5-halocytosines. Again, extensive powder-type spectra simulations and INDO-calculations were necessary to prove that the initial radicals in the latter were indeed cations (I) which deprotonate not at N_1 as is the case for the 5-halouracils but at the exocyclic nitrogen to yield structure (II). This work is being written up presently[2] and has been submitted for a poster at the 7th ICRR in Amsterdam[3].

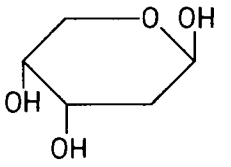
(I) 

(II) 

Another aspect involved the reactions of mobile electrons with 5-halouracils. Since dissociative electron attachment to 5-bromouracil in glasses is mostly pH dependent, it was important to find a system in which the structure and the fate of the electron adduct radical can be studied. Glasses of BeF_2/H_2O only partially fulfill this requirement since the pH of 8M solutions is about 3. Another system included in the investigations which comprise acidic and alkaline glasses in addition[4] was $Be(OH)_2$ which offers an intermediate pH.

In searching for free radical mediated mechanisms of strandbreak induction in DNA, the survey of reactions of H·-atoms in acidic glasses with carbohydrates was continued. For inositol, (III), a detailed comparison between the glass[5] and the OH·-reactions in water[6] could be performed. Furthermore, a newly developed system for studying OH· reactions in aqueous glasses was presented and applied, among others, to inositol and deoxyribose (IV)[7]. A good correlation for the radical structures was found for (III) between both glasses and the solution on one hand as well as for (IV) between the two glasses.

(III) 

(IV) 

Part of this and of work under the previous contract has been presented in invited symposia[8], lectures[9] and in a review[10], dealing with the solid state radiation chemistry of DNA.

Work aimed at the elucidation of sugar radicals in single crystals has been performed in cooperation with W.A. Bernhard (Rochester, New York, USA) during a sabattical term of the head of the research team. The sugar employed was mannose for which ESR-ENDOR-data of three different radicals have been obtained so far.

LITERATURE:

I. 1) Matrix isolation of free radicals from 5-halouracils. 3. Electron spin resonance of base oxidation in aqueous acidic glasses
H. Riederer and J. Hüttermann; J. Phys. Chem. 86, 3454 (1982)

2) π-cations of 5-halouracils and 5-halocytosines in LiCl glasses
M.D. Sevilla, H. Riederer and J. Hüttermann
J. Phys. Chem., to be submitted

3) An ESR-study of the production and reaction of the π-cation radicals of the 5-halouracils and 5-halocytosines.
M.D. Sevilla, H. Riederer and J. Hüttermann
7th ICRR, Amsterdam 1983, submitted for poster presentation

4) H. Riederer, Thesis 1981, University of Regensburg, Germany

5) Free radicals from myoinositol formed by H· reaction in acidic glasses.
J. Krieger and J. Hüttermann; Int. J. Rad. Biol., submitted

6) The selectivity of attack by the hydroxyl radical on myoinositol and the importance of stereo-electronic factors upon radical rearrangement: an ESR conformational-analysis study.
B.C. Gilbert, D.M. King and C.B. Thomas; J.C.S. Perkin II,1980,1821

7) Hydroxyl radicals in aqueous glasses: characterization and reactivity studied by ESR-spectroscopy
H. Riederer, J. Hüttermann, P. Boon and M.C.R. Symons
J. Magn. Res., submitted

II. 8) Symposia: a) Molekulare und zelluläre Mechanismen der Wirkung ionisierender Strahlen.
Gesellschaft für Strahlen- und Umweltforschung, München, 17.3.-19.3.82

b) Electron Spin Resonance of Radicals in Organic and Bio-organic Systems
Royal Society of Chemistry,
Nottingham University, 22.3.-26.3.82

9) Lectures on aspects of the radiation-chemistry of DNA
J. Hüttermann

1) NIH, Washington, Juni 1982
2) Alabama State University, Juni 1982
3) Georgia State University, Juni 1982
4) Roswell Park Memorial Institute, Buffalo, New York, October 1982
5) Oakland University, Michigan, October 1982

10) Review: Solid-state radiation chemistry of DNA and its constituents
J. Hüttermann
Ultramicroscopy 10, 25 (1982).

Progress Report
1982

Contractor:

Universität Münster
Westring 5
D-4400 Münster

Contract no.: BIO-C-470-81-D

Head(s) of research team(s):

Prof. Dr. W. Köhnlein
Institut für Strahlenbiologie
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Hittorfstrasse 17
D-4400 Münster

General subject of the contract:

Effects of ionizing radiation and chemical agents in combination at the molecular level, over the dose range encountered in clinical and industrial use.

List of projects:

1. Combined effects at the molecular level of radiation and chemicals acting specifically on DNA.

Title of project:
Combined effects at the molecular level of radiation and chemicals acting specifically on DNA

Head of project and scientific staff:
Prof. Dr. W. Köhnlein
Institut für Strahlenbiologie, Universität Münster

Among the various hazards the biosphere is exposed to radiation and chemicals play a major role. In the programme of the past year the effects of radiation and chemicals interacting with the DNA have been investigated under three different aspects: position labeling of DNA, 5-halouracil substitution of DNA, and intercalating or binding chemicals. Linear as well as supercircular DNA was used. Different experimental techniques have been employed partly in collaboration with scientists at the Universities of Stockholm (Sweden) and Regensburg (Germany).

1.) For the elucidation of radiation damage in biologically important molecules ^{3}H- and ^{14}C-position labeling was exploited to give information on radiolytical reactions of too low yield for conventional detection methods. Radiation induced tritium cleavage and demethylation were measured with position labeled T2 DNA using thymine-6-^{3}H, thymine-(methyl-^{3}H), and thymine-methyl-^{14}C) for labeling. Radiolytical reactions of the corresponding precursors were also investigated over a wide dose range (10^{2} - 10^{5} Gy) in the presence and absence of radical scavengers. Tritium cleavage follows a linear dose-effect relation in all cases up to 90% release of total activity. G-values were highest for thymidine and lowest for DNA. Demethylation is of special interest because of the carcinogenic efficiency of alkylating agents. Radiolytically released methyl radicals can react with O- and N-positions in DNA leading to alterations with important biological consequences. For the demethylation a linear dose-effect relation was observed only with thymine-(methyl-^{14}C) up to the highest doses employed (10^{5} Gy). For thymidine-(methyl-^{14}C) and DNA-(methyl-^{14}C) after an initial linear increase of demethylation a decrease with a slope of -0.5 in a log-log-plot was found upon further irradiation. Thus indicating that methyl radicals cleaved from thymidine only react with the sugar moiety of the molecule, strengthening the

notion that methylation preferentially occurs at O-positions. Experiments with dimethyl sulfoxide indicate that OH-radicals are responsible for the demethylation.

2.) Position specific alterations in the DNA are produced by the incorporation of 5-halouracils into the DNA. A cooperative international investigation of the nature and fate of radiation induced free radicals in oriented fibers of bromouracil-substituted DNA yielded information of the production of a new 5-bromouracil anion radical. Furthermore the yield of the guanine cation radical was higher in 5-bromouracil DNA than in normal DNA. To extend this work attempts were made to include chlorouracil- and possibly iodouracil-substituted DNA for studying the primary and secondary radiation induced free radicals. All the 5-halouracils will alter the electronic configuration and may influence more or less the transfer of excitation energy in the DNA molecule, thus allowing a systematical search for the sensitization process in DNA. It was the responsibility of this laboratory to work out the conditions for the production of chlorouracil DNA and iodouracil DNA in amounts sufficient for wet spinning, orienting and ESR investigation. The first oriented chlorouracil DNA fibers have been obtained. The conditions for producing DNA highly substituted with iodouracil have been investigated and DNA samples with 35% iodouracil substitution have been prepared already. For the experiments planned, however, even higher substitutions are required.

3.) To investigate the combined effects of radiation and chemicals acting specifically on DNA further experiments were carried out with a number of chemicals to learn more about their effects on DNA. Ionizing radiation was not yet employed. It was confirmed that bleomycin and peplomycin intercalate into the DNA. Phleomycin interacts with DNA by a different mechanism as shown by the altered electrophoretic mobility of supercircular, circular, and linear DNA as well. The interaction of these chemicals including neocarzinostatin with DNA has at least partly been the subject of 4 diplom degree theses which are therefore included into the list of publications.

By continuing and extending these investigations it is hoped to contribute to a better understanding of occupational hazards and their reduction.

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

W. Köhnlein und G. Jung: Neocarzinostatin: Molekulare Wirkungsweise und Perspektiven der klinischen Anwendung. Drug Research/Arzneim. Forsch. 32 (1982) 1474-1479.

O. Merwitz und W. Köhnlein: Gamma-induced demethylation of T2 DNA-(thymidine-methyl-^{14}C) in oxygenated aqueous solution. Proceedings of the 5. Tihany Symposium on Radiation Chemistry. 1982.

F. Schmülling und W. Köhnlein: Induktion von Einzel- und Doppelstrangbrüchen in linearer und superzirkularer DNA durch Phleomycin. Zeitschrift für Naturforschung 37. (1982) 1228-1233.

II. Short Communications, Theses, Internal Reports, Patents..

W. Köhnlein and O. Merwitz: Gamma-induced tritium cleavage from T2 DNA-(thymidine-methyl-^{3}H or -6-^{3}H) in deoxygenated aqueous solution. Jahrestagung der European Society of Radiation Biology, 1982, Bordeaux. Abstract.

J. Plotz: Erhöhung der Strahlenempfindlichkeit durch Aufnahme und Einbau von Basenanalogen in bromuraziltolerierenden Mikroorganismen. Untersuchungen zur Frage der Energieleitung in vivo. Dissertation, 1982. Münster.

G. Lüders: Physikochemische Untersuchungen zur Erhöhung der Selektivität des Antitumorproteins Neocarzinostatin durch Ankopplung an tumorspezifische Vektoren. Diplomarbeit, 1982. Münster.

F. Schmülling: Induktion von Einzel- und Doppelstrangbrüchen in lineare und superzirkulare DNS durch Phleomycin. Diplomarbeit, 1982. Münster.

D. van Leyen: Physikochemische Untersuchung zur gezielten Veränderung des Antitumorproteins Neocarzinostatin. Diplomarbeit, 1982. Münster.

W. Stöhler: Untersuchungen der Zuckerkomponente in der durch Bleomycin degradierten DNA mit chromatographischen und massenspektrometrischen Methoden. Diplomarbeit, 1982. Münster.

Progress Report
1982

Contractor:

Contract no.: BIO-C-356-81-NL

Vrije Universiteit
De Boelelaan 1081
NL-1007 mc Amsterdam

Head(s) of research team(s):

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Natuurkundig Laboratorium VU
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NL-1007 mc Amsterdam

General subject of the contract:

Investigation of the relationship between radiation induced primary damage in DNA and the biological activity of DNA.

List of projects:

1. Relationship between radiation induced primary damage in DNA and the biological activity of DNA.

Title of project nr B10-C-356-81 NL.

Relationship between radiation-induced primary damage in and the biological activity of DNA.

Prof.Dr. H. Loman, Mr J. Woldhuis, Dr. J.B. Verberne, Dr. M.V.M. Lafleur, Prof.Dr. J. Retèl, Prof.Dr. Joh. Blok.

With the objective to contribute to the correlation between radiation-chemical damage and radiobiological effects in DNA, we irradiated the biologically active DNA of the bacteriophage ϕX174, in frozen dilute aqueous solutions under a variety of conditions. After thawing of the samples, the biological activity and sometimes the number of radiation-induced breaks in the DNA are measured. Occasionally the effects of an alkaline treatment on these quantities are determined.
The results reported below refer to frozen solutions with low ionic strength ($\sim 6 \times 10^{-4}$ M) or to systems with high ionic strength (~ 0.1 M).

a) DNA solutions at low ionic strength.

1. Slow freezing, i.e. solidification under nearly equilibrium conditions (which induces [DNA]-inhomogeneities) results in considerable decreased radiosensitivity as compared with rapid freezing of the samples (e.g. in liquid N_2 ($-196^{\circ}C$) on irradiation of deaerated samples at $-20^{\circ}C$. On irradiation of anoxic samples at $-196^{\circ}C$ the dependence of radiosensitivity on the method of freezing tends to disappear.
2. In deaerated rapidly frozen solutions a careful study has been made of the influence of irradiation temperature. A considerably increasing radiosensitivity was observed with increasing temperature (5 temperatures between $-196^{\circ}C$ and $-3^{\circ}C$). Moreover the survival curves show a more or less biphasic nature: a steep initial part turns into a flatter exponential one. Deaerated, slowly frozen solutions show a similar, although less pronounced behaviour. In general, in our earlier work this biphasic nature is often concealed by the scattering of the survival measurements most probably due to not yet understood varying properties of the ice

matrix per sample. Fractionated irradiation has no effect on the biphasic course of the survival curves.

3. Independent of the method of freezing it is shown the OER = 2.0 ± 0.5 both for survival and break production . Furthermore it was confirmed that the contribution of breaks to inactivation of the ϕX DNA amounts to about 40%.
4. In an attempt to analyze the data obtained, a model is being developed of which the main features are that DNA inactivation is due to a) local effect (or 'direct' effect) and b) radicals which only react with the DNA during the process of melting. The prelimenary results of fitting such a model to the data are encouraging.

b) DNA solutions at high ionic strength (∿ 0.1 M).

Increase of the salt concentration in a solution results in an improved homogeneity if the components in the frozen state (see e.g. G. Taborsky, J. Biol. Chem. 245, 1063 (1970)). Therefore a number of experiments were done at an ionic strength of about 0.1 M in order to rule out effects due to inhomogeneities in the radiation experiments. The appearance of the frozen samples becomes independent of the method of freezing (fast or slow). We give the following results without interpretation.

1. The radiation sensitivity of the DNA becomes almost indenpendent of the method of freezing both in oxygenated and deaerated frozen solutions.
2. The radiation sensitivity has increased considerably (by a fact of at least 10) as compared with the low ionic strength experiments.
3. The difference in radiation sensitivity of the DNA in oxygenated and deaerated frozen solutions disappears, i.e. OER ≈ 1.0 (and sometimes even < 1).
4. Although less clear than in low ionic strength systems, the biphasic nature of the survival curves is also frequently observed.

Progress Report
1982

Contractor:

Contract no.: BIO-C-488-82-UK

The Royal Marsden Hospital
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Head(s) of research team(s):

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Prof. G.E. Adams
Physics
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GB-Surrey SM2 5PT

General subject of the contract:

Evaluation of short and long term effects of high dose total irradiation on normal tissues other than the haemopoietic system.

List of projects:

1. Study of dose-response relationships relating to the short and long-term effects of hifh dose total body irradition of normal tissues.

A detailed analysis of the patterns of nausea and vomiting in 107 patients treated with high dose total body irradiation has been made. These patients have been treated with 9.5 - 10.5 Gy total body irradiation using a Cobalt unit at a dose rate of 0.025 Gy per minute; this dose is specified as the maximum lung dose. The dose distribution in other areas of the body varies by ± 5%. A small sub-group of patients were treated using a linear accelerator to the same total dose but at a dose rate of 0.08 Gy per minute. The age and sex distribution is shown in Fig. I. There was an apparent threshold to onset of vomiting at 2 Gy. 38% of patients who vomited during treatment had the onset of their vomiting between dose of 2 and 4 Gy (Fig. II) although the first attack of vomiting was seen in some patients as late as 8 Gy.

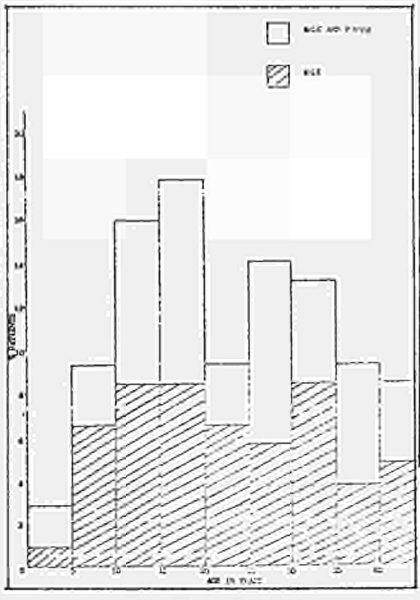

Fig. I

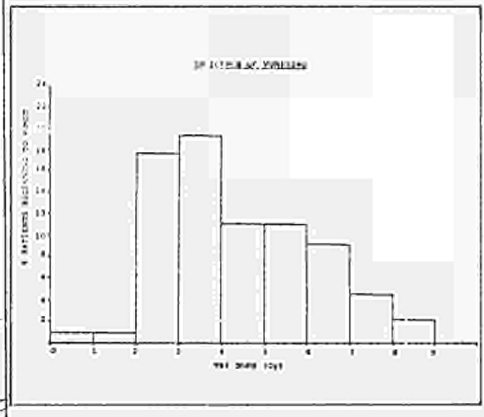

Fig. II

Fig.III shows that 22% of patients experienced no nausea or vomiting during the total body irradiation procedure. Onset of vomiting was earlier in patients treated at a higher dose rate on the linear accelerator suggesting that the threshold is related to the dose received. In the 24 hours following TBI between 10 and 30% of patients experience some nausea and vomiting but this was generally very mild. From days two

to five vomiting was experienced by 70 - 80% of patients although this was again not usually severe. After five days this symptom improved. Nausea and vomiting was also associated with an incidence of mild diarrhoea in 60% of patients which was maximal between days five and eight after irradiation although the mean number of stools passed per day was only two, indicating the modest nature of the problem. These baseline studies provide information for the design of future studies on the effectiveness of specific anti-emetics such as dopamine antagonists.

A remote respiration monitor for use during TBI has been developed as shown in Fig. IV and preliminary data is being collected. Techniques have also been devised for monitoring heart rate, and work is in progress to perfect techniques for monitoring temperature and blood pressure during whole body irradiation.

It is hoped that this basic physiological data will help to elucidate reactions to accidental acute radiation exposures and provide a means for evaluating therapeutic measures which might be taken in such circumstances.

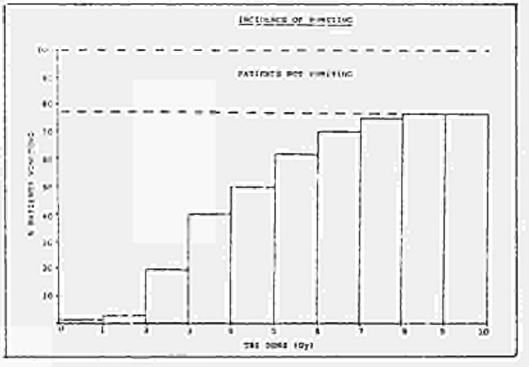

Fig. III

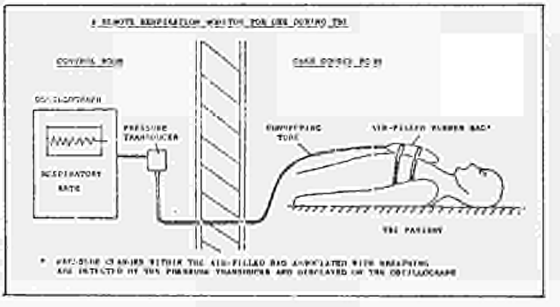

Fig. IV

List of publications in 1982 (TBI)

I. DEPLEDGE, M.H., BARRETT, A.
(1982)
Dose-rate dependence of lung damage after total body irradiation in mice.
International Journal of Radiation Biology 41 (3), 325-334

BARRETT, A.
(1982)
Total body irradiation before bone marrow transplantation: A review.
Clinical Radiology 33, 131-135

BARRETT, A., DEPLEDGE, M.H.
(1982)
Total body irradiation: some factors affecting outcome.
Experimental Hematology 10 (10), 56-63

DEPLEDGE, M.H., BARRETT, A., MORGENSTERN, G. et al
(1982)
Pulmonary oedema with leaky endothelia syndrome.
Experimental Hematology 10 (10), 113

BARRETT, A.
(1982)
Hospital facilities required for the treatment of the seriously irradiated casualty.
In Procedures following major radioactive contamination.
Publ University of Bristol and the Bristol and Weston Health District (T)
pp 64-77

BARRETT, A., JACOBS, A., KOHN, J., RAYMOND, J., POWLES, R.L.
(1982)
Changes in serum amylase and its isoenzymes after whole body irradiation.
British Medical Journal 285, 170-171

BARRETT, A.
(1982)
Total body irradiation (TBI) before bone marrow transplantation in leukaemia: a co-operative study from the European Group for Bone Marrow Transplantation.
In British Journal of Radiology. 55, 562-567

BARRETT, A.
(1982)
Systemic Irradiation in the Management of Malignant Disease - its Value and Limitations.
In Cancer Topics, Vol 4, No. 2.

II. Thesis. "Total Body Irradiation for Bone Marrow Transplantation in Leukaemia".

Progress Report
1982

Contractor: Contract no.: BIO-C-441-81-I

Consiglio Nazionale
delle Ricerche (CNR)
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General subject of the contract:

Molecular mechanisms in radiosensitization by oxygen.

List of projects:

1. Molecular mechanisms in radiosensitization by oxygen.

Title of project nr. 1
Molecular mechanisms in radiosensitization by oxygen.

Head of project and scientific staff: M. Quintiliani, G. Barile, V. Capuano, C. Catena, B. R. Guerra, G. Simone and M. Tamba

The general idea behind the present project is to investigate the possibility of exploiting the genetic control of the oxygen effect on the radiation response of bacterial and mammalian cells for a better understanding of the molecular mechanisms of the oxygen radiosensitization.

The existence of genetic characteristics influencing the level of the oxygen enhancement ratio (OER) is fairly well established in microorganisms, while the existence of a corresponding situation in mammalian cells is supported by, et least, the evidence that mutations interfering with the biosynthesis of glutathione produce cell lines with increased radiosensitivity and drastically reduced OER for cell survival and DNA damage production. (1)

Our investigations were carried out on bacteria and mammalian cells in culture.

Bacterial experiments - Four strains of E. coli K12 were used: a wild type, mutants in RecA and UVRA genes and a double mutant in the same genes. The mutant strains were already reported to exhibit reduced OER for cell survival (2). Survival experiments were carried out in stationary phase of growth in M9 medium. Cells were suspended in .15 M phosphate buffer pH 7 and irradiated with cobalt-60 gamma rays. Next table shows the results

Strains		$D_o N_2$(Gy)	$D_o O_2$(Gy)	OER
AB1157	wild type	196	66	3.0
AB1886	$UVRA^-$	101	50	2.0
AB2463	$RecA^-$	31	14	2.2
AB2480	$UVRA^- RecA^-$	45	24	1.9

In our experimental conditions, the double mutant does not exhibit an OER value as low as reported by previous Aa's. We have not, at the moment, a definite explanation for the discrepancy, however preliminary experiments seem to indicate that it might be due to nutritional factors connected with the use of the M9 medium instead of nutrient broth. Already Ahmed et al (3) have reported that the OER for cell survival in AB2480 strain is dipendent on the amount of aeration of cultures during preirradiation growth, as well as on the stage of growth,

suggesting a possible correlation with the concentration of endogenous scavenging compounds.

A series of experiments was also carried out to investigate the ability of the four bacterial strain to repair sublethal radiation damage produced in the presence or in the absence of oxygen. The technique used was that of administering a given radiation dose in two fractions, separated by varying time intervals during which bacteria were incubated in growth conditions. None of the strains under test showed any evidence of repair of sublethal damage in any irradiation conditions.

Mammalian cell experiments - Mammalian cell experiments mainly consisted of screening tests for OER relative to radiation survival of mutant lines locally available.

Some naturally mutant lines of human fibroblasts, obtained from patients affected by Xeroderma Pigmentosum or Ataxia telangiectasia did not show any significant difference in OER with respect to fibroblasts from normal subjects.

A significant decrease of the OER (from 3.4 to 2.3) was found for radiation survival in an artificially induced UV sensitive mutant line of CHO cells (CHO-43 RO, kindly supplied by M. Stefanini from the "Istituto di Genetica, Biochimica ed Evoluzionistica, CNR). The mutant cells exhibited normal sensitivity to gamma rays in aerobic conditions and reduced sensitivity in anoxia.

References

1. M. Edgren, et al, 1981, 40, 355
2. M. Quintiliani, 1979, Int. J. Radiat. Oncology Biol. Phys., 6, 1069
3. A.H. Ahmed, et al, in "Oxygen and Oxy-radicals in Chemistry and Biology", 1981, Eds. M.A.J. Rodgers and E.L. Powers, Academic Press, New York, London, Toronto, Sydney, San Francisco.

Progress Report
1982

Contractor:

Contract no.: BIO-C-469-D

Hahn-Meitner Institut
für Kernforschung Berlin GmbH
Post box 39 0128
D-1000 Berlin 39

Head(s) of research team(s):

Prof. Dr. W. Schnabel
HMI für Kernforschung Berlin GmbH
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General subject of the contract:

The influence of protecting agents on radiation damage processes in biomolecules.

List of projects:

1. The influence of protecting agents on radiation damage processes in biomolecules.

Title of project No. 1 The influence of protecting agents on radiation damage processes in biomolecules

Head of project and scientific staff: Prof.Dr.W.Schnabel
Dr.U.Gröllmann (in part)
K.-J.Deeg (in part)

The elucidation of the mode of action of protectors strongly depends on the initial assessment of the nature of transient species involved in the development of radiation damage sites in biopolymers. The present studies, devoted mainly to radiation effects in nucleic acids in aqueous solution, were experimentally based on the application of the light scattering detection method in conjunction with pulse radiolysis (16 MeV electrons, pulse duration: 100 ns). This method allows the measurement of changes of the size of macromolecules as a function of time and is, moreover, capable of yielding new information on both the nature of radiation damage sites and the mode of action of protectors. The important results obtained with anoxic systems[1-5] are described as follows: With native calf thymus DNA, crosslinking could be clearly discriminated from double strand breakage[1]. The former proceeded faster than the latter. The influence of ionic strength on both processes was studied. In order to learn more about single strand breaks, experiments with denatured DNA were performed[1]. Two modes of light scattering intensity (LSI) decrease were detected. The rapid mode was attributed to the rate of fragment diffusion. The slow mode, on the other hand, was found to be correlated to the lifetime of free radical sites at the sugar moieties. These radicals can react with protectors present in the system. Systematic investigations on this point were carried out with several ribonucleic acids, poly A, poly U, and poly C[3,4], which exist, at pH 7 to 8, as single stranded molecules and are stable with respect to alkaline hydrolysis. Denaturated DNA appeared to be less appropriate for these experiments, at this stage of the investigation, because of the possibility of partial renaturation. Fig.1 presents, characteristically, the two-mode LSI decrease observed, in this case, with poly A.

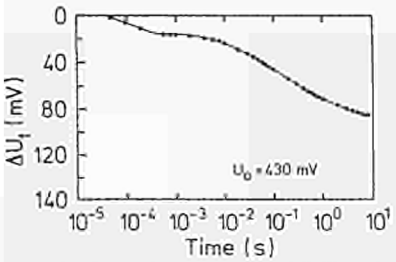

Fig. 1 - Main-chain scission of poly A in N_2O-saturated aqueous solution at pH 7.8 and 23°C. Change of the signal voltage, corresponding to the LSI, as a function of time after the pulse. Abs. dose per pulse: 22 Gy, [poly A]: 0.25 g/l; [$NaClO_4$]: 0.01 mol/l; [phosphate buffer]: 0.005 mol/l.

With the three polynucleotides, a significant acceleration of the slow mode of the LSI decrease, upon the addition of cysteamine or glutathione, was observed. Rate constants of the reaction $P\cdot + RSH \longrightarrow PH + RS\cdot$, determined in these experiments, are of the order of 10^6 l/mol s. It is noteworthy that the extent and the rate constants of LSI decrease are not the same for the three polynucleotides. The differences indicate the influence of the chemical nature of the bases on main-chain rupture. Taking into account that strand breaks are initiated by the attack of OH radicals at the sugar moieties ($OH + PH \longrightarrow H_2O + P\cdot$), it is concluded that, apart from attack at the 4'-carbons, OH attack at carbons in the 1' or 2' position of the ribose moiety contributes essentially to the degradation mechanism.

Results obtained in additional investigations with polynucleotide complexes[5] (self-complexed poly A at pH 4, poly (A+U) and complexes of poly A and Mg^{2+}) corroborated assumptions on double strand breakage in native DNA developed in earlier publications.

Investigations on the oxidative degradation of nucleic acids, which was another subject of intense work, were completed in 1982 as far as certain aspects, especially those pertaining to diffusion-controlled intramolecular reactions, are concerned[6]. This aspect was also treated theoretically[7]. A concise report upon various aspects of this research was given at an international conference in Tokai-mura (Japan)[8].

Related to the work on nucleic acids is a study on the radiation-induced aggregation of ribonuclease[9]. In this case dimerization and multimerization of the enzyme molecules was investigated. The rate constant of dimerization was determined by light scattering measurements. Dimerizations via phenoxyl radicals of tyrosine became feasible from optical absorption measurements.

List of Publications in 1982

I. Publications in Scientific Journals

1) K.Washino and W.Schnabel
Radiation-induced molecular size changes in native and denatured deoxyribonucleic acid under anoxic conditions. A pulse radiolysis study
Makromol. Chem. 183, 697 (1982)

2) K.Washino and W.Schnabel
Radiation-induced molecular size changes in DNA in the presence of p-nitroacetophenone. Pulse radiolysis in conjunction with light-scattering measurements
Int.J.Radiat.Biol. 41, 271 (1982)

3) K.Washino and W.Schnabel
OH radical-induced main-chain scission of poly(riboadenylic acid)
Makromol.Chem.Rapid Commun. 3, 427 (1982)

4) K.Washino, O.Denk, and W.Schnabel
OH radical-induced main-chain scission of poly(ribonucleic acids) under anoxic conditions
Z.Naturforsch. 38c, in print

5) O.Denk, K.Washino, and W.Schnabel
OH radical-induced chemical reactions of polynucleotide complexes
Makromol.Chem., 184, 165 (1983)

6) O.Denk and W.Schnabel
Über die oxidative Hauptkettenspaltung einiger Nukleinsäuren. Pulsradiolytische Untersuchungen
Z.Naturforsch. 37c, 405 (1982)

7) I.A.Raap and U.Gröllmann
The stochastic kinetics of intramolecular reactions in linear polymers
Makromol.Chem., 184, 123 (1983)

8) W.Schnabel, O.Denk, U.Gröllmann, I.A.Raap, and K.Washino
On the reactions of macroradicals in solution. A pulse radiolysis study
Rad.Phys.Chem., in print

9) H.Seki and W.Schnabel
On the free radical-induced aggregation of ribonuclease. A pulse radiolysis study using the light scattering detection method
Z.Naturforsch. 37c, 63 (1982)

II. Doctoral Theses

O.Denk
Zur Kinetik strahlungsinduzierter Reaktionen an Polynukleotiden in Lösung.
Technische Universität Berlin (1982)

U.Gröllmann
Radikalische Abbau- und Vernetzungsprozesse von Makromolekeln in Lösung.
Technische Universität Berlin (1982)

K.Washino
Kinetic and mechanistic investigations on the radiation-induced reactions of DNA and other nucleic acids in solution.
Hokkaido University, Sapporo, Japan (1982)

Progress Report

1982

Contractor:

Max-Planck-Gesellschaft zur
Förderung der Wissenschaften
Postfach 647
D-8000 München

Contract no.: BIO-C-341-81-D

Head(s) of research team(s):

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MPI für Strahlenchemie
Stiftstrasse 34-36
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General subject of the contract:

Chemical studies of chain breaks in DNA induced by gamma-irradiation.

List of projects:

1. Chemical studies of chain breaks in DNA induced by gamma-irradiation.

Title of project nr BIO-C-341-81-D:

Chemical studies of chain breaks in DNA induced by gamma-irradiation

Head of project and scientific staff:

Prof. Dr. D. Schulte-Frohlinde
Prof. Dr. C. v. Sonntag

Dr. M.N. Schuchmann

In a preceding study we observed, that a set of four enzymes which degrade unirradiated DNA to nucleosides does not fully cope with irradiated DNA[1]. It was envisaged to develop a technique to accumulate non-digested material for further analysis. Unfortunately experimental difficulties which we feel might be soon overcome prevented us to present acceptable data. Since some of the enzymatic procedures take considerable time, another project not proposed but directly related to the general subject of our contract has been taken up side by side. This project yielded very interesting results. Very little had been known so far about the decay processes of peroxyl radicals of phosphate esters. In DNA such peroxyl radicals would be formed in the presence of oxygen from the 3'- and 5'-radicals. Trimethylphosphate was used as a model system and the complex reaction sequence elucidated with the help of product analysis as well as pulse radiolysis[2]. It has been found that oxyl radicals play a major role as intermediates in the decay of these peroxyl radicals. This finding supports our earlier interpretation of the mechanistic aspects of the formation of some products identified in irradiated DNA[3,4]. It has been shown that superoxidedismutase has a small but measurable protecting effect in the trimethylphosphate system. It is suggested that in DNA this effect might be considerably larger. Experiments will be carried out to check this hypothesis.

1 M. Dizdaroglu, W. Hermes, D. Schulte-Frohlinde and C. v. Sonntag
Enzymatic digesterion of DNA γ-irradiated in aqueous solution
Separation of the digests by ion-exchange chromatography
Int. J. Radiat. Biol. 33, 563-569 (1978)

2 M.N. Schuchmann and C. v. Sonntag
Radiolysis of di- and trimethylphosphate in oxygenated aqueous solution. A model system for DNA strand breakage
J. Chem. Soc. Perkin Trans II (submitted for publication)

3 M. Dizdaroglu, D. Schulte-Frohlinde, and C. v. Sonntag
Radiation chemistry of DNA, II
Strand breaks and sugar release by γ-irradiation of DNA in aqueous solution. The effect of oxygen
Z. Naturforsch. 30c, 826-828 (1975)

4 M. Isildar, M.N. Schuchmann, D. Schulte-Frohlinde, and C. v. Sonntag
γ-Radiolysis of DNA in oxygenated aqueous solutions: alterations at the sugar moiety
Int. J. Radiat. Biol. 40, 347-354 (1981)

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

Published:

M. Isildar, M.N. Schuchmann, D. Schulte-Frohlinde, and C. v. Sonntag
Oxygen uptake in the radiolysis of aqueous solutions of nucleic acids and their constituents
Int. J. Radiat. Biol. 41, 525-533 (1982)

In print:

G. Behrens, G. Koltzenburg, and D. Schulte-Frohlinde
Model reactions for the degradation of DNA-4' radicals in aqueous solution. Fast hydrolysis of α-alkoxyalkyl radicals with a leaving group in ß-position followed by radical rearrangement and elimination reactions
Z. Naturforsch.

E. Bothe and D. Schulte-Frohlinde
Release of K^+ and H^+ from poly U in aqueous solution upon γ and electron irradiation. Rate of strand break formation in poly U
Z. Naturforsch.

Submitted:

M.N. Schuchmann and C. v. Sonntag
The radiolysis of uracil in oxygenated aqueous solutions. A study by product analysis and pulse radiolysis
J. Chem. Soc. Perkin Trans II

M.N. Schuchmann and C. v. Sonntag
Radiolysis of di- and trimethylphosphate in oxygenated aqueous solution. A model system for DNA strand breakage
J. Chem. Soc. Perkin Trans II

Progress Report
1982

Contractor: Contract no.: BIO-C-362-81-UK

The University of Leicester
University Road
GB-Leicester LE1 7RH

Head(s) of research team(s):

Prof. M.C.R. Symons
Department of Chemistry
University of Leicester
GB-Leicester LE1 7RH

General subject of the contract:

Application of spectroscopic techniques especially e.s.r. to the study of radiation effects in DNA and DNA-protein complexes.

List of projects:

1. Application of spectroscopic techniques especially e.s.r. to the study of radiation effects in DNA, DNA complexes, and related materials.

TITLE OF PROJECT: Application of spectroscopic techniques especially ESR to the study of radiation effects in DNA and DNA-protein complexes

HEAD OF PROJECT: Professor M. C. R. Symons,
Department of Chemistry,
The University,
LEICESTER. LE1 7RH (GB)

A. DNA in solid glasses

Progress has been slight since our previous report. We do not consider that glassy systems will provide the best way ahead for solid-state studies, but we are still trying to find more satisfactory glass-forming materials in the hope of obtaining more concrete information.

B. DNA + Oxygen

Clear ESR signals from superoxide ions have been obtained from oxygenated aqueous systems irradiated at 77 K. Since these are enhanced strongly as the [DNA] is increased the superoxide ions must be formed close to the DNA molecules. On annealing we detect partial conversion into $HO_2\cdot$ radicals prior to the growth of signals assigned to $RO_2\cdot$ radicals. Although these $RO_2\cdot$ radicals are formed primarily by reaction with DNA radicals (R·)

$$R\cdot + O_2 \longrightarrow RO_2\cdot \qquad \ldots. (1)$$

they may also be formed by direct action of O_2^- ions. This interesting possibility is currently being studied. We also have some evidence that $\cdot O_2^-$ ions can add to carbonyl groups:-

$$O_2^- + >C=O \longrightarrow >C\begin{matrix}\diagup OO \\ \diagdown O^-\end{matrix} \qquad \ldots. (2)$$

to give centres resembling $RO_2\cdot$ radicals. This possibility is also being followed up, as are several other interesting facets of the oxygen effect.

During the course of this work it became clear that, in addition to the normal G^+ and T^- centres formed by ionizing radiation, there are relatively low yields (between 1 and 10%) of another radical exhibiting considerable proton hyperfine coupling that could well be a deoxy ribose radical. No firm identification has yet been achieved, so we refer to this as radical X. We are currently endeavouring to obtain trends in the concentration of X with concentration and with oxygen content.

C. DNA + Additives

We have used two classes of additives which bind to DNA, namely metal cations and unsaturated organic compounds. Our aim is primarily to discover effective electron-traps so as to reduce the concentration of T^- and hence to discover the separate rôles that T^- and G^+ centres play in the damage mechanism. However, we hope also to discover additives that are specific hole-traps so that G^+ formation is suppressed. If such additives can be found, then we will explore the results when both are incorporated together.

Metal Cations. - Our results with silver cations remain curious: although we have been unable to detect Ag° or Ag(II) centres by ESR spectroscopy, nevertheless the total yield of $G^{+} + T^{-}$ is clearly reduced, and in particular formation of TH is strongly suppressed.

Similar results were obtained with Hg(II), although in this case, low yields of Hg(I) were detected unambiguously by observing ^{199}Hg hyperfine features at high field. Work using Tl(I) and Cd(II) are in progress.

Of the other ions that we have used, Cu(II) is the most interesting. Very preliminary results suggest that low concentrations of Cu(II) have a marked effect on radical yield, with strong suppression of T^{-} and TH, but also a reduction in G^{+} yield. However, species X appeared to be better defined in these experiments.

Organic Additives. - Our major effort has been directed towards the nitroimidazole drugs which are known to act as good electron-trapping agents. In these systems, clear ESR signals from the imidazole radical anions were observed, together with correspondingly reduced yields of T^{-} and TH. However, our results suggest no major change in the yield of G^{+}. Other organic additives having strong effects on the yields of G^{+} and T^{-} include tetracyanoethylene and 9-bromoanthracene.

D. Strand Break Studies

In all the above studies, "linear" DNA (calf thymus DNA) was used to save time and cost. However, it seemed to us to be essential to obtain an accurate monitor of the systems under study in terms of the yields of single and double strand breaks. Fortunately, we have been able to collaborate with Dr. Paul Cullis of the Chemistry Department and with several biochemists at Leicester who have the latest DNA technology. By using specially prepared cyclic DNA samples coupled with gel electrophoresis we have developed methods for assaying both single and double strand-breaks with considerable accuracy. We are already able to state that strand-breaks do occur efficiently after 77 K radiolysis under our conditions and warming to room temperature, and that these are indeed modified by additives. This work is in its infancy, but ultimately we hope to be able to show how our yields of specific radicals correlate with single and double strand-breaks.

Progress Report
1982

Contractor:

Commissariat à l'Energie
Atomique, CEA
CEN de Grenoble
B.P. n° 85 X
F-38041 Grenoble Cédex

Contract no.: BIO-C-350-81-F

Head(s) of research team(s):

Dr. R. Téoule
Lab. de Radiobiochimie
CEA - CEN de Grenoble
B.P. n° 85 X
F-38041 Grenoble Cédex

Dr. J. Cadet
Lab. de Radiobiochimie
CEA - CEN de Grenoble
B.P. n° 85 X
F-38041 Grenoble Cedex

General subject of the contract:

Study of DNA injury caused by ionizing radiation.

List of projects:

1. Study of DNA injury caused by ionizing radiation.

Title of project nr BIO-C-350-81-F

Study of DNA injury caused by ionizing radiation

Heads of project :

Dr. R. Téoule and Dr. J. Cadet,
Laboratoire de Radiobiochimie, Laboratoires de Chimie,
Département de Recherche Fondamentale, CEA-CEN Grenoble
85 X, F.38041 GRENOBLE CEDEX

The mechanisms of the radiation-induced degradation of 2'-deoxyguanosine in oxygen-free aqueous solutions have been investigated through the isolation and the characterization of the final diamagnetic products resulting from the fate of the transient organic radicals. Special emphasis has been made on the radical reactions regarding the aglycone and the osidic moiety of this nucleoside respectively. The analytical difficulties due to the relatively high polarity of 2'-deoxyguanosine and its related decomposition products have been overcome by using reverse phase high performance liquid chromatography (RP HPLC). The two main nucleoside derivatives which result from radical reactions within the purine moiety have been identified as the α and β anomers of N_7-(2-deoxy-D-erythropento pyranosyl)-2,6-diamino-4-hydroxy-5-formamido pyrimidine on the basis of spectroscopic measurements including ^{1}H NMR, U.V., C.D. and mass spectrometry. A reasonable mechanism for the formation of these modified nucleosides would involve initial hydroxyl radical addition at carbon C(8), followed by hydrogen atom transfer to atom N(7) and subsequent hydrolysis of the resulting diamagnetic compound at the C(8)-N(7) bond. The weakening of the N-glycosidic bond due to the opening of the imidazole ring may explain the rearrangement of the osidic moiety which gives rise to the two more stable α and β pyranoid isomers.

The final products resulting from initial hydrogen abstraction reaction within the sugar ring are quantitatively more important than in the γ-radiolysis of aqueous deaerated solutions of thymidine. This may be accounted for by the high efficiency of the restitution processes regarding the purine base (G(-) 2'-deoxyguanosine ~ 0.6). An interesting reaction which involves the transient formation of a carbon centered

radical at position 4', as demonstrated by using selectively 4'-deuterated 2'-deoxyguanosine, generates the cyclo-5',8-dideoxy-2',5'-guanosine. The fate of the corresponding osid-5'-yl radical leads to the formation of the related 5'-aldehydo nucleoside derivative. It has to be pointed out that the corresponding oxidized nucleoside is not produced by radiolysis of oxygen-free aqueous solution of 2'-deoxyadenosine (in fact, the 5'-yl radical gives rise to a second type of anhydronucleoside through an aromatic intramolecular cyclization reaction). γ-deoxyribonolactone which implies hydrogen abstraction at carbon C(1') in the early stage of its formation has been assigned by comparison of its spectroscopic properties with those of an authentic sample.

These results would provide a better understanding of the rather complicated radiation-induced radicalar events regarding both the purine and the osidic moieties of 2'-deoxyguanosine in oxygen-free aqueous solutions. This study represents also an important step which would facilitate further investigation of more complicated DNA model compounds such as dinucleoside-monophosphates and DNA itself.

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

J. CADET, M. BERGER and L. VOITURIEZ, Separation of radiation and photo-induced 5,6-dihydrothymine derivatives by RP HPLC, J. Chromatogr. 238, 488-494 (1982).

J. CADET and R. TEOULE, Radiation-induced binding of radiosensitizing drugs to DNA in Free Radicals and Cancer (edited by R.A. Floyd) Chapter 6, pp. 183-200, M. Dekker, Inc., New-York, 1982

J. CADET, L. VOITURIEZ and M. BERGER, Separation of nucleic acid components and some of their radiation-induced degradation products by RP-TLC, J. Chromatogr. (in press).

II. Short Communications, Theses, Internal Reports, Patents ...

M. BERGER and J. CADET, Mécanismes de la dégradation radio-induite de la désoxy-2'-guanosine en solution aqueuse désaérée. Journées d'études sur la Chimie des Radiations, Louvain, Belgique, 15-18 June 1982.

M. BERGER, Radiosensibilisation de la thymidine en solution aqueuse desaérée. Thèse de Doctorat de 3e cycle, Grenoble, 10 september 1982.

L.S. MYERS, M.J. SCHWENENSEN, S.S. O'CHESKLEY, H.L. LEWIS and J. CADET, Sensitization of cells by bromide ion : comparative studies with E. coli and CHO cells. Radiation Research Conference, Salt Lake City, USA, 18-22 April 1982.

J. CADET, M. BERGER, L. VOITURIEZ and L.S. MYERS, Radiation-induced degradation of DNA nucleosides in KBr solutions. Radiation Research Conference, Salt Lake City, USA, 18-22 April 1982.

R. TEOULE, Synthetic oligonucleotides and Radiation Biochemistry, Yale University (USA) July 2nd 1982.

Progress Report
1982

Contractor: Contract no.: BIO-494-C-EIR

Trinity College
Dublin 2
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Head(s) of research team(s):

Prof. K.F. Tipton
Dept. of Biochemistry
Trinity College
IRL-Dublin 2

General subject of the contract:

The effects of tritiated compounds on the mouse embryo growing in vitro.

List of projects:

1. The effects of tritiated compounds on the mouse embryo growing in vitro.

Title of Project: The effects of tritiated compounds on the mouse embryo growing in vitro.

Head of Project: M.J. Carroll.

The effects of tritiated compounds on the growth and differentiation in vitro of preimplantation mouse embryos are being investigated. The toxicity of tritium is assessed by observing (1) the differentiation of the cleavage-stage blastomeres into trophoblastic and embryo-precursor cells and (2) hatching of the blastocyst from its zona pellucida. Some of the experiments described were carried out in collaboration with the Biochemistry Group, Joint Research Centre, Ispra.

The earlier work of Snow and Streffer on the toxicities of ^{3}H-thymidine and ^{3}H-water were confirmed and the doses (LD_{50}) of those compounds which caused a 50% reduction in growth of the 2-cell embryo to the blastocyst were, respectively, 0.075 µCi/ml and 250 µCi/ml. Comparison of the effects of ^{3}H-amino acids produced interesting results: ^{3}H-arginine or ^{3}H-lysine at a dose of 0.05 µCi/ml caused a 50% reduction in blastocyst formation, while the LD_{50} for ^{3}H-glutamic acid was 10 µCi/ml. It was speculated that the high toxicities of ^{3}H-arginine and ^{3}H-lysine were due to their selective incorporation into the histone fraction leading to net concentration of tritium in the vicinity of DNA. It was unexpected, therefore, that ^{3}H-tryptophan (which is absent from histones) prevented development of the 2-cell embryo (LD_{50} 0.03 µCi/ml) to the blastocyst and higher doses of ^{3}H-tryptophan arrested cleavage of the 2-cell embryo. The 8-cell embryo was less susceptible to damage and 60% of 8-cell embryos progressed to the blastocyst stage in medium containing 1 µCi/ml of ^{3}H-tryptophan. Both the 2-cell and 8-cell embryo rapidly incorporated ^{3}H-tryptophan and, autoradiographic examination of thin sections of embryos exposed to 1 µCi/ml ^{3}H-tryptophan for 1 h, showed concentration of the radioactivity in the nuclear region of the 2-cell embryo. 2-Cell embryos failed to recover their growth potential after exposure to 1 µCi/ml ^{3}H-tryptophan. The toxicity of ^{3}H-tryptophan was relieved by isotopic dilution with non-radioactive tryptophan but not by tryptophan analogs or intercalating agents, such as acriflavin. The presence of cycloheximide both inhibited the incorporation of ^{3}H-tryptophan into peptide and reduced its toxicity. Exposure of the

2-cell embryo to ^{3}H-tryptophan led to a decrease in the rate of protein synthesis, estimated by the rate of incorporation of ^{35}S-methionine. Current efforts are directed towards understanding the molecular mechanism of this inhibition of protein synthesis and comparing the differential effects of ^{3}H-tryptophan on these processes in the 2-cell and 8-cell embryo. Several stage-specific nuclear and cytoplasmic proteins have been resolved electrophoretically for the early cleavage stages and those directed by maternal mRNA will be identified.

Publications in Scientif Journals, Monographs, Proceedings.

Ultrastructural Studies on the Mouse Embryo: Effects of Exposure to ^{3}H-tryptophan. In: Proc. Electron Microscopy Soc., Dublin. September 198

Progress Report
1982

Contractor:

Univ. Catholique
de Louvain, UCL
Halles Universitaires
Place de l'Université 1
B-1348 Louvain-la-Neuve

Contract no.: BIO-C-358-81-B

Head(s) of research team(s):

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General subject of the contract:

The effects of ionizing radiation on the defence mechanisms against infections.

List of projects:

1. Influence of irradiations on the local intestinal immunity. Prevention and treatment of their effects.

Title of the project: INFLUENCE OF IRRADIATION ON THE LOCAL INTESTINAL IMMUNITY. PREVENTION AND TREATMENT OF THEIR EFFECTS

Head of project and scientific staff: Prof. Hervé BAZIN
Dr L. DE CLERCQ
F. CORMONT

1. EFFECTS OF IRRADIATION ON THE SPLEEN B LYMPHOCYTES

The lymphoid tissue of the spleen is organized around terminal arborizations of the splenic arteries. This white pulp is composed of an inner zone with small lymphocytes and an outer zone containing intermediate sized lymphocytes. In the inner zone, two compartments can be distinguished. The first is called periarteriolar lymphocyte sheat. It contains a great majority of T lymphocytes. The second, the follicles, is formed of B lymphocytes which bear both IgM and IgD surface markers. It may contain a germinal centre. The outer zone or the marginal zone is a broad band of cells which surround the two other compartments, being clearly separated from them by the marginal sinus. The marginal zone contains a majority of non-circulating mu bearing B lymphocytes.

The roles of these different zones namely the marginal zone are still poorly known.

The radiosensitivities of these different cell compartments are unknown since the latter have only been characterized during the past few years. Figure 1 illustrates how the staining with anti-mu or anti-delta antisera allow to distinguish between the three lymphocyte populations of the white pulp.

The effects of ionizing radiations given at high dose rate (0.38 Gy/minute, caesium source) have been studied at doses of 0.38, 0.76, 1.50, 3.50, 5.00, 8.50 and 12.00 Gy. Tissue samples were taken 24 and 72 hours after the irradiation. In collaboration with Drs G. Lemaire, J. Masse and G. Lapaillade of the Département de Protection, Commissariat à l'Energie Atomique, Centre de Fonteney Aux Roses - France, we have also studied OFA outbred rats submitted to a chronic irradiation (cobalt 60) of 0.07 Gy/day for 9,17,22,30,35 and 43 days. Tissue samples have been scrutinized for B lymphocytes by conventional histology, immunofluorescence and peroxydase staining using rabbit anti-mu or delta rat heavy chains. 72 hours after irradiation, qualitative studies have shown that the follicular cell populations seem to be normal at doses up to 3.50 Gy, whereas the great majority of the marginal cells are already eliminated at doses equal or superior to 1.5 Gy. In the case of chronic irradiation,

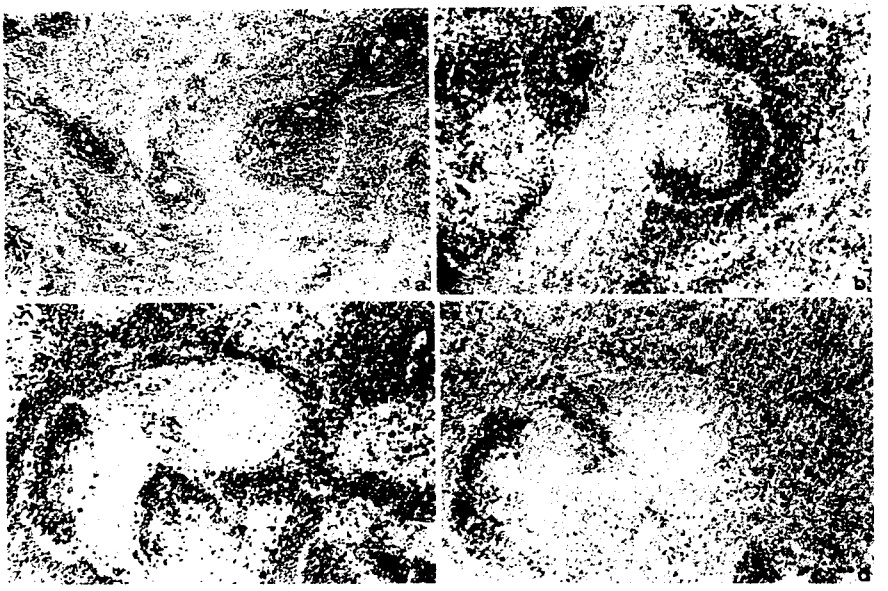

Fig.1. Spleen of OFA rats - a: control rat (HE; x 13) - b: control rat (mu peroxidase staining, x 13) - c: 2.43 Gy irradiated rat (35 days of chronic irradiation) (mu peroxidase staining; x 13) - d: serial section with c, delta peroxidase staining. Note the relative diminution of the follicle lymphocytes (mu positive and delta positive bearing cells) and of the marginal lymphocytes (mu positive, delta negative bearing cells) by comparison to the same cell populations in the control.

qualitative examinations of the different tissues have shown that the three compartments (T and B circulating and B non-circulating lymphocytes) still exist even after forty-three days of continuous irradiation. However, at least the marginal zone seems to be depleted of cells as compared to the controls.

Longer time chronic irradiation will be given to new groups of animals and quantitative studies of the tissue samples will be carried out.

2. CHARACTERIZATION OF NEW MARKERS OF HUMAN LYMPHOID CELLS

We have developed a technology of rat-rat monoclonal antibodies production. The IR983F LOU rat myeloma cell line is the first non-secreting cell line, fully histocompatible with the inbred strain of LOU rats. With this material, we have already obtained a great number of hybridomas against normal or cancerous cells from human origin (in collaboration with Dr A.M. Lebacq). Many monoclonal antibodies of diagnostic and even therapeutic interest have already been obtained.

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings

BAZIN H., PLATTEAU B. and PAUWELS R.
Immunoglobulin serum levels and population of intestinal IgA plasma cells in normal and immuno-deficient (nude - neonatally mu suppressed - neonatally delta supressed) rats.
Cur. Topics in Vet. Medicine and Animal Sci., 1981, 12: 443-457.

BAZIN H., PLATTEAU B., BAKOUR R., JANSSENS M. and WAUTERS G.
Pathogénie des infections après irradiation.2.Rôle des polynucléaires neutrophiles dans la défense des rats irradiés contre Yersinia enterocolitica.
C.R. Soc. Biol., 1982, 176: 402-405.

BAZIN H.
Le système immunologique du tractus intestinal.
Méd. et Hyg., 1982, 40: 2849-2857.

BAZIN H.
Modèles expérimentaux de déficiences immunologiques par irradiations, mutations génétiques ou manipulations.
Sci. Tech. Anim. Lab., 1982, 7: 19-23.

GRAY D., MacLENNAN I.C.M., BAZIN H. and KHAN M.
Migrant mu^+ $delta^+$ and static mu^+ $delta^-$B lymphocyte subsets.
Eur. J. Immunol., 1982, 12: 564-569.

BAZIN H. and LEBACQ A.M.
Physicochemical and Biological properties of rat antibodies.
Transplantation Proceedings (in press).

II. Short Communications, Theses, Internal Reports, Patents...

- Effects of irradiation on the lymphoid immune system. XVII Annual Meeting of the "European Society for Radiation Biology". Bordeaux july 1982, Book of Abstract, in collaboration with MALARBET J.L., LAFUMA J. and PLATTEAU B.
- Effects of irradiation on the spleen lymphoid tissue. Belgium Immunological Society Autumn Meeting. VUB Jette. November 1982, in collaboration with PLATTEAU B., MALARBET J.L. and LAFUMA J.

Progress Report
1982

Contractor: **Contract no.:** BIO-C-351-81-F

Institut National de la Santé
et de la Recherche Médicale
INSERM
Rue de Tolbiac 101
F-75645 Paris Cédex 13

Head(s) of research team(s):

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Rue Wilhem 29
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General subject of the contract:

Molecular mechanisms of X-ray and UV induced cataracts : study of the DNA stability and genetic expression of lens proteins using tissue culture methods.

List of projects:

1. Molecular mechanisms of cataractogenesis.

Title of project : Molecular mechanisms of cataractogenesis

Head of project and scientific staff :

Dr. Y. COURTOIS and Dr. M.F. COUNIS

In the process of pathological or radio- induced cataracts, the initial events concern primarly the lens epithelium. In vivo, the epithelial cells of the germinative region are dividing actively, then elongate into fibers Breaks accumulate in the DNA, the nucleus disappears and specific proteins (crystallins) synthesis occurs on stabilized mRNA.

Our goal is to analyse this differentiation process in vitro in order to evaluate the importance of development and ageing in irradiated lenses.

1) Relationship between DNA breakage and crystallin expression

1 - embryonic chicken lenses of II day old development were used either immediatly or after several days of organ culture.

2 - soluble proteins (including the crystallins) appear more labelled for the same period of labelling after 1 or 3 days of lens culture than for lenses taken just after excision.

3 - no modulation in crystallin synthesis is observed after 1 or 3 days of lens culture but differences in synthesis of high molecular weigh soluble proteins are detected.

4 - DNA breakage by ionizing irradiation is a rapid phenomenom. The irradiation increases the specific radioactivity of all soluble proteins 1 day after the irradiation and does not change the observed modulation of high molecular weight soluble proteins. (Exp.Eye Res. 34, (1982) 861-876).

II) DNA catabolism enzymes

In the fiber cells, the DNA is programmed to break. The X ray DNA repair capacity decreases as terminal differentiation progresses in vitro in II day old lenses or is lacking at 6 days. The DNA metabolism enzymes such as DNA polymerases, thymidine kinase, DNA Ligases) were detected at all the studied stages even when the repair system was lacking or impaired. Could this DNA breakage be correlated with an increase of an endonuclease activity ?

The first results show the presence of an endonuclease in the fiber lens cells, micrococcal like, acting on a supercoiled PM2 DNA as a substrate. (Abstracts. First European Congress on Cell Biology, 1982 and Fifth International Congress of Eye Research, 1982).

III) Chromatin structure

Digestion of lens fiber and epithelia chromatin by micrococcal nuclease has been shown to degrade chromatin into regular fragments containing DNA molecules with multiples of a unit weight DNA (nucleosomes). Different structures of chromatin could explain why differentiating cells could be more sensitive to X rays than dividing or resting cells.

The size of the mononucleosome was measured in active dividing cells of lens epithelia and in post mitotic cells from fibers. It appears to be identical (A.S. Muel - diplôme 1982) in chromatin from both cell types.

List of publications in 1982

I. Publications in Scientific Journals

- M.F. COUNIS, E. CHAUDUN, Y. COURTOIS, J.P. CARREAU, J. JACK and R. CLAYTON
Differential protein synthesis of chick embryonic lenses during in vitro differentiation. Exp. Eye Research, 1982, 34, 861-876.
- M.F. COUNIS, E. CHAUDUN and Y. COURTOIS
Changes in the DNA breakage and crystallin synthesis of embryonic chicken lenses cultured in a tryptophan deficient medium (submitted)

II. Short communications, Thesis

- M.F. COUNIS, A.S. MUEL, E. CHAUDUN, Y. COURTOIS and S.P. MODAK
Nucleosome size and endonuclease activity in embryonic chicken lenses. FEBS meeting on cell function and differentiation Athens, Greece 25-29 April, 1982.
- M.F. COUNIS, A.S. MUEL, E. CHAUDUN, S.P. MODAK and Y. COURTOIS
DNA replication and degradation in differentiation chicken embryonic lens cells. "Réponses induites après introduction de lésions dans l'ADN des procaryotes et des eucaryotes". Toulouse, 5-8 May, 1982.
- M.F. COUNIS, A.S. MUEL, E. CHAUDUN, Y. COURTOIS and S.P. MODAK
Nucleosome size and endonuclease activity during embryonic chick lens differentiation. First European Congress on Cell Biology. Paris, 18-23 July, 1982.
- M.F. COUNIS, A.S. MUEL, E. CHAUDUN, Y. COURTOIS and S.P. MODAK
Ca^{++} dependent deoxyribonuclease in chick embryo lens epithelium and fibers. Fifth International Congress of Eye Research, Eindhoven, The Netherlands, 3-8 October, 1982.
- A.S. MUEL
Taille du nucléosome de la chromatine des cellues de cristallins en différenciation. Université Paris VI, DEA pharmacologie moléculaire, September 1982.

Progress Report
1982

Contractor:

Contract no.: BIO-C-354-81-I

Comitato Nazionale per
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I-00198 Roma

Head(s) of research team(s):

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General subject of the contract:

Radiation damage and recovery of the immune system.

List of projects:

1. Radiation damage and recovery of the immune system.

Title of the project: Radiation damage and recovery of the immune system.

Head of project and scientific staff: Prof. G. Doria
Dr. L. Adorini
Dr. G. Agarossi
Dr. G. Gorini

It is well established that the immune system is not a simple array of independent cells but is rather a complex network of interacting particulate and soluble elements. The antibody response is modulated by signals passed among different types of cells that regulate the intensity and duration of the response after the antigen-induced perturbation of the immune system. Since helper and suppressor T lymphocytes play a key role in immunoregulation their radiation damage is likely to impair surveillance mechanisms against infections and tumour appearance and development. However, the immunological effects of irradiation can hardly be predicted owing to the various antithetic signals involved in immune cell communication.

Our previous studies were addressed to the assessment of the radiosensitivity and recovery of helper T cells. Unprimed helper T cells were found to be very radiosensitive and to recover slowly, as some defects were still detectable 3 months after 200 rad and complete recovery was reached 6 months after 400 rad. Our investigations on the radiosensitivity of primed helper T cells have revealed the existence of two helper T cell subpopulations, one more radiosensitive than the other one. When the population of azobenzenearsonate (ABA)-specific primed helper T cells was analyzed it was found that the different radiosensitivities of the two subpopulations were associated with different modes of action. The more radiosensitive T cell type interacts with B cells through an antigen bridge of physically linked carrier and hapten determinants, while the more radioresistant type does not require

physical linkage of carrier and hapten determinants for effective T-B cell cooperation.

Our more recent studies have been focussed on the radiosensitivity of suppressor activity. Suppressor T cells are induced as a direct or indirect consequence of antigenic stimulation and exert their functions by reducing the amplification signals provided by helper T cells or by acting directly on effector B cells. Thus far, the radiosensitivity of suppressor T cells has not been thoroughly investigated. The scarsity of radiobiological studies in this area reflects the difficulties met in assessing suppressor T cell activity, owing to the fact that helper T cells are also induced together with suppressor T cells in most experimental systems and to the complex mechanisms of antigen-specific immunosuppression. There is general consensus on the notion that suppressor T cells are more radiosensitive than helper T cells. Some experimental data indicate that suppressor T cells are radiosensitive before activation and become relatively radioresistant thereafter. However, according to other results from in vitro studies, activated antigen-specific suppressor T cells seem to be radiosensitive since suppression is reduced by 200 rad and abolished by 400 rad. This conclusion is also supported by results from our laboratory. Suppressor T cells were induced in mice by injecting ABA conjugated to mouse IgG in complete Freund's adjuvant. Two weeks later, spleen cells harvested from these mice, unirradiated or irradiated with 400 rad immediately before sacrifice, were tested in vitro for suppressor activity on the anti-TNP (trinitrophenyl) PFC response of ABA-KLH (keyhole limpet hemocyanin) -primed lymph node cells to TNP-ABA-KLH. Suppression was found to be T cell-mediated and entirely abrogated by 400 rad. These initial studies were extended to investigate the radiosensitivity of antigen-specific suppressor activity in vivo. Suppressor cells were induced by injecting mice with ABA conjugated to syngeneic spleen cells (SC). One week later, these ABA-SC-injected mice and uninjected controls were sacrificed and their spleen

cells were transferred into normal mice which on the next day were immunized with ABA-HRBC (horse red blood cells). Five days after immunization, mice of the two groups were sacrificed and assayed for the number of anti-ABA and anti-HRBC PFC/10^6 spleen cells. Suppression occurred in mice which received spleen cells from ABA-SC-injected donors, reached a maximum of 75% when $50x10^6$ nucleated spleen cells were transferred, and exhibited ABA-specificity as anti-ABA but not anti-HRBC PFC were ever suppressed. The radiosensitivity of suppressor cells induced in 90 day old mice was studied after whole-body exposure of ABA-SC-injected mice and -uninjected controls to 50, 100, 200, 300, or 400 rad of X-rays immediately before sacrifice. The suppressor activity (50%) of transferred $25x10^6$ nucleated spleen cells was not affected by 50-200 rad but was reduced to 70% by 300 rad and to 10% by 400 rad as compared to the suppressor activity of spleen cells from ABA-SC-injected unirradiated controls. Age-related changes in radiosensitivity of suppressor cells were investigated in 20 day and 720 day old mice, ABA-SC-injected and exposed to 50-400 rad of X-rays immediately before sacrifice. The suppressor activity of transferred $25x10^6$ nucleated spleen cells from mice ABA-SC-injected at the age of 20 days was almost completely radioresistant up to 400 rad, whereas suppressor cells induced at the age of 720 days were more radiosensitive than those raised in 90 day old mice. The remaining suppressor activity (S) after each radiation dose (D), as percent of the unirradiated control, fits a bending curve described by $S = \exp. - (aD + bD^2)$. Comparison of a for the three ages investigated indicates that the radiosensitivity of suppressor activity is 10 fold greater in 720 day than in 90 day old mice, in the latter group being 50 fold greater than in 20 day old mice.

Current studies have shown the enormous complexity of immunosuppression. Distinct experimental systems, including the immune response to ABA, have revealed several related but not identical suppressor regulatory pathways involving cells with different phenotypes and soluble factors

with various immunobiological properties. Radiobiological studies on immunosuppression should analyze the relative radiosensitivity of the cellular components involved in the experimental system under investigation to assess radiation damage at the cell population level. This analytical approach may also lead to the understanding of the increased radiosensitivity of the suppressor activity of spleen cells from old animals in which alterations of T cell subpopulations have occurred and accumulated with advancing age.

Publications appeared in 1982

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2. Doria G.
Immunoregulatory cell interactions in the antibody response.
In: Immunogenetics and Immune Regulation, B. Benacerraf (ed), Masson Italia, Milano. p. 107, 1982.

3. Frasca D., Doria G.
Enhanced helper activity in aging mice injected with thymic factors.
In: Immunology and Ageing, N. Fabris (ed), Martinus Nijhoff Publ., The Hague. p. 150, 1982.

4. Doria G., Garavini M., Adorini L.
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5. Adorini L., Pini C., Di Felice G., Doria G., Ricciardi-Castagnoli P. Linee continue di cellule T specifiche per azobenzenearsonato (ABA). II. Analisi funzionale di prodotti antigene-specifici. In: X Convegno Naz. Gruppo di Cooperazione in Immunologia, San Terenzo di Lerici. Riassunti. p. 23, 1982.

6. Ricciardi-Castagnoli P., Robbiati F., Barbanti E., Doria G., Adorini L. Linee continue di cellule T specifiche per azobenzenearsonato (ABA). I. Induzione e caratterizzazione fenotipica. In: X Convegno Naz. Gruppo di Cooperazione in Immunologia, San Terenzo di Lerici. Riassunti. p. 29, 1982.

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9. Doria G., Garavini M., Adorini L. Increased antigen presentation by macrophages from old mice. In: 9th International RES Congress, S. Normann & E. Sorkin (eds), Davos, Switzerland. p. 35, Abstract No. 176, 1982.

10. Doria G., Mancini C., Adorini L. Immunoregulation in senescence. Increased inducibility of antigen-specific suppressor T cells and loss of cell sensitivity to immuno suppression in aging mice. Proc. Natl. Acad. Sci. USA 79: 3803, 1982.

11. Doria G., Agarossi G., Adorini L. Selective effects of ionizing radiations on immunoregulatory cells. Immunol. Rev. 65: 23, 1982.

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13. D'Agostaro G., Ruco L.P., Uccini S., Garavini M., Baroni C.D., Doria G. Defective helper T cell activity in LPS-unresponsive C3H/HeJ mice. Cell. Immunol. 70: 231, 1982.

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Progress Report
1982

Contractor: Contract no.: BIO-C-360-81-B

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Head(s) of research team(s):

Dr. J. E. Dumont
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General subject of the contract:

Thyroid irradiation : Radiobiological consequences of irradiation in cell culture systems and in humans.

List of projects:

1. Radiobiology of the thyroid.
2. Irradiation and the human thyroid.

This work involves the study of the dosimetry, radiobiology, biochemistry and epidemiology of thyroid irradiation. In particular, it is hoped to derive from newly established model systems endpoints that are realistically relevant to the two main long term effects, cancer and hypothyroidism, in circumstances where reliable dosimetric information is available and where the modifying effects of hormones and biochemical features can be clarified. The role of intracellular signals on mutagenesis and carcinogenesis in the thyroid cell is investigated.

Title of project nr 1

RADIOBIOLOGY OF THE THYROID

Head of project and scientific staff : J.E. DUMONT, M.D., Ph.D.

P. ROGER, M.S., J. GOLSTEIN, Ph.D., S. SWILLENS, Ph.D.
A. HEPBURN, M.S., M. SMEKENS, M.S.

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1) DEVELOPMENT OF THE CELL CULTURE SYSTEM :

The radiobiology of the thyroid cell should be studied with accurate dosimetry and using different end points. The obvious experimental model to use is Xray irradiation of a culture system which has retained differentiation characteristics and can be stimulated to grow, such as dog thyroid primary cultures. The end points to be studied will be the various steps of iodine metabolism : iodide trapping, its binding to proteins, thyroglobulin synthesis, cyclic nucleotide metabolism and its response to hormones and neurotransmitters, mutation rates and chromosome rearrangements, growth and cell multiplication and effects on the life span of the cells. As a first step, the control of cell differentiation and growth is being further defined. Dog thyroid cells are now growing and myltiplying in our cultures for ten days. TSH or agents which enhance cyclic AMP accumulation (cholera toxin, forskolin) or epidermal growth factor (EGF) on the one hand, and insulin on the other hand are both necessary to sustain growth and multiplication of the cells. The number of cells can be multiplied by factors of up to 10. During the multiplication, the differentiation characteristics (iodide trapping, epithelial morphology) recede. At the end of the multiplication (which occurs prior to total confluency), these characteristics reappear in the presence of TSH. This process is enhanced by cortisol but depressed by EGF. Thus the system now allows to obtain cell multiplication followed by reinduction of differentiation. However, until now, the lifespan of the cells in vitro is not yet sufficient for reliable testing of the action of irradiation on clonogenic capacity. Confirming results of the Dublin's group, preliminary experiments have shown no effect of irradiation (1, 3, 5 Greys) on the expression of differentiation in stationary cultures. The adaptation of the culture methods to rat thyroid cells, on which the expression of thyroglobulin gene

can be studied, has not been completely successfull. These cells synthesize thyroglobulin and this expression can be enhanced by TSH. However, we have not been able to control repeatedly the multiplication of these cells. We are now trying to adapt our human and rat thyroglobulin cDNA probes for the study of thyroglobulin gene expression in our dog thyroid cell cultures.

2) STUDY OF THE RELATION OF THYROID OXYGEN METABOLISM TO THE EFFECT OF IRRADIATION :

We have now developed a suitable experimental model for the study of the role of GSH peroxidase in thyroid metabolism : the selenium deficient rat. In this animal, the levels of the enzyme are greatly decreased in the thyroid as in other organs, while thyroid hormone synthesis is enhanced. This suggests that inhibition of the cellular H_2O_2 disposal system enhances H_2O_2 accumulation and its consequent action on iodide and tyrosine oxidation in the gland and thus demonstrates the role of GSH peroxidase in the normal thyroid metabolism. The model will now be used in radiobiological experiments.

3) BIOCHEMICAL EFFECTS OF ACUTE IRRADIATION :

The basic theoretical foundations of the interpretation of radiation inactivation of enzyme systems have been reinvestigated. In the case of complex interacting enzymatic systems, it is erroneous to draw simple conclusions on the sizes of the complex on the basis of radiation inactivation data. A new theoretical framework has been proposed for such experiments. We have demonstrated in rat regenerating liver an inverse relation between growth and the level of 2-5 A synthetase. The use of this enzyme as general marker of cell growth and of radiotoxicity will be investigated.

PUBLICATIONS :

1. P. SIMON, S. SWILLENS and J.E. DUMONT
Size determination of an equilibrium enzymic system by radiation inactivation : Theoretical considerations.
Biochem. J. (1982) 205, 477-483.

2. P. ROGER, A. HOTIMSKY, C. MOREAU and J.E. DUMONT
Stimulation by thyrotropin, cholera toxin and dibutyryl cyclic AMP of the multiplication of differentiated thyroid cells in vitro.
Mol. & Cell Endocrinol. (1982) 26, 165-176.

3. P. ROGER and J.E. DUMONT
Epidermal growth factor controls the proliferation and the expression of differentiation in canine thyroid cells in primary culture.
FEBS Letters (1982) 144 n° 2, 209-212.

Title of project nr 2 :

IRRADIATION AND HUMAN THYROID

Head of project and scientific staff : J. MOCKEL, M.D., Ph.D.

P. ROCMANS, M.D., Ph.D., J. VAN SANDE, Ph.D.,
P. GALAND, Ph.D.

Our project on the human thyroid involves four aspects : an epidemiologic cooperative study of groups of patients at risk following thyroid radiation, the definition of cell kinetics in man (adult and newborns), the study of the radiobiology of human thyroid cells in culture, characterization of the biochemical lesion of thyroid nodules.

a) Radiobiology of human thyroid cell in culture - This investigation requires prior progress in project 1. In 1982, preliminary work has been made to scale down the methodology which is successfull for dog thyroid cells, so as to eventually adapt it to small biopsies.However, the results are not yet satisfactory as multiplication of the cells is low and variable from one experiment to another.

b) Definition of cell kinetics in human thyroid - We have developed and used for human samples a thymidine double labeling method which allows the measurement of labeling index and S phase duration and thus the calculation of the cell growth fraction. This method has been validated with controls in vivo in dog (Willems, Lab. Invest., 23, 635, 1970). In 1982 we have applied this method to samples of human thyroid excised at surgery for non thyroid cancers and samples of benign adenomas. Until now, 80.000 cells from 6 different thyroid samples have been counted. In normal thyroids 26 labeled cells have been found, only 2 of which appear to be follicular. These results showing the extreme scarcity of follicular cell division in human thyroid have to be confirmed. They suggest that, as proposed by Pochin, there may not even be one cycle of division for each follicular cell during the whole adult life of man.

c) Characterization of the biochemical lesion of the thyroid nodule - Human thyroid nodules presenting a homogenous pathology have been investigated with regard to their biochemical controls in order to identify the mechanism of their genesis and growth. With regard to hot autonomous nodules,

the main common characteristics of the nodule is a much increased sensitivity of their adenylate cyclase to β-adrenergic agents (11 cases studied) There is no anomaly of basal cyclic AMP levels, response to TSH, prostaglandins, etc.

D) Cooperative epidemiological study of patients at risk after thyroid irradiation - A cooperative study has been initiated with the cooperation of the European Thyroid Association (ETA) and of the Thyroid Group of the European Organization for the Research and Treatment of Cancer (EORTC). This group met in Brussels on September 3rd and 4th 1982. The group reviewed the existent experimental and epidemiologic evidence on the thyroid consequences of high and low dose irradiation. It decided to define the methodology for a large scale European investigation on the effects of low doses of radiation on thyroid. Working papers on each aspect of this methodology (selection of the population to be studied and its suitable controls, dosimetry, protocol of clinical and laboratory examination, policy with regard to the different cases, protocol for pathological examination of tissue samples, organization of data collection for statistical evaluation) are being prepared. They will be discussed and finalized at the next meeting of 1983, so as to be implemented in a common epidemiological study.

PUBLICATION

- J.E. DUMONT, Irradiation and Thyroid Disease, Meeting of the Belgian Biophysical Society, "Colloquium on the Toxicity of Radionuclides", Liège, November 19-20, 1982

Progress Report
1982

Contractor: **Contract no.:** BIO-C-345-80-D

Universität Ulm
Grüner Hof 5c
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Head(s) of research team(s):

Prof. Dr. T. M. Fliedner
Abt. Klin. Phys.u. Arbeitsmed.
Universität Ulm,
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General subject of the contract:

Consequences of radiation exposure : assessment of impairment and restoration of hemopoietic function.

List of projects:

1. Radiobiological mechanisms involved in hemopoietic responses to low level radiation exposure.
2. Blood stem cell determination as a biological indicator of radiation exposure.
3. Reconstitution of radiation induced hemopoietic aplasia by means of blood and fetal liver stem cell transfusions using cell separation and cryo-preservation technics.

Title of project No. 1:

Radiobiological mechanisms involved in hemopoietic responses to low lewel radiation exposure.

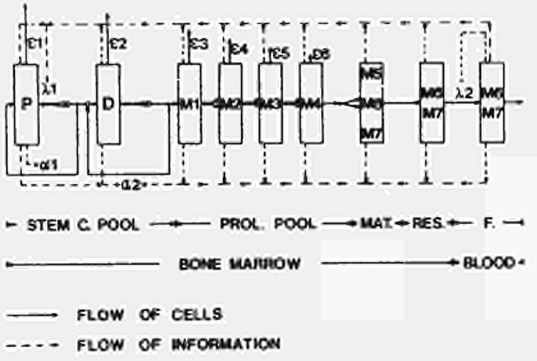
Head of project and scientific staff:

Prof. Dr. T.M. Fliedner (Head) and Doz. Drs. Calvo and Nothdurft associated with Drs. F. Carbonell, G. Grilli, Doz. E.B. Harriss, Ph.D.

In 1982, this project concentrated on the use of simulation models to evaluate the effects of chronic low level radiation exposure on the hemopoietic system and to understand the pathophysiological mechanisms that are responsible for its seemingly high tolerance on the one hand and the leukemic deterioration on the other. The work reconsidered a model proposed by us earlier that was able to simulate hematopoietic effects of external radiation exposure given at a low, a medium and a high dose rate. This model is presented in fig. 1.

Fig. 1:

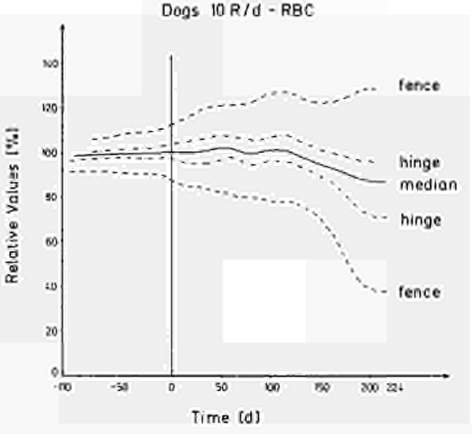
MODEL OF GRANULOCYTOPOIESIS



It is able to simulate the observations, that at low dose rates, the radiation induced cell loss can be compensated for by increased cell production so that the blood concentrations remain normal. It is only when the cell loss in the stem cell compartment exceeds the cell production, that the cell renewal system fails. With this model in mind, it was possible to utilize the data obtained in collaboration with the team on chronic low level irradiation of dogs at the Argonne National Laboratory headed by Dr. T. Fritz and to establish the possibilities and limitations of the hemopoietic system to tolerate chronic low level exposures at different dose rates. At 5 R/day, the red blood cells (RBC) remain at a normal level until 6000 R are accumulated at day 1200. Thereafter, a drop to about 80 % of normal is observed

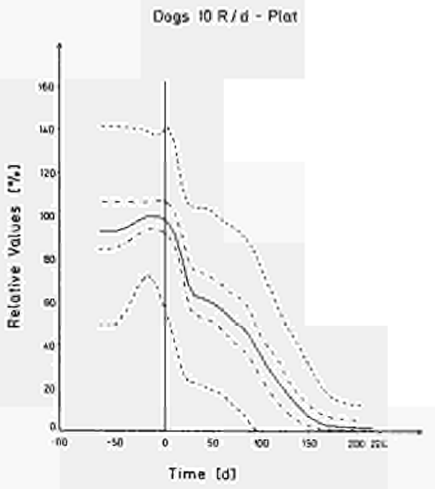
which is retained for some period of time. In the 10 R/day group too few dogs survived that long to arrive at a simular statement. From our studies on the radiation effects on the stem cell compartments (CFU-C) that were reported for 1981, we were interested in the type of response that could be observed during the first 200-400 days. In both groups of dogs, 5 R/day and 10 R/day, the red blood cells remain stable during this initial period (Fig. 2)

Fig. 2:



Of importance are the data on the WBC which are largely determined by the neutrophilic granulocytes. At 5 R/day, there is a marked initial drop followed by a slower rate of decrease, reaching about 35 % of normal values on day 400 compared to 25 % seen at 10 R/day. The platelet count decreases to about 70 % of normal by day 40 in the 5 R/day group. In the 10 R/day group, 3 phases might be distinguished: a first decrease to 60 % in 30 days, then a constant level for some 30 days and a decrease to levels below 20 % of normal by day 110 (Fig. 3). On the basis of consideration that resulted in the proposed model (Fig. 1), and corresponding models for erythropoiesis and and megakaryocytopoiesis, it must be assumed, that the 3 cell systems of relevance (erythropoiesis, granulocytopoiesis and megakaryocytopoiesis) are different in their capacity to tolerate chronic low level exposure. The models will now be adapted further to the situation in the chronically irradiated dog and experiments performed to analyse the biological assumptions associated with the observations.

Fig. 3:



One important biological aspect that has to be considered as a possible mechanism by which the hemopoietic system is able to tolerate a continuous radiation burden, is related to possible changes in the radiation sensitivity of hemopoietic cells due to their increased proliferation rate and to changes in their age distribution in the different compartments. In the previous report of the year 1981 it was documented that in dogs that had accumulated total exposures of about 3200 R at a dose rate of 2.5 R/day the S-phase fraction of GM-CFU was increased from a normal value of 27% to 42%. To study the influence of proliferation and differentiation on radiosensitivity GM-CFU obtained from bone marrow and blood of normal dogs were kept in suspension culture for 3 days under optimal stimulation. At 3 different times of culture samples were taken from the suspensions for the determination of the proliferative state of GM-CFU by means of their S-phase fraction and their radiosensitivity. It was shown that for bone marrow GM-CFU the S-phase fraction increased from 42 % determined on day O to 67% on day 3 of culture. In parallel with this increase in the proliferation rate a significant increase in radio-resistance was found. The D_o-value rose from 0.52 Gy on day O to 0.72 Gy on day 3 in culture. A similar correlation between an increasing S-phase fraction and an increase in radioresistance was obtained for the GM-CFU derived from the blood, when proliferating suspension culture (Fig. 4) The increase in radio-resistance of proliferating blood GM-CFU was even stronger than for bone marrow GM-CFU, since, under

steady state conditions, the former is by far more sensitive than the latter.

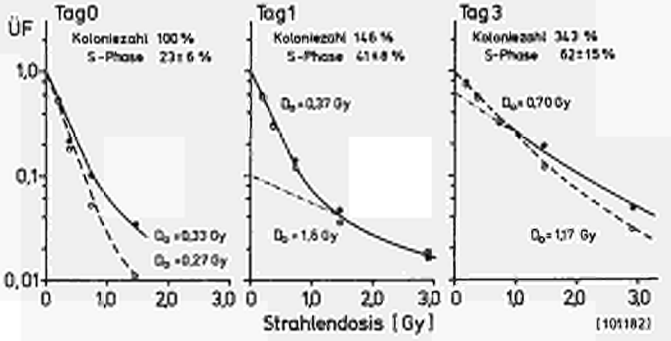


Fig. 4: The increase in radioresistance of blood-derived GM-CFU kept in suspension culture and its correlation to an increasing S-phase fraction. Closed symbols (o) - survival fractions in 7 day-agar cultures, open circles (●) - survival fraction in 10 day-agar cultures

These data obtained from an in vitro model for proliferating GM-CFU will be taken as a basis for a more detailed analysis of qualitative changes in the GM-CFU compartment of dogs under continuous radiation exposure. Such physiological changes may be of principle importance for the radiation tolerance of the hemopoietic system and, therefore, have to be introduced into the models that are applied to the simulations of the radiation response of the stem cell compartments.

Title of project No. 2:

Blood stem cell determinations as a biological indicator of radiation exposure and hemopoietic recovery.

Head of project and scientific staff:

Priv. Doz. Dr. W. Nothdurft and Prof. Dr. T.M. Fliedner together with Drs. F. Carbonell, G. Grilli, G. Pabst, A. Raghavachar and K.H. Steinbach

The basic purpose of this project is to investigate whether and in what way the circulating hemopoietic stem cells - demonstrated as "colony forming units in culture" (CFU-C) indicating the presence of "granulocytic progenitor cells" - can be used to predict the regenerative potential of hemopoiesis in the unirradiated as well as in the total and partial body irradiated mammalian system. Previously, the considerable radiation sensitivity of the circulating CFU-C has been demonstrated, both in vivo and in vitro in dogs as well as in man.

Therefore, in 1982, these studies were continued at the preclinical level. The major line of investigation was to examine the reconstitution of the circulating CFU-C population after total body irradiation and autologous stem-cell transfusion and to investigate whether and in what way the stem cell pool returns to a normal state of functional potential. It was expected to obtain information on the predictive value of blood stem cell (CFU-C) concentrations using the dextran-sulfate mobilization test as a means to establish a return of the stem cell pool to normal.

In normal dogs, the injection of 15 mg DS/kg body weight results in a remarkable increase of circulating CFU-C within 3 hours by a rather constant factor of 13.2 ± 2.9 (average value of test in 8 dogs). When dogs were given a total body exposure of 3 x 6 Gy followed by an infusion of autologous, cryopreserved blood derived mononuclear cells, a recovery of hemopoietic function was observed as reported earlier from our group. It was, however, of importance in this context, that only 3 out of 8 dogs reached a normal proportion of CFU-C in the marrow in the following weeks and months, while in the blood the CFU-C level returned to normal in only 5 out of 8 dogs by day 35 and dropped again to subnormal levels by day 100. It was studied whether this deficit at the stem cell level is also born out in the "dextransulfate mobilization test". As shown in table 1 and 2, the mobilization factor varied as a function of time after irradiation and stem cell transfusion but also with the source of stem cells. While the use of blood derived stem cells resulted in most dogs between day 45 and 110 in a normal increment (Table 1), the use of bone marrow derived stem cells resulted in

Table 1:

MNC mobilization with DS in 3 x 6 Gy whole-body irradiated dogs grafted with autologous peripheral blood derived stem cells.

Dog No.	Day of DS application	MNC / µl blood at time 0	Increase of blood MNC content	
			Factor	Net increase (MNC / µl)
679	pre-irrad.	1927	3.1	4047
	+ 45	690	3.0	1410
7948	pre-irrad.	2310	4.8	8892
	+ 45	685	2.0	662
	+ 110	898	2.2	1099
	+ 225	1011	2.2	1241
80108	pre-irrad.	1629	2.2	1979
	+ 45	976	2.1	1123
	+ 110	1518	1.6	913
80163	pre-irrad.	2230	5.1	9140
	+ 45	874	1.9	783
	+ 110	781	2.3	1033

a subnormal mobilization factor for most time points studied (Table 2). The data lead to the conclusions, that the "mobilization factor" after dextran-sulfate administration is a function of the concentration of CFU-C present in the blood before DS administration. But it has also a significance for the quality of the bone marrow restoration after exogenous injury. Thus, it is encouraging to follow this line of investigation and to explore in what way the number of circulating CFU-C and the mobilization factor correlates with the quality of bone marrow regeneration to be tested by retransplantation experiments in the future.

Table 2:

MNC mobilization with DS in 3 x 6 Gy whole-body irradiated dogs grafted with autologous bone marrow derived stem cells.

Dog No.	Day of DS application	MNC / µl blood at time 0	Increase of blood MNC content	
			Factor	Net increase (MNC / µl)
791207	pre-irrad.	3366	2.6	5385
	+ 45	1156	1.5	556
	+ 110	1281	1.5	608
	+ 225	1592	1.5	815
80197	pre-irrad.	2461	5.7	11,644
	+ 45	966	1.1	69
	+ 110	753	2.2	894
	+ 225	626	1.7	470
80111	pre-irrad.	3642	3.7	10,020
	+ 45	614	1.3	177
80171	pre-irrad.	3332	2.4	4750
	+ 45	640	1.4	305
	+ 110	1016	1.6	627
	+ 225	953	1.5	456

Title of project No. 3:

Reconstitution of radiation induced hemopoietic aplasia by means of blood and fetal liver stem cell transfusions using cell separation and cryopreservation technics.

Head of project and scientific staff:

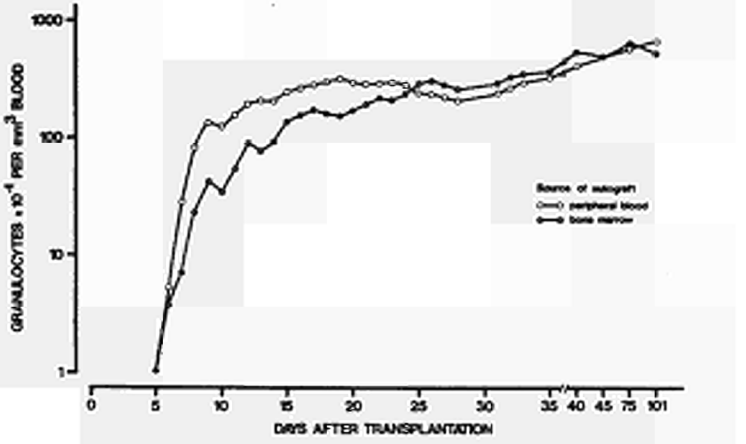
Priv.Doz.Dr. W. Calvo and Prof. Dr. T.M. Fliedner togehter with Drs. F. Carbonell, G. Grilli, W. Nothdurft, O. Prümmer, A. Raghavachar and I. Steinbach

In 1982, this project made significant progress along the following lines First of all, a study was completed on the comparative value of stem cell derived from bone marrow and circulating blood to restore hemopoiesis in a total body irradiated recipient animal. Secondly, the pattern of extramedullary hemopoiesis after stem cell transplantation was characterized that has not been described before as a consequence of stem cell transfus and may well be relevant for the problem of the quality of hemopoietic en graftment after total body irradiation and stem cell transplantation.

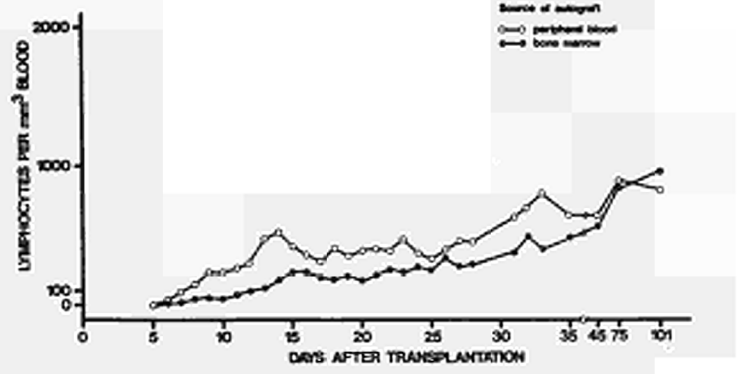
1. Comparison of hemopoietic regeneration after autologous transplantatic of bone marrow or blood derived stem cells

The kinetics and pattern of hemopoietic recovery after supralethal total-body irradiation (TBI) were compared after transfusion of cryopreserved autografts derived from peripheral blood and bone marrow. Fractionated TBI was given in three doses of 6 Gy each at intervals of 48 hrs. Grafts of peripheral blood mononuclear cells (MNC) were collected by means of cc tinuous-flow centrifugation and using the mobilizing agent dextran sulpha Autografts were adjusted to contain equal numbers of committed progenitor cells (CFU-c). Dogs grafted with blood derived MNC (group I) and with MNC from bone marrow (group II) all received about 1×10^5 CFU-c per kg body weight. None of the dogs died from acute radiation toxicity and in all cc sistent hemopoietic engraftment was achieved. Comparing the pattern of re generation of the granulocytes, group I dogs showed a significant regener tory advantage over group II dogs, in particular during the first 20 days after transplantation (Fig. 5). Similarly, lymphoid recovery was more rap in group I until day 14. In both groups, blood lymphocytes remained belc normal values beyond day 100 (Fig. 6). The regeneration patterns of the platelets and reticulocytes revealed no significant differences. These re sults are in agreement with the hypothesis proposed earlier that there ar more pluripotent stem cells per CFU-C in the blood than in the bone marro

. 5:



. 6:



Extramedullary hemopoiesis after stem cell transplantation

considerable interest was the finding, that the transfusion of hemopoie- stem cells into a lethally irradiated recipient dog results not only in characteristic regeneration of bone marrow hemopoiesis but also to the pearance and later disappearance of a wave of hemopoiesis in the normally hemopoietically active spleen. This extramedullary hemopoiesis in close- correlated with the appearance and disappearance of immature hemopoietic lls in the peripheral blood. The mechanisms and the functional significance this transitory hemopoietic activity is not yet well understood. But it of principle interest, that it resembles closely in its pattern the ansitory phase of hemopoiesis in the fetal dog spleen.

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A comparative study of the repopulation potential of autografts from canine blood and bone marrow.
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Progress Report
1982

Contractor:

Istituto di Ricerche
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Via Eritrea 62
I-20157 Milano

Contract no.: BIO-C-355-81-I

Head(s) of research team(s):

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General subject of the contract:

Consequences of radiation exposure and treatment of pathological effects.

List of projects:

1. Characterization of the mode of action of immuno depressive agents.

CHARACTERIZATION OF THE MODE OF ACTION OF IMMUNODEPRESSIVE AGENTS.

F. Spreafico, M.D.; A. Vecchi, Ph.D.; A. Merendino, Biol Dr.; M. Allegrucci, Biol. Dr.; M.A. Canegrati, Biol. Dr.; M. Romano, Biol. Dr.

Istituto di Ricerche Farmacologiche 'Mario Negri' - Via Eritrea, 62
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A first main line of work followed in the past year has been centered on the further characterization, at the cell level, of the mode of action of immunodepressive substances at central lymphoid organs and peripheral non lympoid sites. The main rationale at the basis of this work can be summarized as follows: i) previous work of this group, also supported by this contract, has shown that the various populations and subpopulations of immunocytes present in a given lymphoid organ can vary markedly in their susceptibility to immunomodulators even when possessing closely related structures. In other words, each compound possesses a distinct immunopharmacological profile as discussed in ref. 1 below. ii) immune cells of a given lineage but present in different organs, can be functionally heterogenous. This concept is further supported by our finding that murine macrophages obtained from the lung, spleen and peritoneal capacity markedly vary among them as regards non-specific suppressive capacity, as described in ref. 2 below. This paper also describes that even within an organ (e.g. lung) one can identify subpopulations of cells (i.e. macrophages) differing among them in functional activity, as for instance the capacity to express spontaneous or IFN-stimulated cytotoxicity.

Accordingly, it was of interest to examine whether immunodepressive drugs could differentially affect immune reactivities present at "central" (i.e. blood and spleen) and peripheral organs (i.e. gut and lung). In the mouse it has been shown that indeed with at least certain compounds, immunological effects osserved in the spleen and/or blood do not predict for what occuring at peripheral organs, such as intestine and lung, which are major sites for the first interaction between the host and the environment (e.g. infectious agents). Results so far obtained are fully described in ref. 3, and only a few examples are hereby given. For instance, when NK activity was compared in lung, gut and spleen, Cyclophosphamide was depressive at each of the 3 sites, Azathioprine only in spleen, Hydrocortisone in lung and spleen (but not in gut), and 5FU was depressive in spleen and lung whereas it increased the apparent expression of NK activity in the intestine. With regard to macrophage-mediated cytotoxicity, Azathioprine was depressive in spleen and peritoneal cavity but not in lung, 5FU depressed in lung, peritoneum but not in spleen, Steroids depressed at each site, whereas Doxorubicin was not significantly depressive at any of them. Examples of organ-associated preferentiality in susceptibility to immunodepression were also seen evaluating T cell reactivities.

In conclusion, these data indicate that immunocytes of a given type may vary in their susceptibility to modulation by exogenous agents also depending on their anatomical site of residence. This notion can be of

pratical importance in explaining site-related toxicities of immuno depressants and may serve in the selection of the drugs to be best employed therapeutically. Lastly, these results support the concept that immune changes measured "centrally" (e.g. blood) may not be representative of what occurs peripherally. In the continuation of studies on the characterization of the immunodepressive capacity of plant proteins structurally analogues of Ricin A chain, we have investigated the "in vivo" effects in mice of Gelonin. Single administrations of very low doses (as low as 0.25 mg/kg) of this product were markedly depressive of humoral and cell-mediated reactivities, in conditions in which no other overt toxicity was observable. On the basis of available evidence, the mode of action of this product appears however at least partially different (e.g. B cells are also sensitive) from that described in ref. 4 for the similar proteins MCI and PAP-S. On the basis of these initial data, these vegetal products appear to posses interesting immunomodulatory activity worthy of further investigation.

Medroxyprogesterone acetate has been suggested to posses an immuno protecting activity in the clinic, countering the immunosuppression induced by radiation-mimetic agents. It was of interest therefore to explore this alleged immunomodulatory activity in controlled experimental conditions. In rodents, this drug has however not shown significant immunological activity in conditions showing antineoplastic effect, as described in ref. 5.

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In: Human Cancer Immunology, B. Serrou Ed., Elsevier North Holland Biomedical Press, Amsterdam, in press

2) Moras M.L., Honorati M.C., Spreafico F.: The cytotoxic and suppressive activity of murine bronchoalveolar macrophage subpopulations
J. of the Reticuloendothelial Society, in press

3) Spreafico F., Alberti S., Allegrucci M., Canegrati M.A., Colotta F., Luini W., Pasqualetto E., Romano M., Sironi M., Vecchi A.: On the mode of action of immunodepressive agents
Presented at: Second International Congress of Immunopharmacol. Washington, 5 - 10 july, 1982

4) Spreafico F., Malfiore C., Moras M.L., Marmonti L., Filippeschi S., Barbieri L., Perocco P., Stirpe F.: The immunomodulatory activity of the plant proteins momordica charantia inhibitor and pokeweed antiviral protein
Int. Journal of Immunopharmacol., in press

5) Spreafico F., Filippeschi S., Malfiore C., Moras M.L. and Marmonti L.: Medroxyprogesterone acetate: experimental studies of its antineoplastic activity and its effect on immunological reactivities.
In: Proceedings of the International Symposium on Medroxyprogesterone acetate. Cavalli F. et al. Eds., Excerpta Medica, Amsterdam, pp. 103, 1982

Progress Report
1982

Contractor:

Contract no.: BIO-C-443-81-EIR

Department of Pathology
and Immunology
Regional Hospital
IRL-Galway

Head(s) of research team(s):

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Dr. J. D. Kennedy
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General subject of the contract:

An examination of the role of haematopoietic stromal cells in the regulation of stem cell differentiation in man.

List of projects:

1. An examination of the role of haematopoietic stromal cells in the regulation of stem cell differentiation in man.

Title of Project An examination of the role of haemopoietic stromal cells in the regulation of stem cell differentiation in ma

Head of Project and Scientific Staff: Dr. J F Greally

The purpose of the research is to examine the role of stromal cells in human bone-marrow and thymus in the induction of differentiation in those tissues. The main thrust of the work in the past year was the establishment in the laboratory of in vitro culture techniques for human bone-marrow and thymic epithelium. A secondary objective was the devlopment of an in vivo model in the rat which would allow us to examine the maturation of thymic lymphocytes.

For in vitro assays of bone-marrow differentiation a semi-solid medium methyl-cellulose containing a variety of different conditioning factors was used. These factors included human placental extract, Giant cell tumor (GCT) supernatant factor, PHA stimulated leucocyte conditioned medium, and erythropoietin. In addition bone-marrow was grown in medium containing supernatant from human thymic epithelial cell culture.

Prolific colony forming activity occurred using PHA conditioned medium and also following addition of erythropoietin to the cultures. Poor colony formation occurred following addition of human placental conditioned medium and (GCT) supernatant factor. A method was devised for detailed examination of colony morphology in situ.

Separation of stem cells was carried out using a 2% dextran T 500 separation medium. This was followed by removal of T lymphocytes using sheep red blood cell (SRBC) agglutination which in turn was followed by the treatment of the residual cells with soya-bean agglution (SBA). The remaining cells containing the stem cell fraction were cultured in the presence of thymic-epithelial cell-culture supernatant. No definite evidence of T cell differentiation was observed.

When however the supernant was added to "whole" bone-marrow a marked increase in bone-marrow stromal cell growth occurred within 4-5 days.

In vivo experiments in the rat showed that it was possible to deplete the thymus of all lymphoid tissue by dietary manipulation. These experiments along with others designed to reconstitute the depleted thymus are in progress.

Progress Report
1982

Contractor:

Association Claude Bernard
Avenue Victoria 3
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Contract no.: BIO-C-352-81-F

Head(s) of research team(s):

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General subject of the contract:

Influence of ionizing radiations on the in vitro aging and immortalization of fibroblasts.

List of projects:

1. Influence of ionizing radiations on the in vitro aging and immortalization of fibroblasts.

Project No BIO-C-352-81-F

Title : INFLUENCE OF IONIZING RADIATION ON THE IN VITRO AGING AND IMMORTALIZATION OF FIBROBLASTS

Head of Project : A. Macieira-Coelho

Scientific Staff : B. Azzarone, C. Diatloff-Zito, C. Icard-Liepkalns, C. Bournoutian.

We extended our findings on the effect of low dose rate ionizing radiation on human skin fibroblasts from donors with genetic defects at high risk of cancer, irradiating cultures obtained from two patients with 13 q deletion retinoblastoma. A 62%, 89% and 77% increase in the cumulative population doublings were obtained with one of the cell strains in 3 different groups irradiated with 100, 200 and 300 rad respectively. Cytogenetic analysis showed that in these cell populations there was no selection of abnormal clones. An increase in the doubling potential after 300 rad was obtained with the cells from the other patient, where irradiation was followed by focus formation. Immortalization of the fibroblast-like population however could not be obtained. The results confirm our previous results that full transformation of human fibroblasts with low dose rate ionizing radiation is a rare event. More frequently, as with virus or chemical carcinogens, intermediate steps of transformation are found, consistent with the multistep concept of cell transformation. This type of results, however, is only seen with cells obtained from donors with genetic defects or with cancer. The relationship between the aborted transformation and the pathology of the donor remains for the moment circumstancial.

Our previous results concerning the chromatin changes that coincide with an increased sensitivity of human fibroblasts to ionizing radiation could not distinguish between apurinic sites, single-strand breaks or replication intermediates either due to the presence of slow dividing cells or to a decreased rate of chain elongation. The sedimentation velocity of newly synthesized DNA was followed to distinguish between these possibilities. Results showed that the small molecular weight DNA was not due to slow dividing cells and suggested a defect in

the gap-filling step during the rejoining of adjacent replicons. The centrifugation of chromosomal DNA in sucrose gradients under different conditions of pH and temperature also suggested that most of the small molecular weight is due to breakage of fragile sites during manipulation, which could correspond to a defect in the maturation of the gap-filling step.

Analysis of chromatin under electron microscopy after Miller's spreads revealed that modified chromatin loses its nucleosomal structure and appears as short pieces sometime forming a circle. It has been recently hypothesized that at each cell division small pieces detach and reintegrate into the chromosomes. Our findings could suggest that during aging these pieces fail to reintegrate due to changes in chromatin structure, and thus render the cells more sensitive to low dose rate ionizing radiation.

We also found that these chromatin changes which coincide with an increased radiosensitivity occur in parallel with modifications of the kinetics of cell proliferation of human fibroblasts.

List of Publications 1982

I. Scientific Journals:

F. Puvion-Dutilleul and A. Macieira-Coelho. Ultrastructural organization of nucleoproteins during aging of cultured human embryonic fibroblasts. Exp. Cell. Res. 138 (1982) 423-429.

C. Icard-Liepkalns and A. Macieira-Coelho. Aging and hydrocortisone effects on transient structure of replicative DNA of human fibroblasts. Proc. Soc. Exp. Biol. Med. 170 (1982) 373-377.

F. Puvion-Dutilleul, B. Azzarone and A. Macieira-Coelho. Comparison between proliferative changes and nuclear events during ageing of human fibroblasts in vitro. Mech. Age. Dev. 20 (1982) 75-92.

A. Macieira-Coelho and B. Azzarone. Aging of human fibroblasts is a succession of subtle changes in the cell cycle and has a final short stage with abrupt events. Exp. Cell Res. 141 (1982) 325-332.

II. Communications:

French-Israeli Joint Symposium on Biology of Aging. Brookdale Institute of Gerontology, Jerusalem, February 1982: Changes at the molecular level associated with proliferative changes during fibroblast aging.

First Conference of the Association of Gerontology, Symposium on the Biological Aspects of Ageing, Varanasi University, India, October 1982: Qualitative and quantitative changes occurring in DNA during aging of dividing cells.

Twenty-second Meeting of the American Society for Cell Biology, Baltimore, U.S.A., December 1982: Distribution of DNA between sister cells during serial subcultivation of human fibroblasts.

U.S.-Japan Cooperative Seminar "Pathogenetic Mechanisms in Werner's Syndrome and their Role in Human Aging", Kobe, Japan, December 1982: Changes in chromatin and DNA of fibroblasts during physiologic aging and in Werner's syndrome.

Progress Report
1982

Contractor:

Università di Napoli
Corso Umberto I 35
I-80131 Napoli

Contract no.: BIO-C-353-81-I

Head(s) of research team(s):

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General subject of the contract:

Physiopathology and therapy of radiation induced marrow aplasia : role of glycoprotein hormones modulating early hemopoiesis.

List of projects:

1. Radiation and marrow aplasia. Role of glycoprotein modulators of early hemopoiesis.

Title of project nr 1
Radiation and marrow aplasia. Role of glycoprotein modulators of early hemopoiesis.

Head of project and scientific staff:
Cesare Peschle, M.D. - A.R.Migliaccio, Ph.D. - G.Migliaccio, Ph.D. - A. Covelli, M.D. - F.Mavilio, Ph.D.

A series of murine T-lymphocyte cell lines have been screened for release in their supernatant of activity(ies) inducing in vitro growth of erythroid bursts ("burst-promoting activity", BPA, or "burst-enhancing factor(s)", BEF), granulo-macrophage (GM) colonies (GM "colony-stimulating factor", GM-CSF), pure G or M colonies (G-CSF and M-CSF respectively) or survival of pluripotent stem cells ("stem cell activating factor(s)", SAF) (1,2). Lines have been identified, which release selectively or almost exclusively BPA or GM-CSF, and may or may not also release SAF (3). Supernatants from these lines seemed a most appropriate materials to start purification of BPA, GM-CSF and SAF.

In this regard, we have obtained contradictory result when purifying these factors from supernatants of lectin-stimulated normal murine splenocytes: this seemed to derive, at least in part, from the fact that these supernatants always contained abundant amounts of all the factors to be purified. Similar difficulties in purifying these hemopoietins from crude materials particularly abundant in all of them have been reported by Iscove et al. (4).

The sequence of chromatographic steps adopted included: Con A-Sepharose, Sephadex G150 and DEAE-Sepharose. Preliminary results suggest that SAF and GM-CSF can be purified separately, whereas BPA may perhaps be present in fractions containing either one of these factors.

Once again, it is emphasized that purification of BPA and SAF is a prerequisite to understand the mechanisms regulating the kinetics of stem cells and early erythroid progenitors, under both normal conditions and following radiation.

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3) Migliaccio A.R., et al., in "Recent Advances in Experimental Hematology" Torella V. et al. (eds), Academic Press, New York, in press.
4) Iscove, N.N., J.Cell Physiol., Suppl. 1, 1982, p 65.

Progress Report
1982

Contractor:

Universität Essen
Hufelandstrasse 55
D-4300 Essen

Contract no.: BIO-C-290-81-D

Head(s) of research team(s):

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General subject of the contract:
Investigation on biological dosimetry : Formation of micronuclei in mammalian cells after irradiation with neutrons.

List of projects:

1. Investigation on biological dosimetry : Formation of micronuclei in mammalian cells after irradiation with neutrons.

Titel of project nr BIO-290-81D
Investigation on biological dosimetry: Formation of micronuclei in mammalian cells after irradiation with neutrons.

Head of project and scientific staff:
Prof. Dr. rer. nat. C. Streffer
Dr. med. M. Molls

Im Förderungszeitraum 1982 wurde insbesondere den beiden folgenden Fragestellungen nachgegangen:

1. Welche Kinetik zeigt die Mikronukleusbildung in den Zellzyklen nach Neutronenbestrahlung?
2. Sind Neutronen induzierte chromosomale Schäden, die sich als Mikronuklei manifestieren, reparierbar?

ad 1) Die Embryonen wurden in vitro im 2-Zellstadium in der späten G_2-Phase (33 h post conceptionem) bestrahlt. Die Mikronukleusbildung wurde 15, 29 und 53 Stunden nach Bestrahlung gemessen. Zu diesen Zeitpunkten hatte die große Mehrzahl der bestrahlten Embryonen 1, 2-3 und 3-4 Zellteilungen durchlaufen. Tabelle 1 zeigt, daß die Zahl der Mikronuklei pro Embryo mit zunehmendem Intervall zwischen Bestrahlung und Messung der Mikronukleusfrequenz stieg. Um die Zahl der durchlaufenen Mitosen zu erfahren, war es erforderlich, die Zellzahl der Embryonen zu bestimmen. 15 Stunden nach Bestrahlung geschah dieses durch direkte mikroskopische Beobachtung der Embryonen. Später wurden mit relativ aufwendiger Methode die Zellkerne (Äquivalent für die Zellzahl) individueller Embryonen isoliert und nach Färbung ausgezählt. Die durchschnittliche Zellzahl pro Embryo zu den verschiedenen Untersuchungszeitpunkten ergibt sich ebenfalls aus Tabelle 1.

ad 2) Röntgenbestrahlungsversuche hatten gezeigt, daß die Länge des Intervalls zwischen Bestrahlung 2-zelliger Embryonen

in G_2-Phase und erster Mitose nach Bestrahlung das Ausmaß der Mikronukleusfrequenz beeinflußt. Die "2-Zeller" wurden spät (33 h post conceptionem) und früher (28,5 h post conceptionem) in G_2-Phase mit schnellen Neutronen (7 MeV) bestrahlt. Die Bestimmung der Mikronukleusfrequenz erfolgte 48 h post conceptionem an 4-zelligen Embryonen. Die Ergebnisse der Tabelle 2 demonstrieren einen geringeren chromosomalen Schaden, wenn die Bestrahlung relativ früh in G_2-Phase (Dauer der G_2-Phase: ca. 13,5 h; cytofluorometrisch bestimmt) stattfindet. Aus den Röntgenbestrahlungsversuchen ist uns bekannt, daß der geringere cytogenetische Schaden bei Bestrahlung früh in G_2-Phase einhergeht mit geringeren Störungen der praeimplantativen Zellvermehrung und Blastocystenbildung. Die Ergebnisse sprechen für eine verbesserte Reparatur chromosomaler Schäden während eines längeren Verbleibs der Zellen in G_2-Phase. Neutronenbestrahlung scheint diesen postulierten intrazellulären Reparaturmechanismus nicht vollständig zu blockieren.

Neben den unter 1) und 2) beschriebenen Experimenten wurden Untersuchungen zur Induktion chromosomaler Aberrationen durch Neutronen begonnen. In ersten Ergebnissen deutet sich an, daß mit dieser Untersuchungsmethode Effekte nach sehr kleinen Dosen noch deutlichler nachweisbar sind.

Publikation

Development of cytogenetic effects and recovery after irradiation of preimplantation mouse embryos.
M. Molls, C. Streffer, B. Fellner, U. Weissenborn, M.-L. Steimann, and W. Breipohl. in: Proceedings EULEP Symposium 17th Annual Meeting of the European Society for Radiation Biology, Bordeaux, 1982.

Tabelle 1: Mikronuklei pro Embryo und durchschnittliche Zellzahl pro Embryo nach Bestrahlung mit 7 MeV Neutronen

Dosis (Gy)	15 h p.r.		29 h p.r.		53 h p.r.	
	Mikron.	Zellen	Mikron.	Zellen	Mikron.	Zellen
0	0,07	5,6	0,07	21,5	1,67	67,3
0,12	0,34	5,3	0,82	20,1	2,30	51,9
0,25	0,48	4,9	1,47	18,1	3,10	51,8
0,50	0,68	4,3	1,70	17,5	5,10	40,3
1,00	1,3	4,3	2,30	15,8	6,95	29,2

Tabelle 2: Mikronukleusfrequenz (Prozent) in 4-zelligen Embryonen nach Bestrahlung mit Neutronen in der Mitte (28,5 h p.c.) und am Ende (33,0 h p.c.) der G-2-Phase im 2-Zellstadium

	Dosis (Gy)	0 Mikron.	1 Mikron.	2 Mikron.	3 Mikron.	3 oder mehr Mikron
28,5 h p.c.	0	94,3	5,7			
	0,12	85,0	10,0	5,0		
	0,25	86,6	13,3			
	0,50	85,0	10,0	5,0		
	1,00	67,8	25,0	3,6	3,6	
33 h p.c.	0	94,3	5,7			
	0,12	80,0	16,0	4,0		
	0,25	74,3	17,1	8,6		
	0,50	53,3	26,6	16,6	3,3	
	1,00	59,0	20,4	7,7	10,2	2,5

Progress Report
1982

Contractor:

Contract no.: BIO-C-364-81-EIR

Dublin Institute of Technology
College of Technology
Kevin Street
IRL-Dublin 8

Head(s) of research team(s):

Dr. J. K. Taaffe
Dept. of Physics
Coll. of Technology
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Dr. J.F. Malone
Dept. of Physics
Coll. of Technology
Kevin Street
IRL-Dublin 8

General subject of the contract:

Thyroid irradiation : dosimetry and radiobiological consequences in cell culture systems and in humans.

List of projects:

1. Dosimetric aspects of radionuclides of iodine in the human thyroid and in thyroid cell cultures.
2. Radiobiological response of cultured sheep thyroid cells.
3. Radiobiological aspects of the human thyroid.

Work continued this year on dosimetric studies, studies with thyroid tissues derived from sheep and to a limited extent, with human thyroid.

With regard to dosimetry the main contributions were

(a) a quantitative histological and steriological analysis of established sheep thyroid cultures, and

(b) an initial comparison of a new theoretical derivation for dose due to radioiodine nuclides in thyroid follicles with established methods of determining these values.

The effect of radiation is being studied on cultures of differentiated sheep thyroid cells using three main endpoints - survival, chromosome analysis and transformation. Preliminary results indicate that the survival curves have a high Do and a low extrapolation number, although these are now known to be closer to usual mammalian values than previously thought. Recovery between fractionated doses is also low. Transformation frequency is high and has been detected at doses as low as 2.5Gy of x-rays, but preliminary experiment suggests that after 50Gy of ^{131}I delivered over 1 week transformation is either very low or absent. This has not yet been confirmed by a repeat experiment.

Preliminary radiation survival studies suggest that the human cells are more sensitive to the killing effects of radiation.

Finally we participated in the preparation of a follow up survey for patients exposed to thyroid irradiation during diagnostic medical investigations.

Title of Project No.1: Dosimetric Aspects of Radionuclides of Iodine in the Human Thyroid and in Thyroid Cell Culture.

Head of Project: Dr. J.F. Malone.

Scientific Staff: Dr. J.F. Malone
Dr. O. Redmond
Dr. C. Mothersill
J.K. Taaffe
A. Murphy
K. Maher
N. Corbally

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1. A quantitative histological and stereological analysis of the sheep thyroid cells in culture was performed with a view to providing base-line data that might be employed in dosimetric calculations. The parameters analysed included epithelial cell valume, space valume, follicular lumen valume, fibroblast cell volume, follicular diameter and cell nuclear diameter. Cultures of varying ages from 0 to 44 days were initially examined and a more detailed study was performed on cultures from 8 to 20 days old. An initial set of results are illustrated in the Figure in which the volume of tissue components is expressed as a percentage of the total, and the relevant tissue component diameters are expressed in microns. The relevant properties show a surprising stability during the period from the time when the culture becomes well established until the time when the cells begin to die.

2. Initial calculations of the dose due to ^{131}I to a cell culture were performed for the experiments outlined in Project 2.

3. The revised scheme for calculation of doses due to ^{123}I, ^{128}I and ^{131}I in thyroid follicles devised by Van Best was examined with a view to determining if it had any implications for the dosimetric aspects of this and other projects being undertaken within the programme. This data is of sufficient interest to warrant a comprehensive study.

4. Video displayed ultrasound images were unsuccessfully digitised on a nuclear medicine image processor with a view to quantisation of thyroid dimensions.

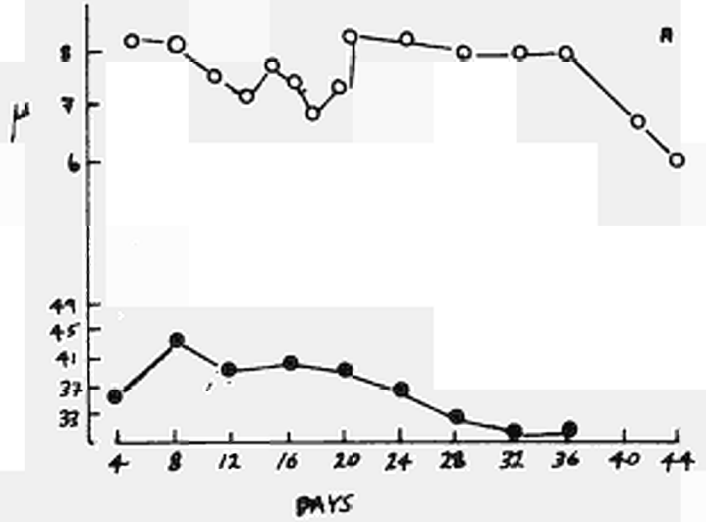

Figure 1A: The nuclear (o) and follicular (o) diameter determined on cultures of sheep thyroid cells over a 44 day culture period.

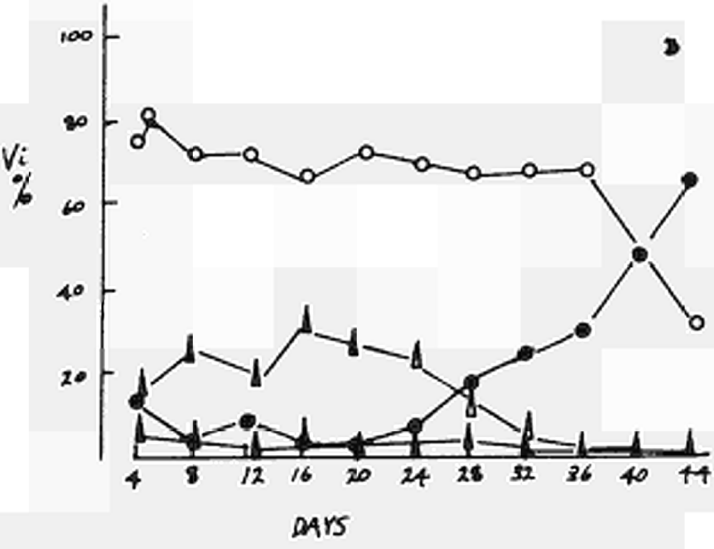

Figure 1B: The volume of the cell culture occupied by Epithelial cells (o), Fibroblast cells (▲), Follicles (△) and empty space (●) measured over a 44 day culture period.

Title of Project 2: Radiobiological Response of Cultured Sheep Thyroid Cells.

Head of Project: Dr. J.F. Malone.

Scientific Staff: Dr. J.F. Malone, Dr. M. Moriarty, Dr. O. Redmond, A. Murphy, Dr. M.J. Cullen, C.B. Seymour, Dr. C. Mothersill, N. Corbally, Dr. J.E. Dumont (Collaborator)

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1. Radiation Survival Curves

Ten day old primary cultures of sheep thyroid are routinely used in radiation experiments. These demonstrate iodine uptake ($\sim 1.5 \times 10^4$ counts per 10^6 cells per minute), T_4 production ($\sim 9\mu$Moles/100ml culture medium). Follicles containing PAS positive material are also well developed at this point. Survival curves have now been constructed for the dose range 2.5 - 30Gy. These give survivals extending to the third decade (0.1%). A number of problems have been encountered particularly at high doses where the survival is very low. The cell numbers required to detect any survival at these doses make interpretation of the results difficult. Improvements in cloning technique now mean that a plating efficiency of 5 - 15% can be obtained for unirradiated controls and this permits accurate survival values to be obtained up to doses of 15.0Gy, where the percentage survival is 0.4 ± 0.06. As can be seen, there is only a small shoulder on the survival curve (n = 1.8) and the Do is quite large (2.12Gy), although these values are closer to normal mammalian values than had been previously thought. This is probably due to the extension of the survival curve to the third decade. Preliminary recovery points were obtained from split-dose experiments where cells were initially irradiated to 7.5Gy and then given another 7.5Gy after a radiation-free interval of 2 hours. Control flasks were irradiated to 15.0Gy. Initial results indicate that recovery is low (a factor of two).

2. Radiation Induced Transformations

An assay for the detection of chemically induced malignant transformations in primary cultures of hamster dermis was developed by Newbold ICRF,

Chalfont-St-Giles, Bucks., U.K. (Personal Communication). This has been adapted in our laboratory for use with the thyroid system. Experiments indicate that transformation can be obtained from radiation doses in the range 2.5 - 10Gy. Lower doses do not lead to observable transformation for several months but transformation can be detected after about 6 weeks at higher doses.

One experiment has been conducted using ^{131}Iodine as the radiation source. The amount of isotope used was calculated to give a dose of approximately 50Gy over a 7 day period to cultures of differentiated sheep thyroid cells. Radiation survival points using a cobalt 60 therapy unit confirmed that the dose obtained by the iodine-treated cells was equivalent in terms of survival to a single dose of the order of 10Gy. Results from the first experiment suggest that the level of transformation induced by this treatment is either very low or absent, since all the cells had died by the fifth subculture.

3. Chromosome Studies

Initial problems with the study of thyroid chromosomes have been overcome and it is now possible to obtain cultures of thyroid cells for analysis with a high mitotic index. The method involves incubation of the cultures with colcemid for 6 hours commencing 24 hours after the first subculture. Using this method the normal sheep karyotype has been established and radiation studies will commence shortly.

4. Functional Endpoints

A number of functional endpoints of improtance in the thyroid gland are being continually monitored. The main ones are iodine trapping, T_4 production and lactate use, all of which correlate well with the degree of differentiated morphology. Experiments to ascertain the effect of irradiation on the longterm function of the cultures are in progress.

5. Longterm Culture

Considerable progress has been made on the longterm culture of sheep thyroid. Cultures can now be maintained for at least 100 days provided a low cell number is set up initially. Efforts were made to control fibrobalst growth in the cultures with a monoclonal antibody against fibroblasts. This led to the elimination of fibroblasts but the organised structure of the culture was also lost.

Title of Project No. 3: Radiobiological Aspects of the Human Thyroid.

Head of Project: Dr. J.F. Malone.

Scientific Staff: Dr. J.F. Malone, A. Murphy, Dr. O. Redmond, C.B. Seymour, Dr. C. Mothersill, N. Corbally

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1. Thyroid tissue was obtained from six patients undergoing surgery. Successful cultures were established from four of these, the remaining two contained large cysts and it was not possible to obtain sufficient tissue from them for a culture. All the successful cultures were from non-malignant tissue.

2. A number of different media and sera were tested for ability to sustain the cells but the culture media used for sheep thyroid was not improved upon.

   Differentiation as shown by follicle formation, PAS positive material, T_4 production and lactate use was similar to that obtained for sheep. Cultures have now been successfully maintained for 43 days.

3. Preliminary attempts to clone human thyroid cultures were hampered by a low plating efficiency, but this has now been improved by using a CO_2 incubator which ensures a more uniform buffering system.

4. Preliminary radiation experiments suggest that human thyroid cells are much more sensitive than sheep thyroid cells but this could be associated with methodology problems or the pathology of the tissue since no normal thyroid was obtained.

   Since tissue specimens from human thyroid operations tended to be small an attempt was made to establish differentiated cultures using an explant technique. Unfortunately, although differentiation occurred initially around the explant the culture became overrun by fibroblasts after about 1 week.

5. Two papers were presented to the Thyroid Irradiation Follow-Up Group Meeting arranged by Dr. Dumont in the late summer '82. Arising from this we agreed to participate in the preparation of the protocol for a formal follow up study with particular reference to dosimetry. A study of the

basic physics of the dosimetry of radionuclides by Van Best may influence the outcome of this study. Hospitals in Ireland who have been involved in thyroidal radioiodine protocols have been contacted to determine the numbers of patients involved over the last 25 years and the extent of records available.

PUBLICATIONS

1. Mothersill C., Seymour C., Malone J.F., (1982). Longterm Cultures of Sheep Thyroid. FEBS Meeting on Cell Differentiation, Athens.

2. Mothersill C., Seymour C., Moriarty M., Malone J.F., (1982). Radiation Induced Transformation in Sheep Thyroid Cells in Culture. Irish Assoc. for Cancer Research Annual Meeting, Cork. (Irish J. Med. Sci. - in press).

3. Mothersill C., Seymour C., Moriarty M., Malone, J.F., (1983). The Detection of Transformation in Cultures of Irradiated Thyroid Cells. Association for Radiation Research Winter Meeting, Salford, U.K. (Int. J. Rad. Biol - in press).

4. Malone J.F., O'Connor M.K., and Hendry J.H., (1982). Glandular Epithelium with particular reference to Thyroid. Cytotoxic Insult to Tissue. Ed. C.S. Potton & J.H. Hendry, Edinburgh Churchill Livingstone, p.p. 181-227.

5. Mothersill C., Seymour C., Malone J.F. (1983). Maintenance of Morphological and Functional Differentiation in Primary Thyroid Cultures for 3 months. Submitted to Nature.

6. Murphy A., Mothersill C., O'Connor M.K., Malone J.F., Cullen M.J. and Taaffe J.K., (1983). An investigation of the Optimum conditions for a Differentiated Culture of Sheep Thyroid Cells. (Submitted to Acta Endocrinologica).

7. Mothersill C., Seymour C.B., Moriarty M. and Malone J.F. The Development of the Transformation Assay for Thyroid Cells. Int. Congress of Radiation Research, Amsterdam, July 1983, (submitted).

8. Maher K.P., Malone J.F., Harley G.D. and McInerney D.P. (1982). Computational requirements for digital fluoroscopy - specification of existing systems and their relationship with nuclear medicine image processors.

9. O'Connor M.K., Malone J.F., Maher K.P., Molloy M., Freyne P. and Malone E.W. (1982). Evaluation of a nuclear medicine computer for digital fluoroscopy. Proc. World Congress on Medical Physics and Bioengineering, p.p. 19-32. Eds: W. Bleifeld, D. Harder, H.K. Lettz and H. Schaldach. (HPBE, Hamburg).

Progress Report
1982

Contractor:

Gesellschaft für Strahlen-
und Umweltforschung mbH
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

Contract no.: BIO-C-344-81-D

Head(s) of research team(s):

Prof. Dr. S. Thierfelder
Inst. für Hämatologie,
GSF
Landwehrstrasse 61
D-8000 München 2

General subject of the contract:

Treatment of short term effects of radiation injury to the lymphohemopoietic system.

List of projects:

1. Recovery from radiation injury after maximal doses of fractionated total and partial body irradiation and bone marrow transplantation.
2. Removal by antisera of anti-host immune reactivity in irradiated bone marrow recipients.
3. Repopulation of the lymphatic tissue after total body irradiation.

Title of project nr 1

Recovery from radiation injury after maximal doses of fractionated total and partial body irradiation and bone marrow transplantation.

Head of project and scientific staff

Priv.Doz. Dr. H.J. Kolb, Dr. U. Bodenberger

Total body irradiation (TBI) and allogeneic marrow transplantation is an effective treatment of leukemia. The purpose of TBI is the elimination of leukemic cells and the suppression of the host's immune response against the allogeneic graft. Higher doses than the presently used 10 Gy may be necessary for complete elimination of leukemia and the suppression of the host's immune response against strongly histoincompatible marrow grafts. TBI in a dose of 22.5 Gy in body midline has been successfully applied to dogs when given with a dose rate of about 4 cGy as fractionated irradiation within seven days. Acute mortality was not different whether 4.5 Gy where given every other day or 1.125 Gy twice daily. Prophylactic administration of oral, non-adsorbable antibiotics improved the survival of acute radiation toxicity, when marrow was given in sufficient amounts. The beneficial effect was not observed when suboptimal marrow doses were given.

Sixtyfour dogs survived acute radiation toxicity after TBI with 12-12.5 Gy in body midline and autologous marrow infusion. They have been observed between 2 months and 6 years after treatment for delayed radiation toxicity. Eight dogs died spontaneously, 4 with infections, 2 with subacute interstitial pneumonitis, one with a soft tissue sarcoma and one with chronic aggressive hepatitis.

TBI with higher doses may not be more effective, if clonogenic leukemic cells are spared by protraction and fractionation to the same degree as normal tissue. We have

irradiated canine marrow "in vitro" with various dose rates and found that CFU-C were inactivated widely independent of the dose rate. Recovery of CFU-C "in vivo" following TBI with sublethal doses was also independent of the dose rate. Hemopoietic interaction following a lethal dose of TBI with marrow obtained after sublethal TBI at different dose rates was also indepent of the dose rate of the first TBI. The effect of fractionation on inactivitation of hemopoietic precursor cells has been studied by radiation of marrow cells in the agar culture plate and in suspension cultures. Preliminary results indicate some recovery in suspension culture. Suppression of the proliferative response in mixed leukocyte culture has been studied by irradiation of peripheral blood lymphocytes "in vitro" prior to culture inhibition of ^{3}H-thymidine incorporation to less than 10 percent of control was observed after 600 R. The dose response curve had a shoulder in lower dose ranges. However, irradiation with different dose rates did not change the suppression of the response. Fractionation in 600 R-fractions appears to be superior to smaller fractions for immunosuppression "in vivo".

Allogeneic marrow transplantation from DLA-incompatible littermates different in one DLA-haplotype resulted in severe graft-versus-host disease, if untreated marrow was given. Insufficient restoration of hemopoiesis was the cause of failure, if the marrow was treated with an absorbed antithymocyte globulin (ATG). Separation of blood lymphocytes with the use of staphylococcal protein A (SpA) induced rosettes and nylon wool filtration allowed the defination of canine T-lymphocytes. These were marked with absorbed ATG and showed specific T-cell functions in MLC. Indirect SpA-rosettes and cytotoxicity were worked out in order to indicate rosette formation and cytotoxicity with primary antibodies unable to bind to SpA or activate complement.

Title of project nr 2

Removal by antisera of anti-host immune reactivity in irradiated bone marrow recipients.

Head of project and scientific staff:

Prof.Dr.S. Thierfelder, Priv.Doz.Dr.H. Rodt

Earlier observations with absorbed polyclonal and lately with monoclonal anti-Th-1 antibodies had revealed that no immunosuppression could be observed after injection into mice. Such mice rejected H-2 incompatible skin grafts in a normal fashion unlike rabbit or rat anti-mouse lymphocyte antibodies. Spleen cells of mice injected with monoclonal anti-T failed to show suppressed graft-versus-host reactions. Graft-versus-host reactions were however suppressed when the anti-T antibodies were applied in vitro for treatment of the donor's marrow. We have recently compared the immunosuppressive effect of 11 monoclonal anti-Th-1 produced in our and in other laboratories. All the antibodies proved immunosuppressive when applied in vitro for reduction of graft-versus-host reactions. They did however not suppress humoral or cellular immunity when injected into mice. C57BL/6 or AKR/J skin grafted on CBA mice pretreated with the various monoclonal antihybridoma antibodies was rejected as quickly as from untreated CBA mice. A treatment schedule was used that prevented skin graft rejection when rabbit ALS was injected. CBA mice that had been sensitized against SRBC developed hemagglutinins no matter whether they had received monoclonal anti-T or not. Rabbit ALS as the positive control prevented SRBC antibody formation. Finally one exceptional antibody was identified, a rat anti-mouse-Th-1 monoclonal antibody (YTS 154.7.7) which delayed the rejection of H-2 compatible and incompatible skin consistantly. Like the

ôther antibodies it fixed complement in cytotoxic and quantitative complement fixation tests. It was the only rat antibody of IgG2b class which we have tested so far. Skingrafts of all mice were still surviving 20 days, at a time when all mice pretreated with the other antibodies had rejected. Compared to rabbit ALS the immunosuppressive effect of YTS 154.7.7. was similar in 2 assays. In a third repitition its immunosuppressive effect on skin grafts was intermediary between rabbit ALS and the other monoclonal anti-T antibodies.

Interestingly the monoclonal antibodies which proved immunosuppressive in vivo was also the most successful antibody to suppress graft-versus-host reactions when applied in vitro to the marrow cells of the donor. It consistantly eliminated acute as well as chronic secondary disease.

Our data indicate that monoclonal antibodies against T antigen differ from polyclonal ALS in important respects. This must be further analyzed in order to avoid failures in clinical application of monoclonal antibodies be it transplantation or as carriers for cytotoxicity or other toxic principles.

Title of project nr 3

Repopulation of the lymphatic tissue after total body irradiation.

Head of project and scientific staff:

Dr.G. Hoffmann-Fezer

Our studies to identify lymphocyte subpopulations in the lymphatic tissue have lead to the use and adaptation of Sternberger's Peroxydase-anti-peroxidase (PAP) method. This immunohistochemical approach has considerable advantages over immunofluorescence as pointed out in the previous report. We applied the PAP method to the identification of cell membrane markers on colonies (CFU) that form in spleen and bone marrow of irradiated mice injected with mouse or rat marrow. For rats we had found - using transfer systems - that hemopoietic stem cells express the Th-1 antigen in contrast to mice. For mice we had raised antibodies which after absorption with spleen cells detected antigens on fetal liver cells and erythropoetic as well as granulocytic precursor cells. Using the PAP method we found that the latter antibodies bound to splenic CFU with stronger labeling of the more peripheral cells which is compatible with the view that colonies form by budding from the vicinity of splenic trabecula in a way that the more mature cells are not at the periphery of the cell colony. Anti-Th-1 was identified using biotinylated monoclonal mouse-anti-Th-1.1 coupled to avidin conjugated with peroxidase. Th-1 expressing cells were quite rare in the colonies. Singular Th-1 positive cells were observed 2-6 days post transplantation outside the colonies near trabecula. This finding is compatible with these cells being stem cells. It is no proof however, because Th-1 occurs also on rat marrow precursor cells other than stem

cells. The lack of Th-1 cells in CFU indicates on the other hand that the numerous Th-1 positive cells normally found in rat bone marrow belong to a maturation stage or pathway which is little represented in CFU colonies.

Publications

Hoffmann-Fezer, G., Lohmeyer, J., Doxiadis, I., Stünkel. K., E.P. Rieber, Kummer, U., Eulitz, M., Thierfelder, S.: Monoclonal antibodies against T-cell antigens studied by immunohistochemistry. Blut 44, 275-288 (1982)

Hoffmann-Fezer, G., Kummer, U., Doxiadis, I., Eulitz, M., Thierfelder, S.: Immunohistochemistry for screening hybridomas supernatants and doublelabeling of cell surface markers. In: Exp. Hematol. Today (Eds.: Baum S, Ledney GD, Thierfelder S) Basel: Karger 24, 187 (1982)

Hoffmann-Fezer, G., Eulitz, M., Kummer, U., Zeitler, H.J., Thierfelder. S.: Nachweis von Zellmarkern im histologischen Schnitt mittels monoklonalen Antikörper. Fresenius Z. Anal. Chem. 311, 349 (1982)

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Rodt, H., Netzel, B., Kolb, H.J., Haas, R.J., Wilms, K., Link, U., Bender-Götze, Ch., Niethammer, D., Wernet, P., Janka, G., Thierfelder, S.: Suppression of GvHD by Antisera: Clinical Results in 20 Patients. Exp. Hematol. Today (Eds.: Baum S, Ledney GD, Thierfelder S) Basel: Karger 147 (1982)

Rodt, H., Bender-Götze, Ch., Haas, R.J., Janka, G., Kolb, H.J., Link, H., Netzel, B., Niethammer, D., Schüch, K., Thierfelder, S., Wilms, K.: Ergebnisse der Inkubationsbehandlung mit Anti-T-Zell Globulin bei der Prophylaxe der Transplantat-gegen-Wirt Krankheit (GvHD). Onkologie 13, 44 (1982)

Rodt, H.: The effect of T-cell specific antibodies on Graft-versus-host disease (GvHD). Recent experimental and clinical aspects. In: Modern Trends in human Leukemia 1982 (in press)

Rodt, H., Thierfelder, S., Kummer, U.: Effect of monoclonal antibodies on GvHD in minor- and MHC-incompatible mouse models. Transpl. Proc. 1982 (in press)

Thierfelder, S., Hoffmann-Fezer, G., Rodt, H., Kummer, U.: Cell Manipulation with Antibodies: Differentiation Effects of Anti-T and ATG. In: Experimental Hematology Today (Eds. S.J. Baum, GS Ledney, S. Thierfelder) Basel, Karger 103-109 (1982)

Thierfelder, S., Hoffmann-Fezer, G.: Cell Surface Markers on Sections of colony forming units and fetal hemopoietic tissue. Exp. Hematol. 10, Suppl. 10, 1982 (in press)

Thierfelder, S., Hoffmann-Fezer, G.: Immunohistochemical staining of haemopoietic precursor cells. In: Haemopoietic Stem Cells, Alfred-Benzon-Symp. Bd. (Eds.: Killmann SA, Cronkite EP, Muller-Berat CN)
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Thierfelder, S., Hoffmann-Fezer, G., Rodt, H., Doxiadis, I., Eulitz, M., Kummer, U.: Antilymphocytic antibodies and marrow transplantation. VI. No Immunosuppression in vivo after Injection of monoclonal antibodies which blocked Graft-versus-Host Reactions and Humoral Antibody Formation in vitro. Transplantation 1982 (in press)

Progress Report
1982

Contractor: Contract no.: BIO-C-357-81-NL

REP-Institutes of the
Organisation for Health Research
TNO
P.O. Box 5815
NL-2280 HV Rijswijk

Head(s) of research team(s):

Prof. Dr. D. W. van Bekkum
Radiobiological Institute
TNO
P.O. Box 5815
NL-2280 HV Rijswijk

General subject of the contract:

Development of safe procedures for transplantation of mismatched bone marrow.

List of projects:

1. Search for optimal conditioning protocol.
2. Influence of microflora on bone marrow repopulation and GvHD.
3. Immune reconstitution after bone marrow transplantation across MHC barriers.

Title of project nr 1: Search for optimal conditioning regimen.
BIO-C-357-N (B)
Head of project and scientific staff: Prof. Dr. D.W. van Bekkum,
Dr. B. Hogeweg, Dr. P.J. Heidt, Dr. G. Wagemaker;
advisor: Dr. H.M. Vriesendorp.

So far, it was found in dogs that lymphocyte-depleted stem cell enriched bone marrow fractions require more intensive conditioning regimens than whole bone marrow grafts. In the case of DLA-matched sibling host/donor combinations, reproducible takes were obtained with either fractionated total body irradiation (TBI) or a single dose of TBI supplemented with Cyclosporin-A treatment. However, in one DLA-haplotype mismatched dog engraftment of stem cell enriched fractions could only be achieved by fractionated TBI at doses (2 x 60 Gy or 3 x 4.5 Gy) which resulted in severe gastro-intestinal toxicity, whereas lasting engraftment was associated invariably with moderate to severe GvHD. To circumvent the GvH-problem in these mismatched combinations, the stem cell enriched fractions will be further depleted of T-lymphocytes by E-rosette sedimentation It was expected that with these T-cell depleted stem cell fractions engraftment will even be more difficult to achieve. Therefore, TBI has been combined with relatively non-toxic conditioning modalities. A large batch of adsorbed and purified rabbit-anti-dog-thymocyte globulin (ATG) has been prepared and tested. The profound immunosuppression resulting from combined treatment of ATG and fractionated TBI (2 x 4.5 Gy) for one haplotype mismatched donor/recipient combination resulted, however, in generalized infections with gram-negative bacteria species. These experiments, therefore, are currently being repeated in gnotobiotic dogs under strict reverse isolation.

Fractionated total lymph node irradiation (TLI) was explored as conditioning for unmodified whole bone marrow grafting from DLA-mismatched unrelated donors and from DLA-identical sibling donors. A regimen of multiple fractions of 1 Gy TLI up to a total dose of 14-19 Gy resulted in rejection of mismatched grafts in the majority of cases. In only 2 out of 12 cases a small proportion of donor type cells was found in the bone marrow. Larger total doses of TLI could not be delivered because of general toxicity of this radiation regimen. Using DLA-identical sibling marrow partial chimerism was obtained following 5 x 2 Gy TLI and, following 10 x 2 Gy TLI complete or nearly complete chimerism was observed. The latter conditioning regimen proved, however, to be too toxic for most animals. These results suggest that TLI cannot be expected to provide additional immunosuppression required for obtaining takes of T-cell depleted mismatched bone marrow grafts. The construction of a dog phantom for dosimetry purposes of TLI and TBI irradiation set-ups has been completed and the dosimetric measurements will soon be finished.

Title of project nr 2: Influence of microflora on bone marrow repopulation and GvHD.
BIO-C-357-N (B)
Head of project and scientific staff: Prof. Dr. D.W. van Bekkum, Dr. P.J. Heidt, Dr. G. Wagemaker.

In mice it was previously demonstrated that the GvHD which could be induced in conventional lethally irradiated recipients by adding small numbers of spleen cells to an H-2 different bone marrow graft, could not be prevented by gastrointestinal decontamination. It was attempted to test whether GvHD induced by H-2 identical spleen cells would be influenced more favourably by gnotobiotic conditions of the recipient. However, using HD2/Rij ($H2^{aq}$) spleen cell grafts into irradiated CBA/Rij ($H2^{aq}$) recipients, it turned out that even as many as 2×10^7 spleen cells did not induce GvHD in conventional animals.

The search for microbial antigens which might trigger GvH reactions following transplantation of incompatible bone marrow was continued and included coli and Klebsiella species. Bacterial cultures were inactivated with antibiotics and then fed to test mice. None of the species tested seemed to be involved in activating the GvH mechanism.

One of the possible drawbacks of the use of gnotobiotic conditions for prevention of GvHD is the interference with a take of the bone marrow graft. It was reported in the literature that Co-trimoxazole can cause hematotoxic side-effects. Since Co-trimoxazole is in use for selective decontamination of the gastro-intestinal tract and is also employed in pediatric bone marrow transplantation patients for the prevention of Pneumocystis carinii pneumonia, we investigated the influence of this drug combination on the regeneration rate of leukocytes after bone marrow transplantation in mice. A significant delay of the regeneration of both the granulocytes and the lymphocytes was observed under Co-trimoxazole treatment. It is now being investigated whether this inhibition can be prevented by the simultaneous administration of Folinic acid.

The effect of gastrointestinal microflora on the appearance of PNA non-agglutinating (PNA^-) Ly 1^+ lymphocytes (the phenotype that was found to carry suppressor activity of GvHD in neonatal thymus) following sublethal whole body irradiation was investigated in C3H conventional and germ free mice. These cells were determined in the regenerating thymus at various intervals up to 35 days after irradiation. No difference between the two groups of mice was observed, which seems to suggest that this type of suppressor cells is not involved in the prevention of GvHD by the germ free state.

Title of project nr 3: Immune reconstitution after bone marrow transplantatio
BIO-C-357-N (B) across MHC barriers.
Head of project and scientific staff: Dr. G. Wagemaker, Dr. P.J. Heidt, Prof. Dr. H. Balner (August, 1982), Prof. Dr. D.W. van Bekkum.

The study on mitigation of GvHD in rhesus monkeys by selectively eliminating lymphocytes from the bone marrow graft by density centrifugation and E-rosette depletion has been continued. It was previously established that reproducible takes could be obtained with stem cell fractions from unrelated mismatched donors by conditioning with 2 doses of 7 Gy separated by 72 hours. The investigation is now in the stage of determining the limiting dose of irradiation for acceptance of such lymphocyte-depleted grafts when limiting cell numbers are given. It appeared that 2 fractions of 6 Gy given with an interval of 24 hours are required for the take of stem cell fractions of completely mismatched donors at a dose of 2.5×10^7/kg, whereas 2 fractions of 5.5 Gy were ineffective. It remains to be determined whether the total dose of 12 Gy can be tolerated without long-term side effects or whether lower doses of irradiation combined with less toxic conditioning agents such as ATG and Cyclosporin-A have preference. This part of the program will be intensified in 1983.
The studies on immune reconstition will be continued as soon as a reproducibl long-term survival has been obtained of monkeys treated with purified stem cells from mismatched donors. In the meantime a large group of RhLA-identical siblings has become available from the breeding program and these will be employed as soon as they have reached the appropriate age (1983) for treatment with compatible stem cell preparations. This has been shown to result in long-term survival and such monkeys are excellently suited for studying the immune reconstitution as they represent a situation which is analogous to current clinical practice of bone marrow transplantatio

Publications

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Progress Report
1982

Contractor:

International Bone Marrow
Transplant Registry, IBMTR
Mt. Sinai Med. Center
P.O. Box 342
USA-Milwaukee WI 53201

Contract no.: BIO-C-520-82-US
BIO-C-471-81-US

Head(s) of research team(s):

Dr. M.M. Bortin
Scientific Director IBMTR
Mt. Sinai Med. Center
P.O. Box 342
USA-Milwaukee WI 53201

General subject of the contract:

Maintenance of the International Bone Marrow Transplant Registry.

List of projects:

1. Maintenance of the International Bone Marrow Transplant Registry.

A. Objectives

The overall purpose of the International Bone Marrow Transplant Registry (Registry) is to aid in improving the success rate of bone marrow transplantation as applied for the treatment of patients with a variety of otherwise incurable diseases. The specific aims are:

1. To maintain a statistical center for the collection, organization and analysis of clinical data provided by bone marrow transplant teams throughout the world;

2. To disseminate the results of clinically relevant analyses of pooled Registry data to bone marrow transplant teams, and to the medical profession for the earliest possible benefit to patients who might be helped by bone marrow transplantation treatment; and

3. To aid in designing, organizing and providing statistical support for controlled, cooperative clinical trials utilizing bone marrow transplantation.

B. Accomplishments During the First Year

1. Maintaining a Statistical Center

Between July 1, 1982 and December 31, 1982 the Registry received reports of 285 patients having a total of 297 transplants. Follow-up reports were received for 113 patients. In addition, 10 new transplant teams started submitting reports, and 9 more stated their intention to do so. This continues to indicate an increase in Registry participation by bone marrow transplant teams.

2. Disseminating the Results of Analyses

Large numbers of reprint requests and inquiries from transplanters continue to be received regarding the Registry report entitled "Factors Associated With Interstitial Pneumonitis After Bone-Marrow Transplantation for Acute Leukemia" that was published in the February 20, 1982 issue of The Lancet.

The Registry Letter to the Editor entitled "Interstitial Pneumonitis: Dose-Rate vs. Total Dose of Radiation" was published in Int J Rad Oncol Biol Phys 8:1815, 1982.

The paper, "Bone Marrow Transplantation for Acute Leukaemia in First Remission" was published in The Lancet ii:1006-1009, 1982.

The manuscript "HLA Antigens in Italian and Non-Italian Caucasoid Aplastic Anemia Patients" by J. D'Amaro, J.J. van Rood, A.A. Rimm and M.M. Bortin has been accepted for publication in Tissue Antigens; it will appear in the March, 1983 issue.

The manuscript, "Bone Marrow Transplantation for Acute Myelogenous Leukemia: Factors Associated with Early Mortality" was accepted for publication in JAMA; it will appear in the March 4, 1983 issue.

The manuscript "Factors Associated with Early Mortality Following Bone Marrow Transplantation for Acute Myelogenous Leukemia: A Report from the International Bone Marrow Transplant Registry" has been accepted for publication in Transplantation Proc; it will appear in the March, 1983 issue.

The manuscript "Pathogenesis of Interstitial Pneumonitis Following Allogeneic Bone Marrow Transplantation for Acute Leukemia" is an invited chapter in the book Biology of Bone Marrow Transplantation (ed. R.P. Gale). It is scheduled to appear in August, 1983.

Abstracts concerning bone marrow transplantation for acute nonlymphoblastic leukemia were published in the programs of the 1982 International Congress of the Transplantation Society and the Annual Meeting of the International Society for Experimental Hematology. An abstract on interstitial pneumonitis in allografted leukemic patients will be incorporated in the program of the UCLA Symposium on the Biology of Bone Marrow Transplantation.

Dr. Bortin presented reports from the Registry regarding bone marrow transplantation for acute leukemia at the International Congress of the Transplantation Society and at the Annual Meeting of the International Society for Experimental Hematology in August, 1983. He is scheduled to present Registry data at a plenary lecture on February 14, 1983 at the UCLA Symposium on the Biology of Bone Marrow Transplantation and at the University of Illinois School of Medicine in May, 1983.

A Registry Newsletter was distributed to more than 300 bone marrow transplanters throughout the world in December, 1982.

Requests for information and analyses from transplanters continued to arrive at the Statistical Center on an average of one to two per week. Raw data, detailed analyses of specific problems and protocol information were provided promptly. Several requests for information per week also were received from patients, relatives of patients and physicians; each request was dealt with promptly.

3. Providing Statistical Support for Clinical Trials

A questionnaire regarding interest in participation in controlled clinical trials was distributed to 71 bone marrow transplant teams to learn the level of interest. The response thus far indicated that 25 teams are very interested and are prepared to enter 200 patients to the studies in the first year. A subcommittee of the Registry will meet in February, 1983 to organize and plan the controlled clinical trials.

III D

SOMATISCHE SPÄTWIRKUNGEN IONISIERENDER STRAHLUNG

LATE SOMATIC EFFECTS OF IONIZING RADIATION

EFFETS SOMATIQUES A LONG TERME DES RAYONNEMENTS IONISANTS

Weitere Forschungsarbeiten zu diesen Themen werden auch in folgenden Tätigkeitsberichten beschrieben :

Further research work on these subjects is also described in the following progress reports :

D'autres travaux sur ces thèmes de recherche sont également décrits dans les rapports suivants :

III A. Feinendegen, L.E.	KFA Jülich	BIO 288 D
III A. Kellerer, A.M.	Univ. Würzburg	BIO 286 D
III B. Busuoli, G./Prodi, V.	CNEN Bologne	BIO 324 I
III B. Clemente, G.F.	CNEN, CSN Casaccia	BIO 323 I
III B. Kirchmann, R.	CEN, SCK Mol	BIO 467 B
III B. Smith, H.	NRPB Chilton	BIO 521 UK
III B. van den Hoek, J.	Landbouwh. Wageningen	BIO 432 NL
III C. Courtois, Y.	INSERM Paris	BIO 351 F
III C. Haag, J.	CEA, CEN Saclay	BIO 347 F
III C. Jammet, H.	Inst. Curie Paris	BIO 348 F
III C. Macieira-Coelho, A.	Assoc. C. Bernard Paris	BIO 352 F
III C. Streffer, C.	Univ. Essen	BIO 290 D
III C. Taaffe, J.K./Malone, J.F.	Coll. Technol. Dublin	BIO 364 EIR
III E. Bootsma, D.	Univ. Rotterdam	BIO 404 NL
III E. Bridges, B.A.	MRC Brighton	BIO 414 UK
III E. Léonard, A.	CEN, SCK Mol	BIO 451 B
III E. Lohman, P.H.M.	TNO Rijswijk	BIO 403 NL
III E. Simons, J.W.I.M.	Univ. Leiden	BIO 407 NL
III E. van der Eb, A.J.	Univ. Leiden	BIO 405 NL
III E. van der Eb, A.J.	Univ. Leiden	BIO 476 NL
III E. Scheer, K.E.	DKFZ Heidelberg	BIO 369 D

Progress Report
1982

Contractor:

Contract no.: BIO-D-376-81-NL

REP-Institutes of the
Organisation for Health Research
TNO
P.O. Box 5815
NL-2280 HV Rijswijk

Head(s) of research team(s):

Dr. P.A.J. Bentvelzen
Radiobiological Institute
TNO
P.O. Box 5815
NL-2280 HV Rijswijk

General subject of the contract:

Fundamental studies on the possible mechanisms of radiation carcinogenesis.

List of projects:

1. Fundamental studies on the possible mechanisms of radiation carcinogenesis.

Title: Fundamental studies on the possible mechanism of radiation carcinogenesis.

Head of project and scientific staff:

P.A.J. Bentvelzen (head), V. Krump-Konvalinkova, J.C. Klein, K.J. van den Berg (scientific staff).

It has been hypothesized that radiation carcinogenesis involves the repositioning of cellular transforming genes. Such a hypothesis might be a challenge to the linear extrapolation theory for calculating the cancer risk at low radiation doses. Several avenues have been used to detect activated transforming genes (also indicated as oncogenes) in radiation-induced tumours.

1. High-molecular weight DNA has been isolated from 32 radiation-induced rat tumours, including 4 pulmonary tumours, 1 skin carcinoma, 1 urether carcinoma, 1 rhabdomyosarcoma and 25 mammary tumours of different histopathological types. Cultures of NIH/3T3 mouse fibroblasts were incubated with a calcium-precipitate of such DNAs and three weeks later examined for morphological transformation. Only DNA of 1 skin tumour and 1 mammary carcinoma was positive in this assay. The resulting clones of transformed cells were injected into athymic "nude" mice and produced tumours within a month at the site of injection.

2. In an earlier study it was found that fragmented cellular DNA from normal mouse and rat cells could transform NIH/3T3 cells, particularly when these cells were preinfected with a leukaemia virus. This enhancing effect of the virus could also be obtained when mouse DNA fragmented were mixed with a cloned Long Terminal Repeat (LTR) of a leukaemia virus. It seems that this proviral element, which contains transcription inducing and enhancing sequences promotes the expression of the oncogene in the DNA samples.
   A striking correspondence was found between the sensitivity of the transforming principle in mouse DNA for restriction enzymes and the recognition sites for these enzymes in the Long Terminal Repeat of a leukaemia virus (see Table 1).

Table 1

Comparison of recognition sites for restriction enzymes with destruction of transforming activity of mouse cellular DNA.

Restriction enzyme	Recognition site in Long Terminal Repeat	Destruction of transformir activity
Ava I	+	+
Bal I	-	+
Bam HI	-	+
Eco RI	-	-
Hind III	-	+
Kpn I	+	+
Pvu II	+	+
Sph I	-	-
Xba I	+	+
Xho I	-	+

All four enzymes, which have cleavage sites in the LTR, also abolish transformation by mouse DNA. Since there are four other enzymes, which abrogate transforming activity, but leave the LTR intact, it is concluded that the transforming principle is a complex of an LTR from an endogenous virus with a cellular oncogene. The expression of this complex is usually inhibited by the action of neighbouring controlling elements. The LTR of an exogenous virus can promote the expression of this complex.

By means of the Northern blotting procedure, it was found that BALB/3T3 cells which had been transformed by mouse DNA expressed a nonviral RNA that hybridized with the LTR of a leukaemia virus. This molecule was not detectable in untransformed cells. However, this RNA species was also present in several radiation-transformed as well as spontaneously transformed lines. It seems that this complex of endogenous LTR and cellular oncogene is important in radiation carcinogenesis.

3. Sera from neutron-irradiated rats were reported to contain antibodies to the mouse mammary tumour virus (M. Imai et al., Gann 72, 1981, 132). By means of a highly sensitive and specific solid phase radioimmunoassay, it could be excluded that sera from rats subjected to different radiation regimens, contain such antiviral antibodies. Using immunofluorescence on fixed cells it was found, however, that the majority of irradiated rats had antibodies to a ubiquitous cytoplasmic antigen in several rodent tumour cell lines, including radiation-induced tumours. Possibly, this antigen is the product of a cellular oncogene.

Publications

K.J. van den Berg, V. Krump-Konvalinkova, P. Bentvelzen and D.W. van Bekkum: Transformation of murine fibroblasts by normal mouse cell DNA fragments: Possible implications for models of nonviral carcinogenesis. In: Biological Carcinogenesis. M.A. Rich and Ph. Furmanski (eds.), p. 193, Marcel Dekker, Basel, Switzerland, 1982.

D.W. van Bekkum and P. Bentvelzen: The concept of gene transfer-misrepair mechanism of radiation carcinogenesis may challenge the linear extrapolation model of risk estimation for low radiation doses. Health Physics 43, p. 231 (1982).

P. Bentvelzen, M.A. Dubbeld, W.H. Koornstra, J.J. Broerse and M.J. van Zwieten: Sera from irradiated rats contain antibodies to a ubiquitous tumour associated antigen. Eur. J. Cancer Clinical Oncol. 19, in press.

Progress Report
1982

Contractor:

Contract no.: BIO-D-375-81-NL

REP-Institutes of the
Organisation for Health Research
TNO
P.O.Box 5815
NL-2280 HV Rijswijk

Head(s) of research team(s):

Dr. J. J. Broerse
Radiobiological Institute
TNO
P.O. Box 5815
NL-2280 HV Rijswijk

General subject of the contract:

Influence of repeated low dose irradiation on mammary gland carcinogenesis.

List of projects:

1. Influence of repeated low dose irradiation on mammary gland carcinogenesis.

Title of project nr. 1:

Influence of repeated low dose irradiation on mammary gland carcinogenesis.

Head of project and scientific staff:
J.J. Broerse, L.A. Hennen, M.J. van Zwieten, A.L. Nooteboom and B.M. van Ham.

Studies on mammary carcinogenesis in experimental animals are important for the risk-benefit analysis of diagnostic procedures involving small doses of ionizing radiation such as employed for mammography. In this connection it should be realized that the doses received in different hospitals show considerable variations (Zuur et al. 1982) and that appreciable dose reduction in routine mammography procedures can be realized.

The on-going programs on mammary carcinogenesis generally refer to the scoring of all tumours regardless of type. In the cooperative studies performed at Rijswijk the tumours are classified according to their histological characteristics (van Zwieten et al. 1982) and dose effect relationships have been reported separately for the benign and malignant lesions. Dose effect relations have been obtained for actuarial incidence (Broerse et al. 1982) for benign tumours (figure 1) and carcinomas (figure 2). The curves for induction of carcinoma after irradiation with X-rays and 0.5 MeV neutrons are parallel on the log-log plot, which implies a nearly constant RBE at the different dose levels.

In order to obtain insight in the effects of repeated low dose irradiations, WAG/Rij and Brown Norway rats have been exposed to single dose irradiation and irradiation with 10 fractions within a 12 day period. The studies are performed with X-rays and 0.5 MeV neutrons, for which the highest RBE values are expected. The following groups each consisting of 40 animals have been introduced into the

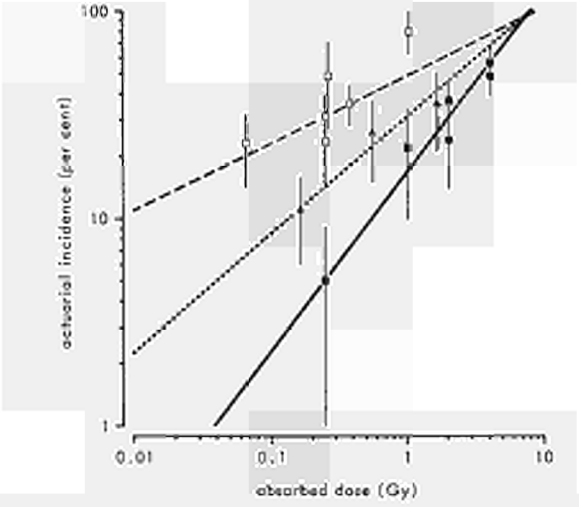

Figure 1: Actuarial incidence of benign mammary tumours in WAG/Rij rats after irradiation with X-rays (■), 15 MeV neutrons (▲) and 0.5 MeV neutrons (□).

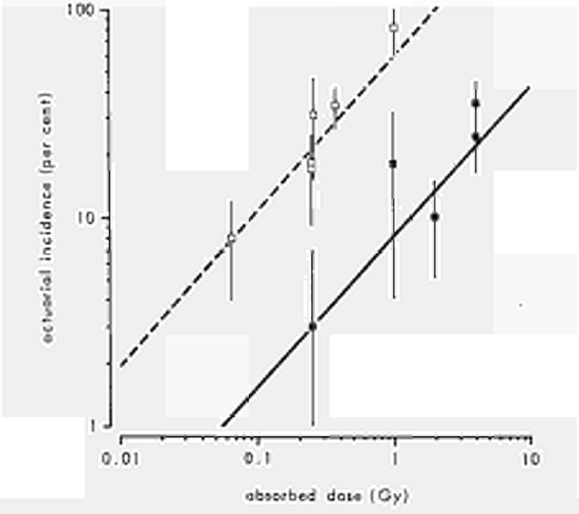

Figure 2: Actuarial incidence of mammary carcinomas in WAG/Rij rats after irradiation with X-rays (■) and 0.5 MeV neutrons (□).

program: a) controls, b) 40 rad X-rays, single dose, c) 10x4 rad X-rays in two weeks, d) 10 rad 0.5 MeV neutrons, single dose, and e) 10x1 rad 0.5 MeV neutrons in two weeks. The latency period for the expression of radiation-induced mammary tumours can be in excess of two years after exposure. In consequence the first tumours will be observed in the course of the coming contract year.

References

J.J. Broerse, L.A. Hennen, M.J. van Zwieten and C.F. Hollander. Mammary carcinogenesis in different rat strains after single and fractionated irradiations. In: Neutron Carcinogenesis, Eds. J.J. Broerse and G.B. Gerber, EUR 8084, Commission of the European Communities Luxembourg, p. 155, 1982.

C. Zuur, J. Zoetelief, A.G. Visser, and J.J. Broerse. Comparison of absorbed doses due to mammography in different Dutch hospitals. In: Proc. World Congress on Medical Physics and Biomedical Engineering 1982, Eds. W. Bleifeld, D. Harder, H.-K. Leetz and M. Schaldach, Hamburg, p. 19.26, 1982.

M.J. van Zwieten, J.J. Broerse and C.F. Hollander. Pathological aspects of mammary carcinogenesis in rats. In: Neutron Carcinogenesis, Eds. J.J. Broerse and G.B. Gerber, EUR 8084, Commission of the European Communities Luxembourg, p. 117, 1982.

Progress Report

1982

Contractor:

Commissariat à l'Energie
Atomique, CEA
Département de Protection
B.P. 510
F-75752 Paris Cédex 15

Contract no.: BIO-D-372-81-F

Head(s) of research team(s):

Dr. J. Chalabreysse
Service Hygiène Industrielle
CEA-CEN de Pierrelatte
B.P. 38
F-26700 Pierrelatte

General subject of the contract:

Study of uranium metabolism and of actual health hazards in the uranium industry (natural and enriched).

List of projects:

1. Study of the pollutant and the conditions of exposure.
2. Study of the metabolism of incorporated uranium and of the health of the workers.

Title of project nr ETUDE DU METABOLISME DE L'URANIUM ET DE L'EFFET SUR LA SANTE DES TRAVAILLEURS DANS L'INDUSTRIE DE L'URANIUM

Head of project and scientific staff : M. ARCHIMBAUD - J. CHALABREYSSE

Description des résultats

1/ ETUDE DES AEROSOLS D'UF4

En 1982 nous avons poursuivi l'étude des poussières d'UF4, recueillies dans une usine de traitement de minerais doublée d'une comparaison avec d'autres composés uranifères classiques du type U3O8, UO2 $(NO3)_2$, UO2 F2 ...

- Nous avons donc orienté notre travail sur l'essai in vitro en multipliant les essais (environ 60) et créant ainsi une gamme assez étendue de paramètres de travail : S (surface de la poussières apparante), P le poids de poussière et t le temps déterminant la critique sans oublier m le poids d'U dissout.

  En réunissant ces résultats nous avons cherché les meilleurs modèles mathématiques possibles existant essayant ainsi de créer un abaque permettant de caractériser tout essai.

- Par ailleurs afin de valider ce test in vitro en tant que test de solubilité, nous avons réalisé des essais avec d'autres éléments uranifères connus pour leur grande ou faible solubilité comme UO2 U3O8 (classe Y insolubles) ou UO2 F2, UO2 $(NO3)_2 6H2O$ (Classe D très soluble) et UO3 (classe W intermédiaire). Les résultats sont tout à fait concordant et renforcent bien l'idée d'un UF4 faiblement soluble. Cette faible solubilité s'explique aussi par l'analyse X et l'ESCAE qui tendent à prouver que la poussière subit au cours de l'essai une transformation et apparait sous forme d'un complexe U - oxyde - Hydroxyde

2/ ETUDE SUR LES TRAVAILLEURS DE COMPOSES URANIFERES

En 1982 de nombreuses analyses ont été effectuées dans les urines et les selles. Elles ont permis l'analyse de quelques dossiers particuliers :

- Chez un sujet ayant été exposé à toutes les poussières de l'usine,

  on constate :

  . que la chute de l'uranium dans les urines est significative ($p < 0,05$) entre le dosage précédant la "mise au vert" et la période de "mise au vert".

  . qu'il en est de même dans les selles avec une très forte signification ($p < 10^{-30}$).

. *Par contre, on ne retrouve pas de corrélation entre les dosages dans les urines comparés aux dosages dans les selles.*

. *Les taux de créatinine entre ces deux périodes ne sont pas significativement différents.*

- *Chez les autres sujets, une analyse rapide semble confirmer ces premières impressions.*

Lecture détaillée préliminaire des courbes d'évolution pour certains postes

A première vue, il semble apparaître un certain retard de l'apparition de contamination dans les excrétats par rapport aux prélèvements d'atmosphère.

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

-SURVEILLANCE RADIOTOXICOLOGIQUE DES TRAVAILLEURS : LE DOSAGE DE LA CREATININE DANS LES URINES-
CAMARASAJ - CHALABREYSSE J - TEULON F - RONGIER E

(Radioprotection, 1982, 17 (1), p.3-11)

II. Short Communications, Theses, Internal Reports, Patents. . .

Surveillance radiotoxicologique des travailleurs : le dosage de la créatinine dans les urines.

J. CAMARASA - J. CHALABREYSSE - F. TEULON - E. RONGIER - Médichem. 14 15 16 IX 1982

Progress Report
1982

Contractor:

Fondation Bergonié
Centre Rég. Anti-Cancéreux
Rue de Saint-Genès 180
F-33076 Bordeaux Cédex

Contract no.: BIO-D-371-81-F

Head(s) of research team(s):

Dr. J. F. Duplan
Unité de Rech. Radiobiologie
Expérimentale et Cancérologie
Rue de Saint-Genès 180
F-33076 Bordeaux Cédex

General subject of the contract:

Genetic recombination as a mechanism of induction of leukemia upon irradiation and its impairment.

List of projects:

1. Occurence of a leukomogenic recombinant in radiation induced tumours. Kinetics of expression of endogeneous C57BL virus and localization of the viral expression in different tissues.

Title of project nr 1

Occurence and role of leukemogenic recombinants in radiation-induced tumours.

Head of project: Dr J.F. Duplan

Scientific staff : B. Guillemain, E. Legrand, T. Astier, R. Daculsi.

Previous studies carried out within the framework of the present contract led to the assumption that B tropic viruses produced by recombination of N and X endogenous C57BL viruses were not leukemogenic per se but needed to undergo a second recombinational event with the endogenous viruses in order to become pathogenic. To study this hypothesis a cloned B tropic virus 1223B was injected into newborn mice ; it induced 7 % of thymic lymphomas (latency 374 days), 21 % of spleen T cell tumors (314) and 71 % of various B and null cells lymphoid tumors (455 d.). Several lymphomatous thymus, spleens and lymphnodes were cultured and were shown to yield viruses which could differ from the injected 1223B virus. One isolate T896 was studied in more details as it was inducing 42 % of spleen T cell tumours in less than 200 days when injected into newborn C57BL mice. Four viral clones were obtained from this isolate. Two (Cl_2 and Cl_3) were rather similar to 1223B ; the third (T896/Cl_1) was dual tropic but it showed no capacity to produce foci on mink cells (MCF) or to transform cells in vitro ; in addition it did not induce any leukemia when injected into newborn mice. The fourth clone T896/Cl_4 was strictly B tropic but different from 1223B by its ability to form XC plaques ; it produced more than 30 % of spleen T cell leukemias in less than 200 days when injected into newborn mice. These findings supports the view that the causative agent of the T cell leukemia is not the 1223B viral clone but a new recombinant T896/Cl_4. Further immunological and biochemical studies will allow to characterize this clone.

Besides the role played by the viral recombination the following experiments stress the role of radiation in the interaction between cells and viruses. As previously indicated 1223B is a B-tropic cloned virus which produces mostly extra-thymic lymphomas. This virus was inoculated 1 day before or 1 day after a series of 2 exposures to 175 rads of X-rays. 50 % of thymic lymphomas developed in the group which was injected with the virus previous to radiation exposure and 12 % were recorded in the other group. The controls which had been irradiated but not inoculated with the virus developed 8 % of thymic lymphomas. It should be considered a) that 1223B is not leukemogenic unless subject to a genetic recombination, b) that even after recombination it does not induced thymic lymphoma in non irradiated animal, c) that it must be injected before exposure of the mice to radiation to express a transforming effect on the thymus. Thus it might be assumed either that a recombinant is formed in irradiated mice which is thymotropic or that 1223B recombine preferentially with the genome of a thymic cell population which survives radiation exposure and actively participate in the endoregeneration of the thymus. It is interesting to note that in most experiments carried out in the past the cell-free extracts from radiation-induced thymomas were assayed for thymotropic activity in normal newborn mice and not in irradiated animals. It might well be that the present findings are not restricted to a specific virus and that, in general, viruses which induce extra-thymic lymphomas in normal mice can also produce thymic lymphosarcomas in irradiated animals.

LIST OF PUBLICATIONS IN 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

DACULSI, R., ASTIER, T., LEGRAND, E. and DUPLAN, J.F.

Terminal deoxynucleotidyl Transferase Activity in the Regenerating Thymus of X-irradiated mice.

Thymus 4, 45-55, 1982.

ASTIER, T., GUILLEMAIN, B., LAIGRET, F., MAMOUN, R. and DUPLAN, J.F.

Serological Characterization of C-type Retroviruses Endogenous to the C57BL/6 Mouse and Isolated in Tumours Induced by Radiation Leukaemia Virus (RadLV-Rs).

J. Gen. Virol. 61, 55-63, 1982.

LEGRAND, E., GUILLEMAIN, B., DACULSI, R. and LAIGRET, F.

Leukemogenic activity of B-ecotropic C-type retroviruses isolated from tumors induced by radiation leukemia virus (RadLV-Rs) in C57BL/6 mice.

Int. J. Cancer 30, 241-247, 1982.

STEVENSON A.F.G., DACULSI, R. and MONIG H.

Haematological Studies on ^{90}Sr-^{90}Y-toxicity : II. Femoral CFU-s kinetics and Mitogen Response of Spleen Cells.

Radiat. Environ. Biophys., 1982, 20, 275-287.

II. Short Communications, Theses, Internal Reports, Patents...

LEGRAND E., DACULSI R. and DUPLAN J.F.- Inhibition of radiation-induced leukemogenesis by hemopoietic suspensions of different origins. INSERM, Unité 117, Bordeaux. XVIIème Réunion annuelle de la Société Européenne de Radiobiologie, Bordeaux Juillet 1982.

LEGRAND E., GUILLEMAIN B., DACULSI R. et LAIGRET F.- Activité leucémogène des rétrovirus écotropes de type B isolés des tumeurs induites par le RadLV-Rs chez les souris C57BL/6. Forum de Cancérologie, 7 juin 1982. Paris. Bulletin du Cancer, 1982.

LEGRAND E., DACULSI R., ASTIER T. and DUPLAN J.F.- Effect of Bone Marrow and Spleen CFUs on radiation induced leukemogenesis in C57BL/6 mice. International Workshop organized by EULEP, Ulm, 3-5 Décembre 1982.

DACULSI R., ASTIER T., LEGRAND E. and DUPLAN J.F.- Terminal deoxynucleotidyl transferase activity in the regenerating thymus of X-irradiated mice. International Workshop organized by EULEP, Ulm, 3-5 Décembre 1982.

MAMOUN R.Z., ASTIER A., GUILLEMAIN B.- Viral Expressions in Bovine Cells. Fifth International Symposium on Bovine Leukosis, Tubingen, October 19-21, 1982.

GAUTHIER T., LAUNAIS M., GUILLEMAIN B., ASTIER T., MAMOUN R. and BOUSICAUX A.- Evaluation of ELISA, AGID, RIA and EPI Tests in the Diagnosis of BLV Infektion. Fifth International Symposium on Bovine Leukosis, Tubingen, October 19-21, 1982.

Progress Report
1982

Contractor:

Contract no.: BIO-D-474-81-UK

Medical Research Council
MRC
Park Crescent 20
GB-London W1N 4AL

Head(s) of research team(s):

Dr. S. B. Field
Cyclotron Unit - Biology Dep.
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Hammersmith Hospital
Ducane Road
GB-London W12 OHS

General subject of the contract:

Non-stochastic effects of radiation on normal tissues relevant to radiotherapy and radiation protection.

List of projects:

1. Cell kinetics and radiation damage.
2. Vascular injury and late radiation damage.

Title of Project No. 1.

Kinetic models for expression of radiation-induced functional impairment in normal tissues.

Head of project and scientific staff:
Dr. S. B. Field, Dr. A. S. Michalowski, Dr. T. E. Wheldon.

Using mathematical models, computer simulation studies have been carried out to identify factors influencing the development of functional impairment for two types of tissue organization viz: 'Type H' which have strict demarcation of stem cells and functional cells, and 'Type F' which are composed of cells which are capable both of proliferation and of tissue-specific function (e.g. liver and kidney parenchyma). It is tentatively proposed that 'Type H' and 'Type F' organization is responsible for 'acute' and 'late' tissue response respectively. The present studies demonstrate that responses of 'Type H' and 'Type F' tissues are differently influenced by various factors (see Table I). For example, proliferation of 'doomed' cells, a phenomenon whose existence is often ignored, contributes to the severity of functional impairment in 'Type H' tissues by retarding stem cell repopulation, but reduces the severity of impairment in 'Type F' tissues since 'doomed' cells retain the ability to function and their limited proliferation contributes to maintenance of functional capacity.

The relationship between clonogenic survival and functional impairment has been studied in these postulated different tissue types. The relationship is more complex in 'Type F' tissues and the parameters of the clonogenic cell survival curve cannot reliably be deduced from data on functional impairment. Specifically, the limited proliferative ability of 'doomed cells' confers on 'Type F' tissues a degree of protection of functional capacity, which is more effective at lower doses. This dose-dependent protection is unrelated to survival properties of clonogenic cells but could be erroneously interpreted as implying a wide shoulder or large β/α ratio for the survival curve for the 'target cells' of the tissue.

These model studies indicate the potentially important role of factors other than clonogenic survival in determining the severity of radiation-induced functional impairment. They also lead to possible new approaches to the protection of tissues from radiation injury, e.g. by post-irradiation modification of cellular proliferation.

Radiation injury to the oesophagus has been studied, as an example of an 'H type' tissue. Pathological lesions of the alimentary tract were examined microscopically. The incidence of ulcerative oesophagitis (UO,

iscontinuity of the epithelial lining of the oesophagus accompanied by cute inflammatory reaction in the underlying layers of the wall) was found o increase from 0% to 100% during the 7th and 8th day, and subsequently to ecrease to 10% by day 14. Loss of body weight preceded the onset of oesohagitis. The maximum average loss was 17% at day 9 and 10. The changes in ody weight were mild and did not reflect precise timing of UO, suggesting n influence of another concomitant pathology. Indeed, eighty one per cent f the mice were found to have a duodenal ulcer (DU) at all times, not nfrequently accompanied by ulcer(s) of the small and, rarely, large intestne. The results show that radiation-induced UO is not a fatal condition. hanges in animal body weight do not seem to be due exclusively to UO. The se of the mortality end-point for assessment of 'radiation oesophagitis' s meaningless and should be discontinued.

In a separate series of experiments, dose effect curves were generated or the incidence of UO at 9 days, i.e. at the peak. Irradiation of the ntire thorax or mediastinum gave 50% UO with 23 Gy. This was increased to 4 Gy if either the upper or lower mediastinum only was irradiated. The ccompanying lesions of the gastro-intestinal tract were again seen and nalysed in detail.

ABLE I

	Type H	Type F
ighly sensitive omeostatic egulation of opulation size	beneficial (earlier recovery following less severe impairment of function)	deleterious (earlier and more severe functional deficit)
eliberate stimulation f cell proliferation fter irradiation	assists repopulation	precipitates avalanche ('recall' reactions seen in the clinic)
imited sub-clonoenic) proliferation f radiationterilised cells	usually of minor consequence	as a rule critically protective

List of publications in 1982

Wheldon, T.E., Michalowski, A.S. and Kirk, J. (1982)
The effect of irradiation on function in self-renewing normal tissues with differing proliferative organization. Br. J. Radiol. 55, 759-766.

A. Michalowski and J. Burgin (1982)
Duodenal ulcers as an abscopal effect of thoracic irradiation in mice. In: Progress in Radio-Oncology II. Ed. K.H. Kärcher et al., Raven Press New York. pp. 105-110.

Wheldon, T.E., Michalowski, A.S. and Kirk J. (In press).
The relation of clonogenic cell survival to functional capacity of irradiated normal tissue. Br. J. Radiol. (Abstract).

Michalowski, A., Uehara, S., Yin, W-B., Burgin, J. and Silvester, J.A. (in press).
Alternative types of duodenal ulcer induced in mice by partial X-irradiation of the thorax. Radiat. Res.

Title of Project No. 2.

Vascular injury and late radiation damage.

Head of project and scientific staff:
Dr. S. B. Field, Dr. Marilyn P. Law.

Radiation induced vascular damage may lead to connective tissue changes, subsequent death of parenchymal cells and replacement fibrosis. The aim of the present project is to test this hypothesis by measuring vascular changes and fibrosis in rodent kidney.

Vascular function was measured in rats using various short lived positron emitting isotope tracers (^{82}Rb or $^{15}O_2$ for blood flow: ^{11}CO labelled erythrocytes for blood volume) visualised by a positron camera developed at the Rutherford Laboratory (Bateman et al. 1980). The data has been analysed using computer techniques. Rubidium-82 infused into the femoral vein gave the best images of the kidney. Activity in a number of longitudinal planes was corrected for activity in adjacent planes. Using the corrected scans an index of blood flow was calculated as the activity in each kidney divided by that in the heart. To test the sensitivity of the method one kidney was heated and the decrease in the blood flow index did depend on the duration of hyperthermia as expected. Although this technique would enable serial measurements to be made on individual animals, a major disadvantage is that the camera is not on this site. Also the computing time required is long and the resolution is poor. Consequently more conventional tracer techniques such as those using labelled microspheres or hippuran are currently being pursued in mice. These methods involve sacrificing the animals so that serial measurements are not possible. However, the use of short lived radiotracers will enable us to measure collagen in the same animal after the radioactivity has decayed. These studies led to the development of a technique for irradiating individual kidneys in the mouse without partial nephrectomy. Small 'D' shaped fields can be used to expose either anaesthetised or conscious animals. Mice have survived the early post treatment period despite the inclusion of small regions of the gastro-intestinal tract in the field. The first animals died at 2-3 months after higher doses (18-24 Gy) to both kidneys. Pathological examination revealed a high incidence of intestinal ulcer suggesting some form of abscopal effect which must be considered in conjunction with late effects in kidney.

Radiation induced vascular injury in the kidney may cause hypertension.

Blood pressure (BP) measurements were made on the tail artery of mice us
a cuff/detector. In this method the tail is surrounded by an inflatable
cuff which is blown up to occlude the tail arteries and the applied pres
is measured by a pressure transducer. The tail artery is illuminated by
controlled light source and a photoelectric pulse detector responds to
changes in blood density caused by the arterial pulse. By recording the
and reappearance of pulse signals simultaneously with cuff pressure, BP
be determined.

CFLP and C_3HKM mice submitted to the tail cuff procedure without
obvious agitation and measurements were made on unanaesthetized animals.
Administration of ether or Sagatal produced an unacceptable reduction in
BP compared with that of the unanaesthetized animal. However, valium
administered an hour before measurement produced no significant BP chang
and was therefore used to quieten the more excitable animals. BP has be
measured in groups of CBA mice which had received bilateral kidney radia
of 13 Gy and 24.5 Gy 10 months previously. Preliminary results show tha
these mice had a mean BP 20mm Hg higher than that of the unirradiated
controls.

Reference:

Bateman, J.E., Connolly, J.F., Stephenson, R. and Flesher, A.C. 1980.
The development of the Rutherford Laboratory MWPC Positron Camera.
Nuclear Instruments and Methods, 176, 83-88.

Progress Report
1982

Contractor:

Gesellschaft für Strahlen- und Umweltforschung mbH.
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

Contract no.: BIO-D-366-81-D

Head(s) of research team(s):

Prof. Dr. W. Gössner
GSF Inst. Biologie
Abteilung für Pathologie
Ingolstädter Landstrasse 1
D-8042 Neuherberg

General subject of the contract:

Pathogenesis of late somatic effects of radiation.

List of projects:

1. The dependence of radiation induced osteosarcoma risk on dose, time and quality of radiation.
2. The pathogenesis of radiation induced bone tumours.
3. The role of various endogenous and exogenous factors with regard to radiation induced late effects.

The research work carried out during 1982 has continued on long-term studies of the late effects of short-lived bone-seeking radionuclides. The major research objective was the evaluation of the radiation-induced osteosarcoma risk.

The present work is subdivided into three projects:

1. The dependence of radiation-induced osteosarcoma risk on dose, time and quality of radiation
2. The pathogenesis of radiation-induced bone tumours
3. The role of various endogenous and exogenous factors with regard to radiation-induced late effects

In 1982 the work of project 1 was concentrated on the osteosarcoma risk after the protracted internal β-irradiation with ^{177}Lutetium at low dose rate and after single injections of ^{239}Neptunium in comparison to ^{177}Lutetium at the same skeletal dose level with regard to the influence of its long-lived daughter nuclide ^{239}Plutonium. In addition new experiments with combined incorporation of short-lived (^{227}Thorium) and long-lived (^{227}Actinium) radionuclides have been started.
In project 2 virus isolates from radiation-induced osteosarcomas of different mice strains have been further characterized. In addition the influence of different dose rates and of the strain of mice on the induction of endogenous C-type retrovirus expression after internal irradiation has been studied. The frequency of non-neoplastic proliferations in the skeleton during the latency period of radiation-induced osteosarcomas has been further analysed.
The research of project 3 is focused on age and strain disposition and on the influence of substances with effects on skeletal tissue (Cadmium, β-Aminopropionitril, Indomethacin) on RNA/DNA metabolism and the immune system (Daunomycin, 5-Azacytidine, ALP).

' PROJECT No. 1:

ɔndence of radiation-induced osteosarcoma risk on dose,
l quality of radiation

PROJECT AND SCIENTIFIC STAFF:

ıer, U. Linzner, A. Luz, W. A. Müller, E. Schäffer

ɔacted administration of ^{177}Lutetium
ıitter, half-life 6.7 d)

ɔt 533 days after start of an experiment with female
mice, 4 weeks of age.

ɔy repeated i.p. injections of 0.208 mCi/kg (i.e.
ɔtal dose rate less than 20 rad/day).

ɔsarcoma incidence:

ɪCi/kg (mean skeletal dose 14 Gy)/12 weeks	4% (4/100)
ɪCi/kg single injection	3% (6/200)
ɪCi/kg (mean skeletal dose 28 Gy)/24 weeks	6% (3/50)
ɪCi/kg single injection	4% (2/50)

ɔesent the data demonstrate that lowering of the dose
does not decrease the osteosarcoma risk.

le injection of ^{239}Neptunium
ıitter, half-life 2.3 d)

lt 490 days after start of an experiment with female
mice, 4 weeks of age.

ɔsarcoma incidence as compared to ^{177}Lu-experiment:

skeletal dose 14 Gy	
mCi/kg ^{239}Np	7.5% (15/200)
mCi/kg ^{177}Lu	2 % (4/200)
skeletal dose 56 Gy	
mCi/kg ^{239}Np	4 % (2/50)
mCi/kg ^{177}Lu	6 % (3/50)

ɪifference of the osteosarcoma incidence for both
ɔnuclides in the group with 14 Gy is significant
ɛss than 0.01). This indicates an increase of the
ɔsarcoma risk by the continuous weak internal background
ɪiation of the daughter nuclide ^{239}Pu in the ^{239}Np-group
ɔwer mean skeletal dose.

3. Experiments with combined incorporation of short-lived and long-lived radionuclides

3.1. Combined incorporation of ^{227}Ac (β-emitter, half-life 22 years) and its short-lived daughter nuclide ^{227}Th (α-emitter, half-life 18.7 d)

Start of a new experiment with female NMRI mice with i.p. injection of 0.05 µCi/kg ^{227}Ac at age of 10 weeks, evaluation of the incorporated activity by whole-body measurement, i.p. injection of different activities of ^{227}Th at age of 12 weeks.

There were 50 animals per group:
Untreated, ^{227}Ac; 0.5, 2 and 5 µCi/kg ^{227}Th combined with and without ^{227}Ac.

The schedule will be repeated in 1983 with the same number of animals.

3.2. Combined incorporation of 0.05 µCi/kg ^{227}Ac (i.e. low level α-irradiation by daughter nuclide ^{227}Th) and ^{177}Lu

Result 639 days after start of an experiment with female NMRI mice, 4 weeks of age.

Osteosarcoma incidence:

0.05 µCi/kg	^{227}Ac	1%	(1/50)
5 mCi/kg	^{177}Lu	8%	(6/75)
5 mCi/kg	^{177}Lu plus ^{227}Ac	17%	(13/75)
10 mCi/kg	^{177}Lu	29%	(29/100)
10 mCi/kg	^{177}Lu plus ^{227}Ac	17%	(17/100)

At present no significant synergistic effect of both radionuclides has been observed in these experiments.

4. Experiments with Protactinium

First pilot experiments were performed with ^{233}Pa, a β-emitting actinide with a half-life of 27 days and a maximum β-energy of 0.6 MeV. In the nuclear technique ^{233}Pa plays a certain role as daughter product of ^{237}Np, the latter being produced in Uranium-fuel rods by the reaction ^{238}U (n,2n) $^{237}Np \xrightarrow{\alpha} {}^{233}Pa$. ^{233}Pa is also formed in a still higher yield after neutron irradiation of thorium.

Since ^{233}Pa is a β-emitter with a half-life, which is comparable to the - by us thoroughly investigated - α-emitter ^{227}Th it may serve to elucidate special question of RBE with respect to osteosarcoma induction. The first results of distribution studies after injection of ^{233}Pa as citrate form into 4 week old female NMRI mice showed 1 and 7 days after incorporation more than 60% of injected amount in the skeleton, 1.5% in the liver.

TITLE OF PROJECT No. 2:
The pathogenesis of radiation-induced bone tumours

HEAD OF PROJECT AND SCIENTIFIC STAFF:
W. Gössner, V. Erfle, A. Luz, K.-H. Marquart, W. A. Müller, A. B. Murray, E. Schäffer

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1. Radiation (^{224}Ra, ^{227}Th) induced osteosarcomas of different strains of mice were found to express infectious retroviruses with the following frequency (retrovirus positive tumours/tumours examined): BALB/c (8/8), C57BL/6 (2/3), C3Hx101/F_1 (1/1), NMRI (6/12) and CBA (0/2). Twenty-two permanent cell cultures were established from these 26 tumours and 15 of these osteosarcoma cell lines were found positive for retrovirus expression. Two virus isolates, which have been found to be oncogenic after injection into new-born mice, were further characterized:
OS-2 virus (osteosarcoma cell line, C3H x 101/F_1 strain mouse) and OS-5 virus (osteosarcoma transplant cell line, NMRI strain mouse) showed "in vitro" ecotropic host range. OS-5 virus induced lymphomas (76%), osteopetrosis (33%) and osteomas (19%) whereas OS-2 virus induced only osteopetrosis and osteomas.

   The tryptic peptide maps of the major core protein p30 of these two viruses showed the same migration pattern as the p30 of AKV, an endogenous non-oncogenic retrovirus. Distinct differences were seen in comparison with the p30 peptide maps of FBJ osteosarcoma virus or Rauscher MuLV. RNase T_1 fingerprints of OS-2 and OS-5 virus showed enzyme resistant oligonucleotides homologous to the AKV RNase T_1 fingerprint with additional oligonucleotides which were not specific for AKV. Some of these oligonucleotides were typical for highly leukemogenic viruses isolated from spontaneous AKR leukemias and were located in the envelope-gene and the promotor-gene regions of the viral genome.

Serological analysis of the core and envelope proteins with monoclonal antibodies revealed almost identity of the core proteins of OS-2 and OS-5 virus with those of AKV, but a clearly distinguishable pattern of antigenic sites of the envelope proteins between the osteosarcoma viruses and AKV. The data indicate that these two virus isolates are a mixture of an AKV-related virus and another endogenous retrovirus which represent an AKV-related recombinant virus which evolved during the development of the osteosarcomas. It will be now possible to isolate and characterize those parts of the proviral genome which are mutated by radiation and which seem to be crucial for the transformation process.

2. We investigated the influence of different dose rates and of the strain of the mice subjected to internal irradiation upon the induction of endogenous C-type retroviruses during the latency period. Some of these retrovirus isolates were further characterized by biochemical and biological means.

The application of 0.06 µCi ^{224}Ra/kg 3.5 days to young female mice resulted in an osteosarcoma incidence of 14% whereas the injection of 0.5 µCi ^{224}Ra/kg/3.5 days results in an osteosarcoma incidence of up to 98%. If the latter protocol is applied to mice of the BALB/c, C57BL/6 and NMRI strains the latency period of the induced osteosarcomas is significantly different between the BALB/c strain mice showing the shortest (160 days) and the NMRI strain mice showing the longest (340 days) latency period (C57BL/6: 300 days). The individual osteosarcoma incidences reached the same level.

In order to further elucidate the relationship between the level of activated retroviruses in the early latency period and the estimated osteosarcoma risk we irradiated 4 weeks old female BALB/c, C57BL/6 and NMRI mice with 0.06 and 0.5 µCi ^{224}Ra/kg every 3.5 days 4 and 8 times resp. Six animals represented one experimental group.

The activation of infectious endogenous ecotropic and xenotropic retroviruses was investigated 3.5 days after the last injection in spleen, bone marrow and bone tissue. In BALB/c mice ecotropic retrovirus was activated mainly in bone marrow and bone tissue. Xenotropic retrovirus was spontaneously activated in all three organs of un-irradiated mice. In the organs of irradiated animals a dose dependent increase of activated infectious virus particles was detected. In C57BL/6 mice activated eco-tropic retrovirus was detected only in irradiated animals. A four- to twenty-fold higher virus activation was found in the high dose group as compared to the low dose group. The activation of xenotropic retrovirus also showed dose dependency with three- to fifty-fold differences in the two irradiated groups.
In NMRI strain mice only one animal showed ecotropic virus activation in bone tissue and spleen. Three mice showed xenotropic virus activation in spleen and bone marrow.
A number of these early virus isolates were injected into new-born NMRI mice. One of the first viruses injected, the ecotropic retrovirus isolate of NMRI mice, induced lymphomas (latency period 6 months) after injection into new-born female NMRI mice. These data indicate, that the radiation-induced changes in the proviral genome can take place very early in the latency period. This would offer the possibility to realize crucial mutations for carcino-genesis in irradiated individuals at an early stage on a molecular basis.

3. Frequency of non-neoplastic proliferations in the skeleton during the latency period of radiation-induced osteosarcoma.
Between day 200 and 600 after incorporation of 5 µCi/kg ^{227}Th in female CBA mice, 4 weeks of age, groups of animals were killed at random. Histological sections of humeri, femora, tibiae and the vertebral column were examined. The frequency of animals with non-neoplastic proliferations

(similar to the hot phase of Morbus Paget) and osteosarcoma as compared to the rate of (clinically detected) osteosarcoma in a parallel running long-term experiment (see project 3) is shown in Figure 1.

Spontaneous non-neoplastic proliferations in CBA mice occur with low frequency as compared to other strains of mice studied in this laboratory. The frequency of cases with this lesion is 20-30% beyond 400 days after start of experiment. Like in a former experiment with C3H x 101/F_1 mice the frequency of cases with non-neoplastic proliferations increases after internal irradiation and reaches values of nearly 90% irrespective of the fact of the relatively low sensitivity of CBA mice for osteosarcoma induction (see project 3). Osteosarcoma cases were detected with a frequency of about 10% in the internally irradiated animals. There is no significant difference in the frequency of detection of osteosarcoma in groups with detailed histological investigation of the skeleton and in groups with selection of clinical obvious osteosarcoma cases. This indicates a rapid growth of osteosarcoma. The increase of non-neoplastic proliferations runs parallel to the first observation of osteosarcomas and exceeds the frequency of osteosarcoma considerably. Therefore it does not appear to be obligatory that these non-neoplastic proliferations proceed to neoplasia.

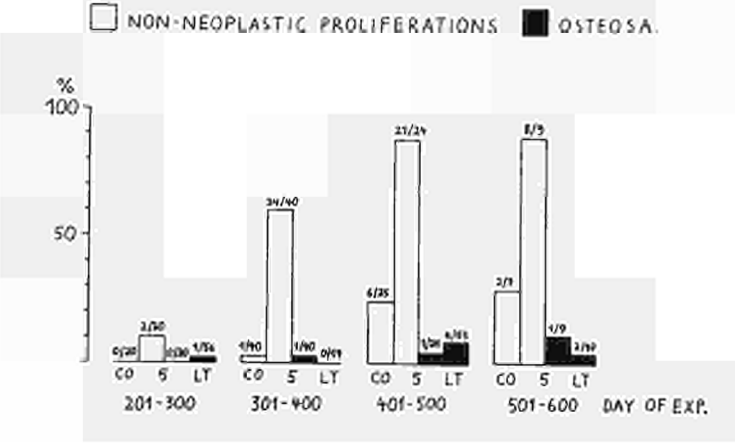

FIGURE 1

Frequency of cases with non-neoplastic proliferations in the skeleton and frequency of cases with osteosarcoma in random samples of female CBA mice after incorporation of ^{227}Th at age of 4 weeks.

CO = untreated control animals
(no osteosarcomas were observed)

5 = 5 µCi/kg ^{227}Th
(cases with non-neoplastic proliferations and cases with osteosarcomas)

LT = osteosarcoma rate in a parallel running long-term experiment (see Figure 3)

TITLE OF PROJECT No. 3:

The role of various endogenous and exogenous factors with regard to radiation-induced late effects

HEAD OF PROJECT AND SCIENTIFIC STAFF:

W. Gössner, A. Luz, W. A. Müller, E. Schäffer

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1. Study of the influence of endogenous factors on the osteosarcoma induction by internal irradiation

1.1. Age disposition

Final data were analysed of an experiment comparing the late effect of ^{227}Th injected in 1 month and 12 months old female NMRI mice. Tumour incidence was corrected for competing risks after Miescher et al. The result is qualitatively different in the two dose ranges studied (Figure 2).

After incorporation of 1 µCi/kg the cases with osteosarcoma were observed earlier (within 600 days) and with higher incidence (more than 50%) in the 12-months group as compared to the 1-month group (30% osteosarcoma incidence beyond day 700 of experiment).

After incorporation of 5 µCi/kg the 12-months group reached only 5% osteosarcoma incidence within its life span and did not exceed the incidence of the 1-month group at the same time. At later times the 1-month group reached an osteosarcoma incidence of more than 60%. This observation is only partly explained by a radiation-induced earlier occurrence of non-bone-tumours (mainly malignant lymphoma, lung tumours and neoplasms of the ovary) in the older age group. There were still 16 survivors in the older age group at day 400 of the experiment.

1.2. Strain disposition

1.2.1. Comparison of the osteosarcoma induction in female BALB/c and CBA mice, final evaluation

800 days after incorporation of 5 µCi/kg ^{227}Th the following osteosarcoma incidence has been observed:

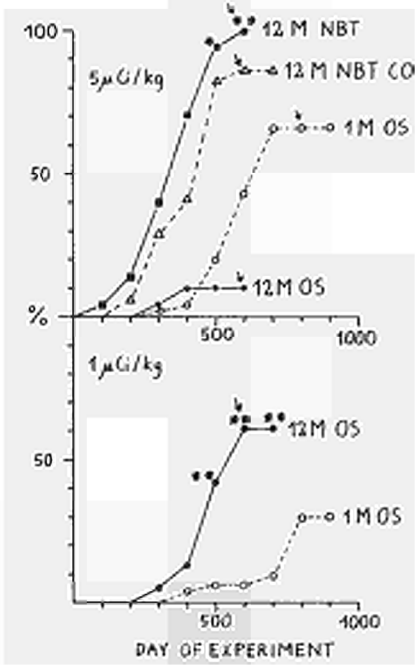

FIGURE 2
Cumulative tumour incidence (corrected for competing risks after Miescher et al.).
Long-term experiment after incorporation of 1 or 5 µCi/kg ^{227}Th in female NMRI mice at age of 1 month (1 M) or 12 months (12 M).
CO = untreated control animals
OS = osteosarcoma
NBT = non-bone-tumours
Arrow = less than 10 survivors
* P < 0.05 ** P < 0.01 logranktest

BALB/c: 53% (26/49)
CBA: 24% (14/58)
P less than 0.01 for difference.

The osteosarcoma incidence corrected for competing risks according to Miescher et al. is shown in Figure 3. For the whole observation period the logranktest reveals a significance of P less than 0.001 for the difference between both strains.

Tumour virological data of both strains were already described in the report of 1981.

Spontaneous non-neoplastic proliferations in the skeleton at 24 months of age were more frequently observed in BALB/c (9/12) than in CBA mice (2/10).

1.3. Genetic monitoring

In the meantime 3 groups of female C3H mice out of a nucleus biochemically monitored in the Department of Mammalian Genetics were under long-term observation. In the first group (born in VIII/81) one mammary gland tumour was observed.

2. Influence of chemicals administered during the latency period of radiation-induced osteosarcoma in mice

2.1. Administration of substances with effects on skeletal tissue

2.1.1. Administration of a low-level Cadmium-containing diet

Female CBA mice received 5 µCi/kg ^{227}Th at age of 4 weeks. Half of the animals received a diet with 25 ppm Cadmiumchloride during day 120 to 291 of the experiment.

Osteosarcoma incidence on day 683 of the experiment:
Untreated 0/50; Cadmium 0/50;
^{227}Th-group 6% (3/50); ^{227}Th + Cadmium group 6% (3/50).

2.1.2. Administration of β-Aminopropionitrile (BAPN)

Start of a new experiment. Female BALB/c mice, 6 weeks of age, received 5 µCi/kg ^{227}Th. Half of the animals will receive a diet with 250 mg/100 g BAPN during months 4, 6, 8, 10, 12, 14, and 16 of the experiment.

2.1.3. Inhibition of bone resorption by administration of Indomethacin

Start of a new experiment. Female BALB/c mice received 5 µCi/kg ^{227}Th at 16 weeks of age. Starting with the 13th week of the experiment half of the animals will receive 20 µg/ml Indomethacin in the drinking water.

2.2. Administration of substances with effect on RNA/DNA metabolism and/or the immune system

2.2.1. Administration of Daunomycin during the early latency period of ^{227}Th-induced osteosarcoma

Female BALB/c mice, 4 weeks of age, received 5 µCi/kg ^{227}Th and half of the animals were treated with 6 weekly i.p. injections of 1 mg/kg Daunomycin, starting 1 day after incorporation of the ^{227}Th. The final evaluation of the experiment with correction of the osteosarcoma incidence for competing risks according to Miescher et al. showed even in the log-ranktest no significant difference between the ^{227}Th-group and the ^{227}Th plus Daunomycin group. Both groups reached a cumulative osteosarcoma incidence of 81% 800 days after start of the experiment.

2.2.2. Administration of 5-Azacytidine

Female BALB/c mice received 5 µCi/kg ^{227}Th at age of 12 weeks. In the week before the ^{227}Th incorporation one half of the animals received 3 i.p. injections of 1 mg/kg 5-Azacytidine (inhibition of the DNA-methylation!).

2.2.3. Modulation of the immune system

2.2.3.1. Stimulation by LPS

Final evaluation of data after whole-body measurement showed that the effect of LPS reported in 1981 was not correct and can be explained by differences in the dose burden between the ^{227}Th and ^{227}Th plus LPS group of mice.

2.2.3.2. Administration of an alkyl-lysophospholipid (ALP) during the latency period of radiation-induced osteosarcoma

Female BALB/c mice received 5 µCi/kg ^{227}Th at 4 weeks of age. Half of the animals received ALP (compound "ET-18-O-CH3") on 6 days per week by stomach tube, starting in the 4th month of the experiment. The osteosarcoma incidence on day 389 of experiment is 12% (6/49) for the ^{227}Th group and 6% (3/49) for the ^{227}Th plus ALP group. A similar treatment could not inhibit the development of spontaneous lymphoma in AKR mice and radiation-induced lymphoma in C57BL/6 mice.

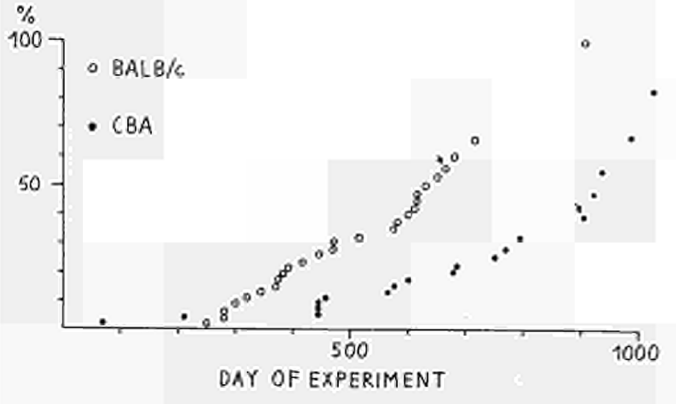

FIGURE 3
Cumulative osteosarcoma incidence (corrected for competing risks after Miescher et al.) after incorporation of 5 µCi/kg ^{227}Th in female BALB/c and CBA mice, 4 weeks of age.
Arrow = less than 10 survivors.

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Health Physics, in press

Müller, W.A., Luz, A., Schäffer, E.H., Gössner, W.:
The role of time-factor and RBE for the induction of osteosarcomas by incorporated short-lived bone-seekers.
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EULEP-Newsletter 32, 10-12 (1983)

Progress Report
1982

Contractor:

Gesellschaft für Strahlen- und Umweltforschung mbH.
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

Contract no.: BIO-D-461-81-D

Head(s) of research team(s):

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Institut für Biologie
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Ingolstädter Landstrasse 1
D-8042 Neuherberg

Prof. Dr. A.M. Kellerer*
Prof. Dr. H. Spiess*

General subject of the contract:

Epidemiological studies of the radiation carcinogenesis and its biophysical basis.

List of projects:

1. Late effects in Ra -224 treated patients.
2. Epidemiological studies on Ra - 224 treated patients.
3. Epidemiology of experimental radiation carcinogenesis.

* This research programme is carried out in coordination with the "Institut für Medizinische Strahlenkunde der Universität Würzburg", Prof. Dr. A.M. Kellerer and the "Kinderpoliklinik der Universität München", Prof. Dr. H. Spiess.

This collaborative research program includes two epidemiological studies on the ^{224}Radium patients, and a project that is directed towards the further development of the statistical tools and towards the linkage between epidemiological studies and animal experiments on radiation carcinogenesis.

Project 1 is still in the relatively early phase where nearly half of the ankylosing spondylitis patients treated with ^{224}Ra have been at risk less than 12 years. The long latent times for bone tumours known from project 2, and the possibility that these latent times may be further increased for the low doses, make a continued follow-up in project 1 necessary for any definite statement on increased bone tumour rates. The observation of three malignant skeletal tumours but of no osteosarcomas among the new ^{224}Ra patients is of particular interest as it indicates that not only the frequency but also the spectrum of radiation-induced skeletal malignancies may depend on the dose of ^{224}Ra.

While project 1 is approaching the period where any elevated rate of bone tumours may become apparent, project 2 has gone through the maximal expression of radiation-induced bone tumours, so that the emphasis is now on the study of soft tissue tumours and of other late effects of ^{224}Ra.

Project 3 is largely directed towards the development of statistical tools that are applicable both, in epidemiological studies and in large scale animal experiments on radiation carcinogenesis. Furthermore the analysis of the effects of radon in rats has been supplemented by a comparison to the effect of fission neutrons and by the derivation of efficiency ratios.

TITLE OF PROJECT No. 1:
Late effects in ^{224}Ra-treated patients

HEAD OF PROJECT AND SCIENTIFIC STAFF:
Prof. Dr. W. Gössner, Dr. R. R. Wick

1982 the low dose group of ankylosing spondylitis patients, treated intravenously with repeated injections of ^{224}Ra, consists of 1426 patients from 9 hospitals in the FRG (Table 1). Another group of 74 patients from 4 hospitals has not yet been contacted for different administrative reasons. In addition,the control group of patients not treated with radioactive drugs or X-rays has been enlarged by another 968 patients in 1982. For this reason, observation in the control group is partly incomplete.

Among the 1426 patients with a mean skeletal α-dose of 65 rad and a mean time at risk of 15.8 years, three cases of malignant tumours in the skeleton have been recorded for a skeletal dose of less than 90 rad. Two of these three cases were tumours of the bone marrow and the other was a fibrosarcoma, while Spiess observed almost exclusively osteosarcomas and fibrosarcomas in his group of patients treated with higher doses of ^{224}Ra (project 2).

The observation of bone tumours in this study is still incomplete because the appearance times of bone tumours range from 3.5 - 25 years with a maximum at about 10 - 12 years and many patients have not yet passed this time range (Figure 1).

The incidence of liver and kidney diseases as well as other soft tissue lesions will be studied in the future according to the findings of project 2.

TABLE 1

Summary of ankylosing spondylitis patients (December 1982)

	Exposure group	Control group
Total number of patients	1426	1556[+]
Patients contacted recently	732	368
Records still in work (no current address etc.)	176	925
Patients not addressed recently	155	89
Deceased patients	363	174
Remarkable causes of death		
Malignant skeletal tumours	3	0
Leukaemia	4 (+1L)	5
Anaemia, panmyelophthisis	7 (+5L)	2 (+1L)
Kidney diseases	47	22
Liver diseases	20	17

+) includes 968 newly recorded patients of the control group Bad Bramstedt

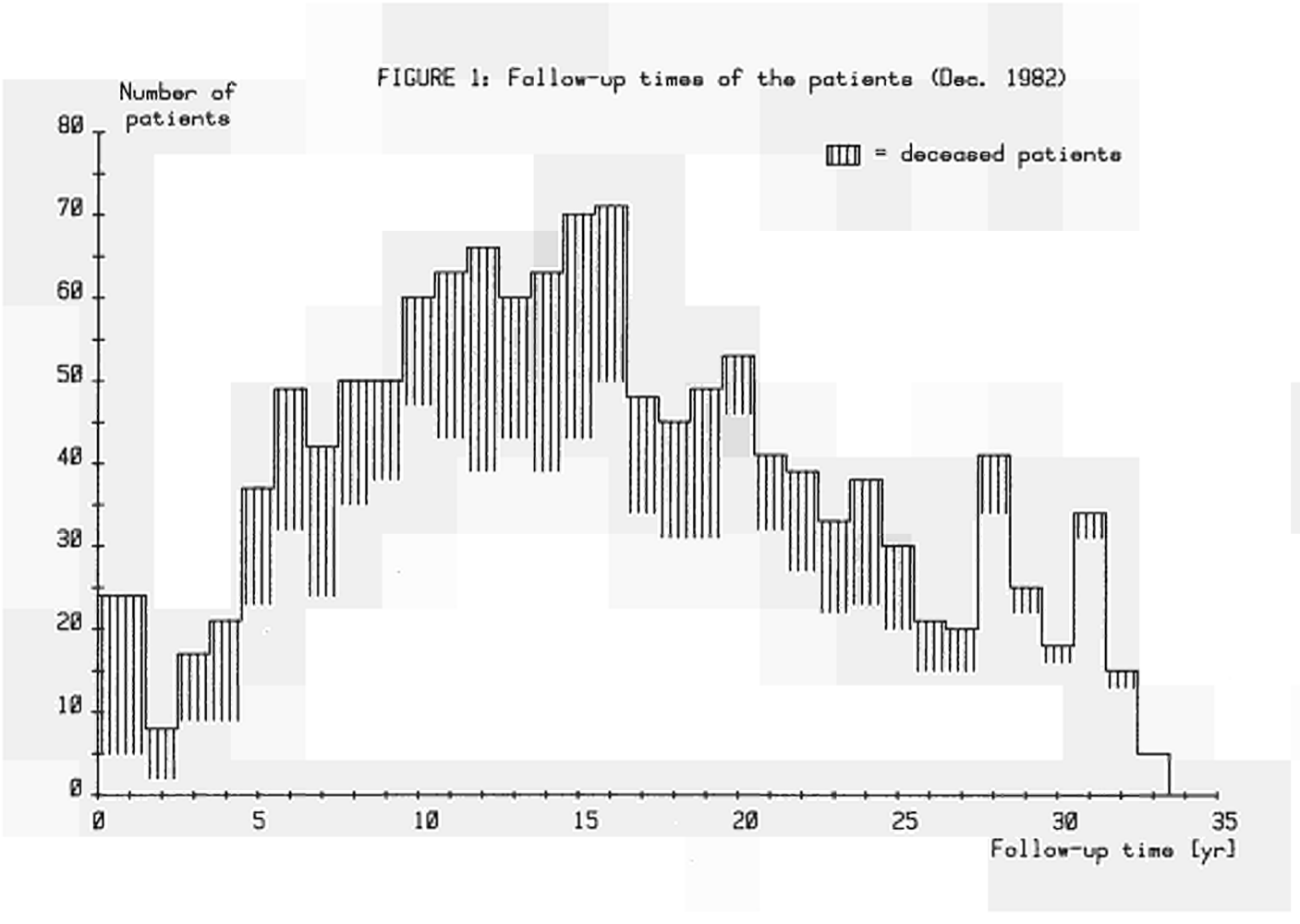
FIGURE 1: Follow-up times of the patients (Dec. 1982)
Number of patients
= deceased patients
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
Follow-up time [yr]

TITLE OF PROJECT No. 2:
Epidemiological studies on ^{224}Ra-treated patients

HEAD OF PROJECT AND SCIENTIFIC STAFF:
Prof. Dr. H. Spiess

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1. Skeletal diseases in ^{224}Ra patients

Among 218 juveniles and 680 adults given multiple injections of ^{224}Ra, the calculated skeletal doses averaged 1111 and 210 rads, respectively. The endosteal doses 0-10 µm from bone surfaces are about 7.5 times higher than the skeletal doses. As of 1982, bone sarcomas have occurred in 36 of the patients injected as juveniles and 18 of the adults. The appearance times ranged from 3.5 to 25 years after the first ^{224}Ra injection. The last bone sarcoma appeared in 1974. No carcinomas of the head sinuses have been reported among any ^{224}Ra patients, although many such carcinomas have appeared among persons with skeletally-deposited ^{226}Ra.

2. Soft tissue diseases in ^{224}Ra patients

We are following the health of 218 juveniles and 680 adults who received repeated intravenous injections of ^{224}Ra about 30 years ago, mainly for the treatment of tuberculosis or ankylosing spondylitis. The cumulative dosage averaged 29 µCi ^{224}Ra/kg for the juveniles and 15 µCi ^{224}Ra/kg for the adults. As of April 1982, deaths were recorded for 81 of the patients injected as juveniles and 357 of the adults. Soft tissue diseases among the living and dead patients included: Cancers of soft tissue (10 juv. and 62 adults), leukaemias (0 juv. and 3 adults), kidney diseases (8 juv. and 54 adults), liver diseases (2 juv. and 24 adults), and cataracts (11 juv. and 27 adults). Some, but certainly not all, of these disease cases might have been caused by the soft tissue irradiation from ^{224}Ra and its decay products.

TABLE 2
Summary of the ^{224}Ra patients (December 1982)

	Age at first injection		
	1-20 y	Adult	Total
Traced patients	218	680	898
Deaths	81	357	438
Average injected ^{224}Ra [μCi/kg]	29	15	18
Average skeletal dose [rad]	1111	210	429
Skeletal diseases			
Bone sarcoma	36	18	54
Exostosis (benign)	28	0	28
Growth retardation	50	0	50
Tooth breakage	24	16	40
Soft tissue diseases			
Cancer of soft tissue	10	62	72
Leukaemia	0	3	3
Kidney disease	8	54	62
Liver disease	2	24	26
Cataract	11	27	38

TABLE 3
New cases since 1981

Deaths	4	2	6
Tooth breakage		1	1
Cancer of soft tissue	1		1
Kidney disease	2	1	3

TITLE OF PROJECT No. 3:
Epidemiology of experimental radiation carcinogenesis

HEAD OF PROJECT AND SCIENTIFIC STAFF:
Prof. Dr. A. M. Kellerer, Dr. D. Chmelevsky, Dr. U. Mäder

Work in this project was aimed at a synthesis of experimental and epidemiological investigations of carcinogenesis by different radiations. Earlier, high neutron RBE values at low doses have been established for mammary tumours in two strains of rats. To obtain reliable data at very low doses, new methods of analysis were developed in the past year. Closely related has been the attempt to achieve a coherent approach that permits the comparison of results from different laboratories. At the seminar organized by the CEC in Rijswijk we have presented a synopsis of concepts and methods.

A further principle aim of the project has been the derivation of numerical data on radiation carcinogenesis. The finding on high neutron RBEs for mammary tumours has been complemented by the analysis of radon and fission neutron induced neoplasms in the Sprague-Dawley rat (joint project with Dr. Lafuma et al. at CEN-FAR) and a comparative analysis of the broad osteosarcoma study of Gössner, Luz et al. at GSF/Neuherberg.

1. Comparative analysis of pulmonary neoplasms in the male Sprague-Dawley rat induced by radon and by fission neutrons

After the completion of the radon inhalation studies, most of the work during the past year has gone into the evaluation of the current experiment by Dr. Lafuma et al., CEN-FAR, on radiation carcinogenesis by fission neutrons in male Sprague-Dawley rats. We have utilized the experiment to obtain the equivalence ratios between radon and fission neutron induced pulmonary neoplasms. In a dose range between 10 mGy and 2 Gy of fission neutrons the equivalence ratio is approximately

3 WLM/mGy. Related experiments with γ-radiations have been initiated.
The investigation of the other neutron induced neoplasms is only partly completed. One of the major aspects studied in the past year has been the methodological problem of the separation of lethal and non-lethal neoplasms that require entirely different statistical methods. Preliminary results indicate that sarcomas and carcinomas follow markedly different time courses; there are also apparent differences in the dose-effect relations.

2. *Statistical investigations on the incidence of osteosarcomas and its dose and dose-rate dependence*

In a preliminary part of this project the mortality corrected tumour rates and tumour incidences for the osteosarcoma experiments by Gössner and Luz at GSF Neuherberg had been computed. A further step in 1982 was the comparison of theoretical models that are utilized for the description of the time course and the dose dependence of the tumour incidence. Specifically we compared the *log-normal model* that has largely been utilized by Luz et al., with the *Weibull model* that may be the most common mathematical formulation in radiation carcinogenesis studies. From the comparison of individual fits to the data of the different experimental groups it was concluded that the two models are equally suitable. Apart from a conceptional difficulty with the log-normal model with tumours that appear early, there is no statistical reason for preferring one of the two models.

In a third step of this investigation an attempt was made to apply the two models in a simultaneous fit to all experimental groups. In such simultaneous fits the number of free parameters is substantially reduced and a coherent dose-effect relation can be obtained. Both for the log-normal model and the Weibull model there is only one free parameter for each group in addition to one common parameter. It is remarkable that the simultaneous fits to the two basic models achieve roughly the same maximum likelihood. The result makes it

possible to use in addition to the log-normal model the alternative description, and to compare the osteosarcoma studies with other radiation carcinogenesis investigations that are more frequently treated in terms of the familiar Weibull model.

PUBLICATIONS:

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Health Physics, in press

Progress Report
1982

Contractor:

REP-Institutes of the
Organisation for Health Research
TNO
P.O. Box 5815
NL-2280 HV Rijswijk

Contract no.: BIO-D-301-81-NL

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Prof. G.W. Barendsen
REP-Institutes of the
Organisation for Health Research
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General subject of the contract:

Dosimetric and experimental studies on lung-tumour induction by inhalation of radon.

List of projects:

1. Dosimetric and experimental studies on lung-tumour induction by inhalation of radon.

Title of project:

Dosimetric and experimental studies on lung tumour induction by inhalation of radon.

Head of project and scientific staff:
Dr.Ir. B. Hogeweg and Prof.Dr. G.W. Barendsen

The application in building materials of industrial restproducts (for example phosphogypsum and fly-ash) with increased concentrations of natural radioactive nuclides as well as the decreasing trend of the ventilation rate as energy conserving measure, can result in increased levels of the radon aerosol concentration of indoor air. On the basis of a risk factor deduced from epidemiological data of miners, it is generally concluded that the elevated radon levels in houses can result in an increased potential risk of lungtumour induction for their residents. However, the validity of the application of this riskfactor for the public is questionable since exposure levels and exposure conditions for residents are greatly different from those of miners. It is likely that the value, calculated on the basis of this riskfactor, will present an overestimation for the increase of the potential risk of the general public.

It is the aim of this study on lungtumour induction by radon inhalation under indoor conditions to gain, on the basis of experimental and dosimetric studies, a better understanding of the various factors which determine the risk of radon inhalation at low indoor levels. For the experimental part WAG/Rij rats will be exposed daily in an exposure chamber to relatively low concentrations of radon-daughter products under indoor conditions.

According to the report UNSCEAR 1977, in many houses the activity ratio of the alpha particle emitting nuclides Rn, RaA, RaB and RaC ranges from 10/6/3/2 to 10/9/6/4. It can be demonstrated that this condition for the radon daughter equilibrium in the exposure chamber, corresponds to a ventilation rate of the chamber of 1 h^{-1}.

In chronic exposure chambers ammonia will be produced by the action of urease-positive bacteria on urine and faeces. There are publications

showing that in such exposure chambers the ammonia concentration will rise beyond acceptable levels. Since according to Broderson et al (1976) exposure of rats to enhanced levels will increase the severity of rhinitis, otitis media, tracheitis and pneumonia characteristic of murine respiratory mycoplasmosis, it will be evident that for the study of lungtumour induction, control of the concentration level of ammonia in the chamber is important.

In order to study the ammonia concentration in the exposure chamber as a function of time, this concentration was measured at regular intervals with Dräger tubes (type Ammonia 5/a). Furthermore the influence of bacteriostatic cageboard and the addition of Aqualloy granules and foam in the chamber on the ammonia concentration as a function of the time were measured. Aqualloy is the trade mark of a synthetic hydrogel and is a mixture of styrene-maleicanhydride and polyvinylacetate. Aqualloy is reported by Van Bekkum et al. (in press) to be an ammonia reducing additive.

In the exposure chamber, having a volume of 2.5 m^3, a number of 192 WAG/Rij male rats (the maximum number for which the chamber was designed) were exposed simultaneously to the inside-air. The ventilation rate of the chamber was adjusted to 1 h^{-1}. The results of the ammonia concentration measurements for the different conditions are presented in the figure. This figure shows that for the unprepared tissue paper the bacteriostatic cageboard alone and in combination with Aqualloy foam, the ammonia concentration is gradually increasing with time and that there is a steeprise in the concentration after about 30 to 40 hours. For Aqualloy granules, the figure shows, that the concentration remains constant at a level of about 1 ppm for a period of about 50 hours and after that period rises steeply.

The results demonstrate that with the additive of the Aqualloy granules, the ammonia concentration can be reduced over a relatively long period of time to a low level and that for animals exposed to radon no cooperative action of ammonia has to be expected.

References:

Broderson, J.R. et al. Am.J.Pathol. 85, 115 (1976).
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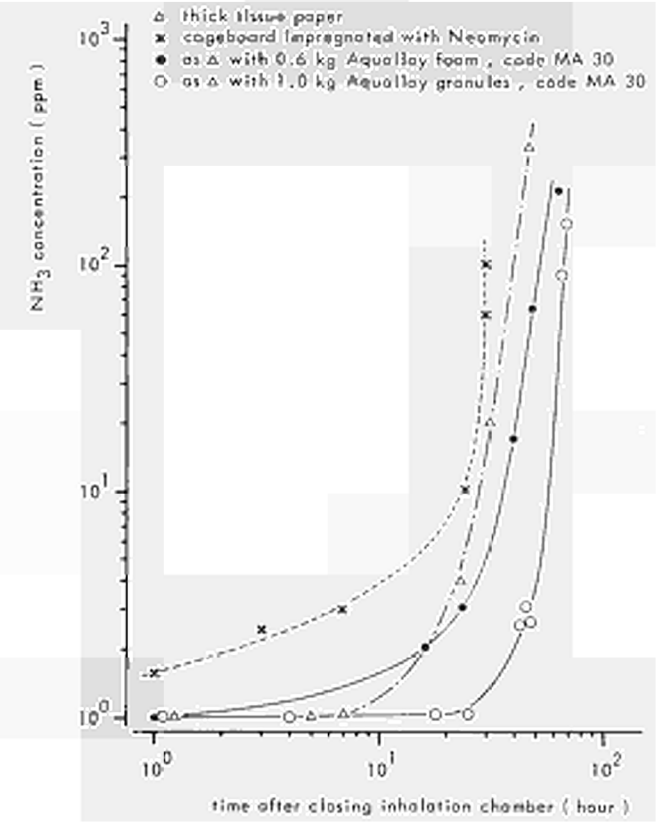

Ammonia concentration in the radon exposure chamber for different types of cageboard additives.

Progress Report
1982

Contractor:

Contract no.: BIO-D-382-81-UK

Churchill Hospital
Research Institute
University of Oxford
Headington
GB-Oxford OX3 7LJ

Head(s) of research team(s):

Dr. J. W. Hopewell
Churchill Hospital
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General subject of the contract:

Pathogenesis of early and late radiation reactions in normal tissues.

List of projects:

1. Measurement of early and late effects of irradiation in pig skin.
2. Biological effects of non-uniform irradiation on pig skin.
3. Wound healing in irradiated skin.
4. Pathogenesis of late vascular damage in the hamster cheek pouch.

Title of project nr 1: Measurement of early and late effects of irradiation in pig skin

Head of project and scientific staff: J.W. Hopewell, C.M.A. Young, R. Hamlet, M. Robbins

When establishing radiation protection criteria for the skin it is essential to examine whether factors such as the age of the subject are important. In this type of investigation the validity of any laboratory animal model must be questioned because of the difficulty of extrapolating the age scale of another species to that of man.

Although it is often argued that pig skin is the best laboratory animal model for human skin it was decided in this instance to compare the effects of age on the radiation response of both the pig and the rat.

In pig skin the effect of single doses of radiation on animals aged 12-14, 35 and 52 weeks was examined. At 52 weeks of age pig skin can be considered to be mature on the basis of a number of physiological and morphological measurements (Young and Hopewell, Microvas. Res. 20, 182, 1980). After single doses of x-rays pig skin exhibits a characteristic two wave reaction. The first wave after 3-9 weeks was characterised by a minimal to moderate erythema while the second wave (10-16 weeks) resulted in the appearance of a dusky/mauve erythema followed by the development of dermal necrosis after higher doses. The proportion of skin fields showing these two types of reaction after irradiation with doses of 1800-2340cGy in animals of different ages are listed in Table 1.

Table 1: Effects of age on the reaction of pig skin to single doses of x-rays (16cm² field)

Dose (cGy)	Age (Weeks)	Dusky/mauve reaction	Dermal necrosis
1800	12-14	4/10(40.0)	0/10(0)
	35	6/8(75.0)	0/8(0)
	52	8/10(80.0)	1/10(10.0)
2070	12-14	16/18(88.9)	10/18(55.6)
	35	8/8(100.0)	4/8(50.0)
	52	15/17(88.2)	8/17(47.1)
2340	12-14	14/14(100.0)	14/14(100.0)
	35	8/8(100.0)	8/8(100.0)
	52	10/10(100.0)	9/10(90.0)

The percentages of fields showing a specific reaction are given in brackets

The 50% effect dose for dermal necrosis did not appear to be significantly influenced by age. However, for the more subjective endpoint, of dusky/mauve erythema, a somewhat larger number of fields on animals aged 35 and 52 weeks appeared to show the reaction as compared with that of 12-14 week old pigs.

In rats animals aged 7, 14 and 52 weeks were used. A 14 week old rat is often considered to be a young adult. After irradiation with single doses of x-rays only a single wave of reaction was observed. This is equivalent to the first wave of reaction in the pig. The reaction of rat skin to irradiation can be assessed by the severity of moist epithelial desquamationin foot skin after irradiation with doses 2500cGy. The area of foot showing moist breakdown and the duration of desquamation increase with increasing dose.

Dose effect curves for the response of the skin in rats of different ages were obtained by calculating the area under the curve of each severity/time response curve (Figure). The skin of 14 week old rats was significantly more sensitive to radiation than that of 7 and 52 week old animals.

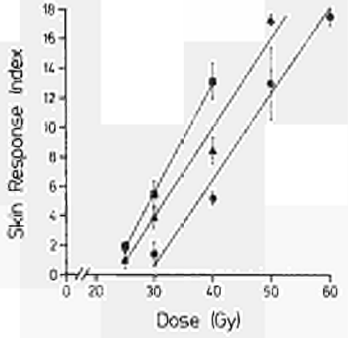

Figure: Dose-response curves for animals irradiated at 7(▲) 14(■) or 52(●) weeks of age. The response index is derived by calculation of area under the curve of tissue response with time

The reasons for a distinct age change in the radiation reaction of rat skin and no marked age effect in pig skin is not understood. This may be related to the different tissues being irradiated or could reflect important species differences. Although human data is extremely scanty no marked difference was observed in the acute response of young and old patients to radiotherapy (Rubin and Casarett, Clinical Radiation Pathology, 1968).

Title of project nr 2: Biological effects of non-uniform irradiation on pig skin

Head of project and scientific staff: D.M. Peel and J.W. Hopewell

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The project has continued with investigations involving irradiation from thulium-170, a beta emitter of maximum energy 0.97MeV and range in tissue of 3.2mm. The results have been compared with those from irradiations with the higher energy emitter, strontium-90, which have been previously described.

Circular skin fields from 2 to 19mm diameter have been irradiated with thulium-170, and the skin changes recorded during the first sixteen weeks after irradiation. The skin reactions which were seen differed from those observed after strontium irradiation. Strontium-90 produced a two-wave radiation reaction similar to that produced by x-irradiation, a first wave epithelial response and a second wave dermal response. Thulium-170, with its lower energy beta emission, produced only the first epithelial phase of the reaction, the dose to the deep dermis being too low to produce a dermal response.

The epithelial response observed was similar to that seen after strontium, erythema accompanied by moist desquamation at the higher doses. A clear difference in response was seen from the different sized strontium sources (Figure 1). This was not seen after thulium irradiation, however, where the same degree of reaction was observed at a given dose from the 19, 9 and 5mm thulium sources. It was only when the source size was reduced to 2mm diameter that the reaction was diminished. Following thulium irradiation, 50% of the fields showed moist desquamation after 80 Gy from the 19, 9 and 5mm sources compared with 240 Gy from the 2mm source (Figure 2).

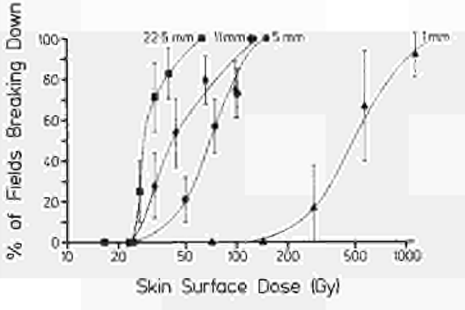

Figure 1: Percentage of skin fields showing epithelial breakdown as a function of skin surface dose from strontium-90 Irradiation (22.5, 11.0, 5.0 and 1.0mm diameter field sizes)

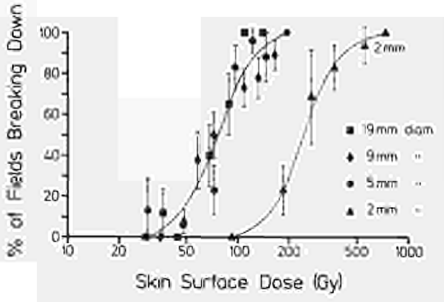

Figure 2: Percentage of skin fields showing epithelial breakdown as a function of skin surface dose from thulium-170 irradiation (19, 9, 5 and 2mm diameter field sizes)

It is proposed that the lack of field size effect seen after the lower energy thulium irradiation can be explained by the fact that the irradiated epidermis is repopulated by epithelial cells surviving at the bases of the hair follicles throughout the irradiated field (Figure 3). This is in contrast to repopulation after strontium irradiation, which is mostly from the edges of the field and hence a field size effect is seen. It is only when the field size is reduced to 2mm (the approximate distance between hair follicles), and repopulation from the edges of the field adds significantly to that from the hair follicles, that the reaction becomes less severe after thulium irradiation.

This hypothesis is supported by the further observation that strontium skin reactions were more severe than thulium skin reactions, particularly in the larger field sizes where epithelial cell survival in the hair follicles is most important (Figures 1 and 2).

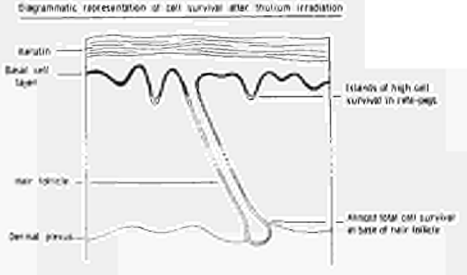

Figure 3: Diagrammatic representation of the site of cell survival in pig skin after thulium-170 irradiation. Surviving cells participate in the rapid repopulation of the damaged basal layer

Title of project nr 3: Wound healing in irradiated skin
Head of project and scientific staff: C.M.A. Young and J.W. Hopewell

---

In the repair of skin lesions produced as a consequence of radiation exposure random based pedicle skin flaps raised from irradiated skin have been shown to be of little value; the length of such flaps that will remain viable is extremely limited. This was clearly illustrated in pedicle skin flaps raised on the flank of pigs, the flaps were only based on small segmental blood vessels (Young,Plast. Recon. Surg. 79, 455, 1982; Hopewell, In: Radiation Biology in Cancer Research, Raven Press, N.Y. 1980)

More recently studies have been initiated to examine the effects of single doses of x-irradiation of the survival length of myocutaneous flaps. Such flaps are based on a single major artery and vein supplying a subcutaneous muscle block. The myocutaneous flaps used in these investigations were based on a small triangular muscle located in the upper part of the hind leg of the pig. A skin field 16 x 4cm was marked out down both hind legs of each pig. The field was positioned so that the top lay approximately 4cm dorsal to the vascular pedicle on which the flap was to be based.

Skin fields on the left hind leg were irradiated with single doses of 1800cGy. An additional caudal extension to the under part of the field was included to ensure that the whole of the future vascular pedicle was irradiated.

Skin flaps were raised from the 16 x 4cm fields at 6 and 12 weeks after irradiation. A flap was formed by incising the skin around all four sides of the field. All subcutaneous vascular connections were severed but for those over the small muscular pedicle. The flap was then sutured back on to its original bed. Control unirradiated flaps were raised on the opposite leg.

The length of the flap that was viable could be measured two days after surgery. The effect of radiation both at 6 and 12 weeks prior to surgery was to reduce the mean viable length of flaps to approximately half that of normal myocutaneous flaps (Table). This reduction in survival was similar to that recorded in random pattern flaps on the flank of pigs after this dose (Hopewell, 1980).

The effects of raising flaps at longer time periods after irradiation and of irradiating only the pedicle of a future flap are now to be investigated.

Table: Myocutaneous flap viability (cm) 2 days after surgery

Time after irradiation (weeks)	Normal flaps	irradiated flaps	irradiated/normal
6	8.5	4.0	0.47
	15.0	5.1	0.34
	17.5	6.5	0.37
	14.0	10.5	0.74
Mean ± SE	13.8 ± 1.9	6.5 ± 1.4	0.48 ± 0.09
12	19.0	12.0	0.63
	18.5	8.5	0.46
	18.1	8.8	0.49
	12.3	4.4	0.36
Mean ± SE	17.0 ± 1.6	8.4 ± 1.6	0.49 ± 0.06

Title of project nr 4: The pathogenesis of late vascular damage in the hamster cheek pouch

Head of project and scientific staff: J.W.Hopewell, Y. Gunn and D.Campling

Radiation induced changes in the vascular system are believed to play an important role in the pathogenesis of late radiation damage in normal tissues. In order to separate vascular changes from secondary damage to the parenchyma it is helpful to use a simple model system. The cheek pouch of hamsters, which consists of an epidermis and a thin supporting layer of muscle, collagen and fat represents such a simple model system. It is also possible to make comparative measurements between irradiated and controls by utilising both pouches in the same animal, thus reducing errors. The effects of single doses of 250kV x-rays, in the range 500-2500cGy, have been studied. Animals were observed for a maximum period of 2 years after irradiation.

In an earlier report the results of quantitative morphological studies were presented which illustrated that there was either a generalised vasodilation or a selective loss of capillaries in the vasculature after irradiation. After the higher doses this occurs at two distinct and separate times, 3 and 7 months post-irradiation. Currently a direct measurement of vessel diameters are being made with a digitiser, which should clarify which of the aforementioned effects are occurring.

In order to investigate the pathogenesis of these changes and to ascertain the cell types involved autoradiographic studies are continuing. Thirteen groups of animals have been irradiated with the same range of doses as were used in the morphometric studies. The morphological data are being used as a guide to plan sacrifice times.

The first group of animals were labelled prior to sacrifice, by six local injections of 0.2 μCi of ^{3}H-Thymidine given at 12 hourly intervals directly into the cheek pouch connective tissue. The labelling indices (L.I) of endothelial and smooth muscle cells were assessed in all vessel types. Vessel lengths and diameters are also being measured in the observed

sections. The results for the preliminary series of animals, killed 3-4 months after irradiation, are listed in the table below.

Table: Range of labelling indices (%) in smooth muscle and endothelial cells in animals killed 3-4 months after irradiation.

Dose cGy		Control		Irradiated	
		Smooth muscle	Endothelium	Smooth muscle	Endothelium
15	(3)	0.0	0.0-0.66	0.0-0.28	0.0
20	(2)	0.0-0.15	0.0-0.16	0.0	0.0
22.5	(5)	0.0-0.36	0.0-0.16	0.0	0.0-2.46
25	(4)	0.0-0.12	0.0-0.49	0.0-1.28	1.12-11.94

The current number of animals in each group is given in parenthesis.

It is clear from these results that the normal labelling indices for these two cell types is very low. However, after irradiation the labelling index of endothelial cells indicates that they are capable of a large increase in their proliferation rate.

In more recent investigations intraperitoneal injections of ^{3}H-Thymidine were used in order to avoid any irritant effects that might be produced by local injection. This material should provide a baseline for more detailed studies of cell-turnover times.

Publications in 1982

I. Scientific papers

Hopewell, J.W. and Young, (1982) The effect of field size on the reaction of pig skin to single doses of x-rays. Brit. J. Radiol. 55, 356-361

Hopewell, J.W.(1982) Persistent and late occurring lesions in the irradiated feet of rats: their clinical relevance. Brit. J. Radiol. 55,574-578

Peel,D.M., Hansen, L.S., Coggle,J.E., Hopewell, J.W., Charles, M.W. and Wells, J. (1982) Non-stochastic effects of different energy beta emitters on pig and mouse skin. In: Proc. Third Int. Symp. Radiation Protection, Inverness pp. 543-548

Wells, J., Charles, M.W., Peel, D.M., Hansen, L.S., Hopewell, J.W. and Coggle, J.E. (1982) Non-uniform irradiation of skin: Criteria for limiting non-stochastic effects. In: Proc. Third Int. Symp Radiation Protection, Inverness pp. 537-542

Young, C.M.A. and Hopewell, J.W. (1982) Functional and morphological changes in pig skin after single and fractionated doses of x-rays. Int. J. Radiat. Oncol. Biol. Phys. 8, 1539-1547

Hamlet, R. and Hopewell, J.W. (1982) The radiation response of skin in young and old rats. Int. J. Radiat. Biol. 42, 573-576

Young, C.M.A. (1982) The re-vascularisation of pedicle skin flaps in the pig : A functional and morphological study. Plast. Reconstr. Surg. 70, 455-464

II. Short communications

Young, C.M.A. and Hopewell, J.W. (1982) The effects of x-rays irradiation on the blood flow and re-vascularisation of free skin grafts. Int. J. Microcirc. Clin. Exp.1, 338

Hopewell, J.W. (1982) Biological bases of radiation protection standards. J. Soc. Radiol. Protec. 2, 10-11

Hopewell, J.W. and Young, C.M.A. (1982) The effect of field size on the reaction of pig skin to single doses of x-rays. Brit. J. Radiol. 55, 936-937

Young, C.M.A. (1982) Skin grafting in previously irradiated sites. Brit. J. Radiol. 55, 944

Young, C.M.A. (1982) Blood flow in pedicle skin flaps. Int. J. Microcirc. Clin. Invest. 1, 338

Progress Report
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Contractor: Contract no.: BIO-490-D-UK

Churchill Hospital Research Inst.
University of Oxford,
Headington,
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Head(s) of research team(s):

Dr. J.W. Hopewell, Ph.D.
Churchill Hospital Research Inst.
University of Oxford,
Headington
GB-Oxford OX3 7LJ

General subject of the contract:

The pathogenesis of late radiation damage in the central nervous system.

List of projects:

1. The pathogenesis of late radiation damage in the central nervous system.

Title of project nr 1: The pathogenesis of late radiation damage in the central nervous system

Head of project and scientific staff: J.W. Hopewell, J.H. Wilkinson, and T.K. Yeung, in collaboration with Dr. H.S. Reinhold, TNO Radiobiology Institute, The Netherlands

In order to assess radiation induced changes in the vascular architecture of the rat cerebral cortex, histological sections from animals perfused with saline and a blue-dye/latex/fixative mixture were examined. Factors such as the total vascular density, blood volume index and vessel wall surface area index were evaluated, in addition to vessel diameters.

Animals were killed at various times after irradiation with doses of 1770-3000cGy. The examination of all dose groups up until 39 weeks after treatment have now been completed.

Assessment of the total blood vessel count, blood volume index and vessel wall surface area index indicated a decrease in the vascularity of the cerebral cortex 15 weeks after irradiation with doses of 2000-3000cGy (Figure 1-3). These parameters had returned to, or were above, control levels by 39 weeks. In the two groups of animals examined after 39 weeks, that were irradiated with doses of 1770 and 2200cGy the vascularity appeared to be increased relative to controls.

The measurement of blood vessel diameter showed that the decline in the vascularity at 15-28 weeks could be mainly attributed to a loss of small vessels, $<8\,\mu m$ diameter (Figure 4).

The timing of this decrease in the vascularity of the cortex was consistent with the appearance of occlusive changes in arterioles which have been observed in the brain and other normal tissues at approximately this time interval after irradiation (Hopewell, 1982).

Reference:

Hopewell,J.W. Radiation effects on vascular tissue : In Cytotoxic Insult to Tissue, Eds. Potten, C.S. and Hendry, J.H., Churchill Livingstone, Edinburgh, 1982. pp.228-257

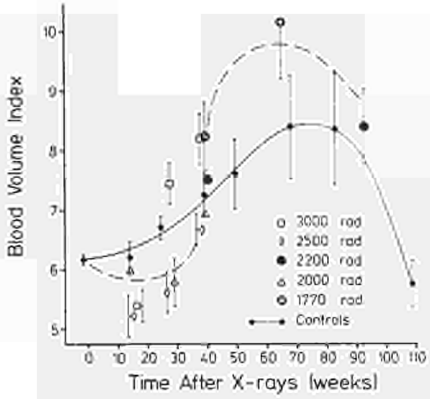

Figure 1: Time-related changes in the blood volume index in the cerebral cortex of irradiated and control rats

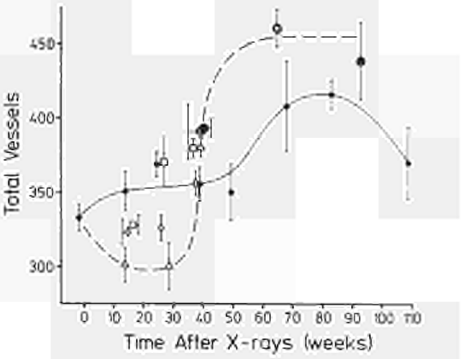

Figure 2: Time-related changes in the number of blood vessel fragments in the cerebral cortex of irradiated and control rats

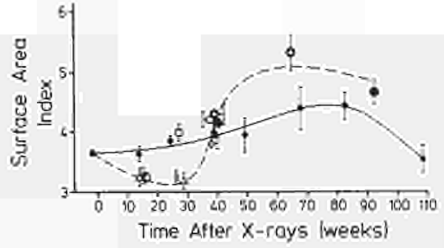

Figure 3: Time-related changes in the blood vessel wall surface area index in the cerebral cortex of irradiated and control rats

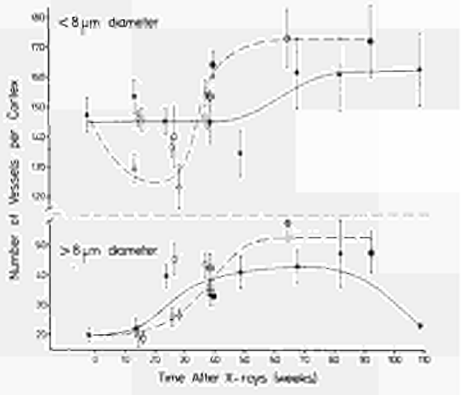

Figure 4: Time-related changes in the number of blood vessels with a 'small' (<8µm) diameter or a 'large' (>8µm) diameter in the cerebral cortex of irradiated and control rats

Progress Report
1982

Contractor:

Medical Research Council
MRC
Park Crescent 20
GB-London W1N 4AL

Contract no.: BIO-D-442-81-UK

Head(s) of research team(s):

Dr. S. Hornsey
Cyclotron Unit
MRC
Ducane Road
GB-London W12 OHS

General subject of the contract:

Radiation damage to cells and tissues at dose levels relevant to radiotherapy and protection.

List of projects:

1. Sensitivity of central nervous system in normal and dystrophic rodents.

Sensitivity of central nervous system in normal and in dystrophic rodents

Dr. Shirley Hornsey

Histology of irradiated rat brains

The death of rats following irradiation of large volumes of the brain was accompanied by loss of neuroglial cells in the subependymal plate, presumably leading to white matter necrosis. However, histological evidence of white matter necrosis in dying animals was not always found. A more common finding was demyelination in the 5th cranial (trigeminal) nerve. That damage to this nerve is a major contribution to the death of the animal is supported by evidence of reduced feeding resulting from injury to both motor and sensory roots of the trigeminal nerve. Animals lose weight and the teeth lengthen due to reduced nibbling. Facial lesions suggest that damage to the trigeminal nerve roots is general.

Residual damage in spinal cord

Residual damage in spinal cord, assessed as the dose reduction in the ED_{50} for a second treatment, increases with the increase in dose of the primary treatment. The increase in residual damage rises sharply when the primary treatment exceeds 25% of the ED_{50} dose for the damage under examination. For the type of damage to spinal cord associated with neuroglial cells the residual injury is the same after X rays or neutrons. For the type of damage associated with the vascular system, residual damage after X rays is related to the fraction of ED_{50} dose in the same way as for neuroglial damage. However, as the ED_{50} for vascular damage in the spinal cord is lower than for neuroglial damage this means that for a given dose the residual vascular damage will be greater than for residual neuroglial damage.

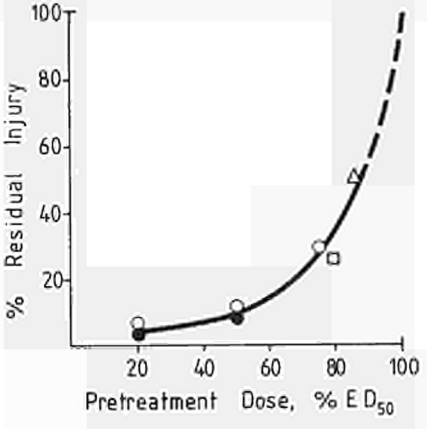

Fig. 1. The reduction in an X-ray test dose as a function of previous X-ray or neutron treatment. The reduction in dose is shown as a percentage of the dose required to produce the same level of damage in previously untreated animals and is called % Residual Injury. Glial-related damage: pretreated with X rays ○, □; pretreated with neutrons ●. Vascular-related damage: pretreated with X rays △.

Cycle time of glial cells in spinal cord

Single i.p. injections of tritiated thymidine were given to 3 month old rats which were then killed 1 hour to 120 days later. Each animal was perfused with ice-cold fixative, and the cervical cord removed. Plastic 1μ sections have been prepared in which the small number of labelled cells present can be detected using dark ground illumination. Cell identification and grain-counts can then be carried out using transmitted light. The correct exposure times required to enable accurate grain-counting have been established, and these measurements will provide an assessment of the biological half-time of the label. Previous work has suggested an intermitotic time (T_i) of about one month for glial cells in spinal cord, while the T_i reported for glial cells in brain is about 20 hours. The present experiment has been designed to confirm whether or not this important apparent difference between the cord and brain glial populations is real.

Radiosensitivity of mutant mouse strains

Radiosensitivity of the duodenum and jejunum in two mutant mouse strains has been measured. The mutant strains carrying the neurological lesions causing lurching or sprawling, only survive as heterozygotes. The lesion in the Lurchers transmitted by the semi-dominant gene Lc is associated with structural defects in the brain, particularly to the cerebellum, which is smaller than normal, and where the Purkinje cell layer is absent and the deep myelinated fibres appear irregular. Electron microscopy shows irregularities in the cell membrane, nuclear membrane and endoplasmic reticulum of the Purkinje cells. A recurring feature of the Lurcher is abnormal blood vessels which appear thickened and irregular (Caddy and Biscoe 1975). The lesion in the sprawling mutant mice is transmitted by the dominant gene Swl and is associated with a deficiency of myelinated axons in sensory roots, dorsal columns of the spinal cord and the peripheral nerves and is probably due to the failure in maturation and myelination of the sensory axons (Duchen, 2975).

No significant increase in sensitivity of the intestinal epithelium to 250 kV X rays was observed between the heterozygote mice carrying either the lurching or sprawling genes compared with their siblings not carrying the dominant genes. Tests on the sensitivity of the skin epithelium are still to be made.

References:

Caddy, K.W.T. and Biscoe, T.J. Brain Research 91, 276-280, 1975.
Duchen, L.W. Neuropathology & Applied Neurobiology, 1, 89-101, 1975.

Publications:

Hornsey, S., Myers, R. and Warren, P.
Residual injury in the spinal cord after treatment with X rays and neutrons. Brit. J. Radiol. 55, 516-519. 1982.

Rogers, M., Myers, R., Jenkinson, T. and Hornsey, S.
Histology of the irradiated rat brain in the first post-irradiation year. Brit. J. Radiol., 55, 208-212. 1982.

Progress Report
1982

Contractor:

Gesellschaft für Strahlen-
und Umweltforschung mbH.
GSF
Ingoldstädter Landstrasse 1
D-8042 Neuherberg

Contract no.: BIO-D-365-81-D

Head(s) of research team(s):

Prof. Dr. H. Kriegel
Abt. für Nuklearbiologie
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Ingoldstädter Landstrasse 1
D-8042 Neuherberg

General subject of the contract:

Decorporation and interruption of transfer of radionuclides;
especially of radioactive alkalines and alkaline earthes.

List of projects:

1. Decorporation and interruption of transfer of radionuclides;
   especially of radioactive alkalines and alkaline earthes.

Title of project nr BI-D 365-D (B)

Head of project an scientific staff: Prof. Dr. H. Kriegel
Dr. W.E. Kollmer, GSF
Dr. D. Berg, GSF
Dr. W.H. Müller, CEC

If individuals professionally exposed to radiation accidentally take up radioactive isotopes above the permissible level it is necessary that measures be taken to achieve an enhanced elimination, especially if the isotope is of a high radiotoxicity. In case of alkaline earths cryptand - 222 was especially efficient. A kinetic study with Sr-85 was undertaken to determine the influence of the mode and time of administration of cryptand - 222 on its decorporating efficiency.

Generally male NMRI mice were alloted to experimental groups with 5 animals each. All animals received an intravenous injection of 0.5µCi Sr-85. One group (controls) remained without application of the cryptand. The animals in the other groups received a single intraperitoneal, subcutaneous or intramuscular injection of cryptand-222 either 5 or 10 minutes after the administration of Sr-85. The doses of the cryptand was chosen between 100 and 200 µg per kg body weigth. Subsequently the total body radioactivity was repeatedly measured up two weeks after the administration. At the end of the experiment all animals were killed by an over dose of ether. The animals which had received the cryptand intraperitoneally were exsanguinated in ether anesthesia and the femur, liver and kidneys were recovered by dissection. The radioactivity of these organs was measured in an γ- counter.

The most important results of the investigations can be generally summarised and demonstrated with the data of an experiment

entered in Table 1. In the whole body measurements an enhanced decorporation of >30% was observed in the animals treated with 222. It was also shown that this effect was restricted to the first day after the administration of cryptand - 222. Thereafter the decline in the controls and the treated animals proceeded in parallel. The effect of the i.p. injected cryptand-222 on the content of Sr-85 in the individual organs is demonstrated in Table 1. The efficiency of the different modes of application of 222 is compared. The substance exhibited has its highest effectivity after intraperitoneal administration. The intramuscular dose was the least effect.

Summary:

The results of these experiments demonstrate that a single dose of the cryptand-222 exerts its decorporating effect exclusively within the first day after its administration irrespective of its application 5 or 10 minutes after the uptake of Sr-85. The intraperitoneal injection was roughly 25 % more effective compared to the intramuscular injection. The investigations will be continued.

Publications in 1982:

MÜLLER, W.H.: Removel of radio-barium by cryptand-222 from mammals. EULEP-Newes-Letters 30, 28-38 (1982)

Table 1

Distribution of ^{85}Sr in different organs with and without cryptand-222
% of injected radioactivity pro g

Time (h) post inj.	Blood			Femur			Liver		Kidney	
	^{85}Sr*	$\frac{}{^{85}Sr+222°}$	(%)	^{85}Sr*	$\frac{}{^{85}Sr+222°}$	(%)	^{85}Sr*	$\frac{}{^{85}Sr+222°}$	^{85}Sr*	$\frac{}{^{85}Sr+222°}$
1	1.08	0.85	21.3	36.61	23.27	36.4	0.69	0.70	1.39	2.41
24	0.04	0.04	0	36.71	22.91	35.8	0.02	0.02	0.05	0.06
48	0.03	0.03	0	32.66	23.87	26.9	0.02	0.01	0.03	0.03

The increased concentration of radioactivity in the kidney relative to the controls demonstrates the enhanced excretion of Sr-85 by this organ, due to an increased mobilisation of the radioisotope during the effective period of cryptand-222 (1d after its administration)

* ^{85}Sr: 0.5µCi Sr-85

° $^{85}Sr+222$: 0.5µCi-85 and 200µM/kg Cryptand-222, i.p., 5 min. post inj. of Sr-85

Table 2

Effectivity of decorporation with different modes of administration. Whole body radioactivity as % of the controls.

Days after administration	i.p.	s.c.	i.m.
1	22.85	20.45	17.05
2	23.09	18.80	14.07
6	24.02	20.34	12.54

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Contractor:

Commissariat à l'Energie
Atomique, CEA
Dépt. de la Protection DPS
B.P. n°6
F-92260 Fontenay-aux-Roses

Contract no.: BIO-D-370-81-F

Head(s) of research team(s):

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Serv. Radiopath. et Toxicol.
CEA-CEN de Fontenay-aux-Roses
B.P. n°6
F-92260 Fontenay-aux-Roses.

General subject of the contract:

Study of the biology of the Pu-tributylphosphate complex; metabolism and possible decorporation.

List of projects:

1. Metabolism of the Pu-239 tributylphosphate complex.

ETUDE DE LA BIOLOGIE DU COMPLEXE Pu-TRIBUTYLPHOSPHATE. METABOLISME ET DECORPORATION EVENTUELLE

CONTRAT n° BIO - D - 370 F (S.D.)

Les études du devenir biologique du plutonium après inhalation du complexe Pu-TBP ont été poursuivies chez le singe, ainsi que les essais thérapeutiques basés soit sur l'emploi du DTPA, soit sur l'emploi du lavage pulmonaire.

1. Devenir biologique du Pu inhalé à l'état de complexe Pu-TBP

Le précédent rapport annuel faisait état des critiques envers notre esttimation de la charge osseuse basée sur la multiplication par 10 de la charge des deux fémurs. Lors de sacrifices à 4 jours et 90 jours la détermination de la charge osseuse a été effectuée par comptage de tous les os. Les résultats du tableau 1 montrent que la charge des fémurs est assez fiable pour la détermination de la charge du squelette entier 90 jours après une telle contamination.

Le tableau 2 représente l'état actuel des résulats obtenus après inhalation de Pu-TBP.

2. Essais thérapeutiques par le Ca-DTPA

La faible efficacité du DTPA reportée sur le rapport précédent a été confirmé par l'emploi de dose plus faible de DTPA calcique, 15 $mg.kg^{-1}$ dose recommandée chez l'homme. Les résultats obtenus montrent que la partie éliminée du poumon ne semble pas être influencée par la concentration en DTPA. Celle-ci par contre affecte les dépôts osseux et hépatiques (tableau 3).

Des résultats partiels montrent que l'injection journalière de Zn-DTPA, mieux toléré aux fortes doses que le Ca-DTPA, ne permet pas d'espérer une amélioration.

	ANIMAL 272 SACRIFICE 90 J	ANIMAL 275 SACRIFICE 90 J	ANIMAL 284 SACRIFICE 4 J
FEMURS	9,2	10,4	7,4
HUMERUS	7,7	8,3	6,4
CRANE	18,7	11,9	15,6
RADIUS + CUBITUS	9,89	5,4	5,7
MAINS		4,0	6,2
TIBIAS + PERONNES	8,72	6,8	5,4
PIEDS		4,9	5,1
OMOPLATES	5,4	6,0	4,7
CLAVICULES	0,87	0,80	0,6
BASSIN	29,4	16,8	11,4
COLONNE VERTEBRALE		11,7	13,6
COTES + STERNUM	7,3	8,5	8,7
QUEUE	2,8	6,6	8,1

Tableau 1 : Répartition en pourcent de la charge squelettique après inhalation de ^{239}Pu-TBP.

3. Lavage pulmonaire

L'efficacité moyenne du traitement par lavage pulmonaire (figure 1) est obtenue à partir des résultats de babouins traités à dix reprises bilatéralement aux jours 1, 4, 11, 18, 25, 32, 39, 46, 53 et 60 jours après la contamination respiratoire. Les charges déposées variant de 5 à 22 nCi. L'efficacité moyenne est égale à 60% de la charge pulmonaire retenue, soit un résultat très voisin de celui obtenu avec l'oxyde de plutonium (58%) (figure 1).

Les séances de lavage 5 à 10 n'amenant qu'un gain moyen de 7%, le protocole retenu pour la dernière phase de l'expérimentation (traitement associant DTPA et lavage pulmonaire) ne comprendra que 5 lavages.

Le bilan comparatif effectué respectivement 15 et 130 jours après la contamination sur des animaux non traités ou ayant subi une série plus ou moins importante de lavages pulmonaires indique pas de modification dans la translocation du plutonium (tableau 4).

Tableau 2 : Inhalation de ^{239}Pu-TBP, résultats exprimés en pourcent de la charge inhalée.

	Sacrifice (jours)			
	3	15	30	90
POUMONS	69,4	79,5	60,4	55,9
TRACHEE	0,22	0,13	0,06	0,027
GANGLIONS THORACIQUES	0,22	0,49	0,58	1,76
SANG	1,3	0,51		
FOIE	0,35	0,97	1,26	2,38
OS	0,33	1,3	2,95	11,95

	Témoins	Ca-DTPA 150 mg.kg^{-1} (10 injections)	Ca-DTPA 15 mg.kg^{-1} (10 injections)
POUMONS	69,2	59,7	56,6
TRACHEE	0,045	0,042	0,17
GANGLIONS THORACIQUES	0,86	0,67	0,56
FOIE	1,1	0,083	0,87
SQUELETTE	3,9	0,31	1,69
FECES	25	21,9	27,5
URINES	0,6	17,2	12,5
Rapport FOIE/POUMONS	0,015	0,0053	0,015
" SQUELETTE/POUMONS	0,056	0,014	0,030

Tableau 3 : Traitement par le DTPA après inhalation de ^{239}Pu-TBP. Le sacrifice est effectué à 30 jours. Les résultats sont exprimés en pourcent de la dose inhalée.

	Sacrifice (jours)			
	15 JOURS		130 JOURS	
	3 LAVAGES	TEMOINS	10 LAVAGES	TEMOINS
POUMONS	43,3	79,5	18,0	59,6
GANGLIONS THORACIQUES	0,6	0,49	0,7	2,4
FOIE	0,3	0,97	0,9	3
SQUELETTE	2,3	1,3	5	9,5
LIQUIDE DE LAVAGE	39	-	43,6	-

Tableau 4 : Effet du lavage pulmonaire sur la "translocation" du plutonium (résultats exprimés en poucent de la dose inhalée).

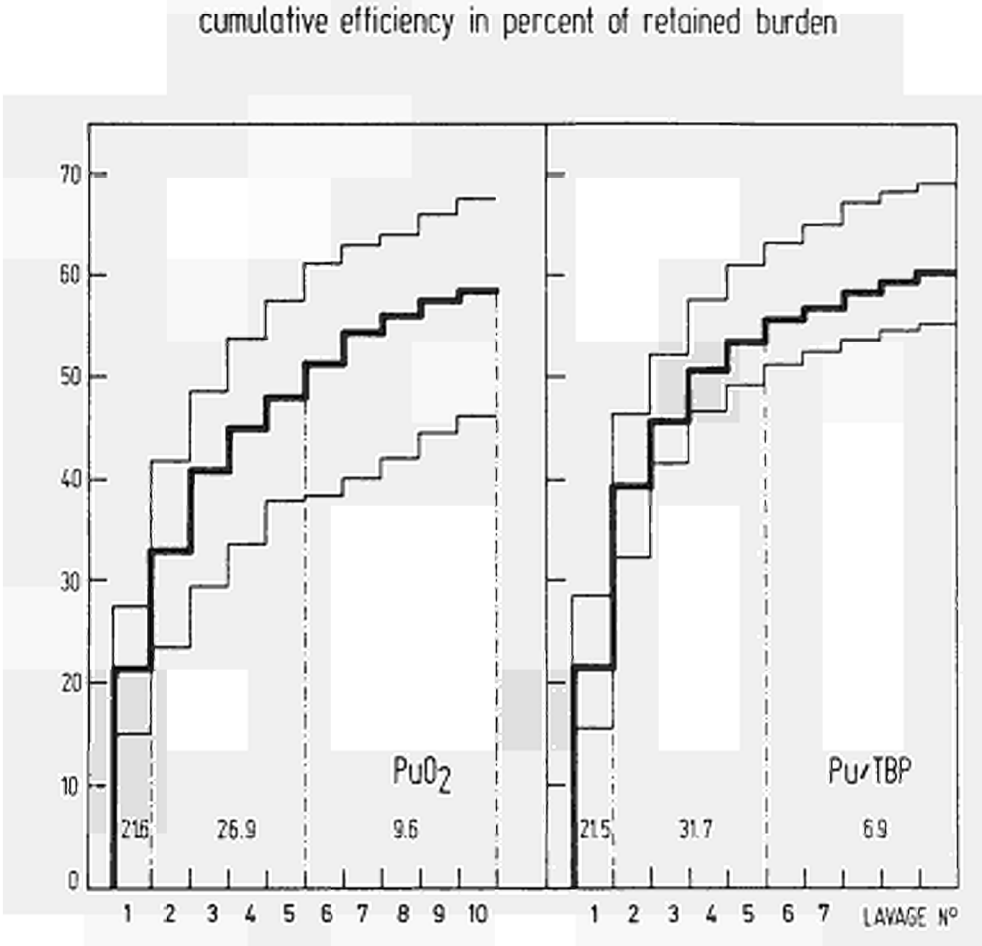

Figure 1 : Efficacité comparée du lavage pulmonaire après inhalation de PuO_2 ou du complexe Pu-TBP.

Progress Report
1982

Contractor:

Contract no.: BIO-D-383-81-UK

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General subject of the contract:

Radiation damage from inhaled particles in the rodent lung.

List of projects:

1. The effects of exposure of mice to Am-241 aerosols.
2. The effects of exposure of mice to maintained lung burdens of plutonium-239 dioxide.

Title of Project No. 1: The effects of exposure of mice to Am-241 aerosols.

Head of Project and Scientific Staff: B. E. Lambert, J. E. Coggle, D. A. Scott, S. R. Moores, A. Black, K. Danielak.

This work, which was delayed because of an outbreak of Sendai virus in the SAS/4 mouse breeding colony, was restarted using BALC/c mice.

During late 1981 and early 1982 groups of mice have been exposed to $^{241}Am(NO_3)_3$ aerosols in two regimes and kept for late effect studies:-

(a) In this study groups of (57) 6 week old mice have inhaled aerosols of $^{241}Am(NO_3)_3$ to produce initial alveolar depositions (IADs), as measured at 1 day, in the range 0.1 - 5 nCi. These mice have been assigned to three groups, i.e. either killed in groups at regular intervals for the determination of lung retention and translocation, or killed at 1 year for the assessment of lung tumour incidence, or left to live their lifespan followed by a full histopathological investigation.

Already, as expected, distinct differences have been established for deposition and retention between $^{239}PuO_2$ (see previous contract) and $^{241}Am(NO_3)_3$. Thus, the rate of loss of ^{241}Am from the lungs was much faster than for ^{239}Pu; 70% was removed with a half time of about 7 days, 28% with a $T_{\frac{1}{2}}$ of about 24 days and the remaining 2% with a $T_{\frac{1}{2}}$ of about 270 days. The rate and extent of translocation and uptake into liver and bone was also proportionately greater, e.g. at 1 week after inhalation the liver contained about 30% and the skeleton about 50% of the initial lung content. This rapid translocation to bone has prompted a study of distribution of ^{241}Am amongst different bones and a careful search for bone pathology. Early results suggest that the concentrations in most bones are similar with, however, the skull bones significantly lower. Some of the mice in the early tumour study (1 year) have been killed but no results are yet available of lung tumour incidence.

(b) Mice in this part of the experiment were re-exposed twice following an initial inhalation which produced an IAD of ^{241}Am of about 3.5 nCi. The re-exposures were carried out at 6 and 16 weeks to re-

establish a lung burden of about 3 nCi, as determined by a trial experiment in order that the dose rate to the lung should follow the same pattern as that provided by a single IAD of $^{239}PuO_2$ of about 2.5 nCi (which produced a maximum tumour response). Mice in these groups will, again, be either killed at 1 year (early 1983) or kept for their lifespan for tumour induction studies.

Title of Project No. 2: The effects of exposure of mice to maintained lung burdens of plutonium-239 dioxide.

Head of Project and Scientific Staff: B. E. Lambert, J. E. Coggle, D. A. Scott, S. R. Moores, A. Black, K. Danielak.

This project, which again was delayed by an enforced change of mouse strain, has just started using female, 6 week old, BALB/c mice. These mice have been exposed (late 1982) in groups of 57 to inhalation of aerosols of $^{239}PuO_2$ of AMAD 0.8 μm (σ_g 1.2). These groups will be re-exposed at intervals of two months to re-establish an initial alveolar deposition (IAD) of about 3 nCi (a level which gave a maximum tumour response after a single inhalation) and then killed at 1 year for the enumeration and classification of lung tumours. In addition, the free alveolar macrophage population will be estimated, at the two monthly intervals, by lung lavage.

The first tumour incidence results will be available in November 1983.

These experiments will be repeated with mice at 12 weeks of age (from March 1983) and 6 months of age (from July 1983).

List of Publications 1982

II. Short Communications, Theses, Internal Reports, Patents

"The Metabolism of Americium-241 in Mice Following Exposure by Inhalation or Injection". Thesis presented for the degree of M.Sc. (University of London) by K. J. Kittridge, 1982.

Progress Report
1982

Contractor:

Contract no.: BIO-D-447-81-UK

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General subject of the contract:

Radiation damage from inhaled particles in the rodent lung.

List of projects:

1. Late effects of exposure of mice to Pu-239 dioxide.

Title of Project No. 1: Late Effects of Exposure of Mice to Pu-239 Dioxide

Head of Project and Scientific Staff: B. E. Lambert, D. A. Scott

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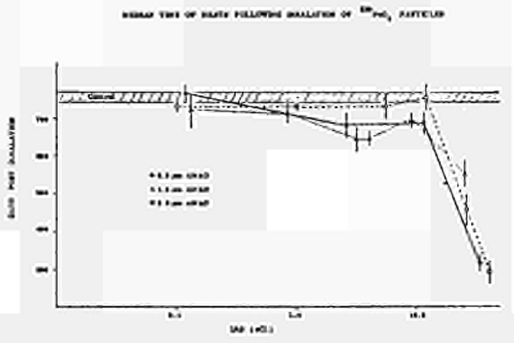
This work was an extension of contract 252-77-1 BIO UK which concerned the long term effects of inhalation of $^{239}PuO_2$ aerosols. The additional time has enabled more survival data to be accumulated and analysed but much of the long term pathology has yet to be confirmed by histology.

Groups of mice have been exposed by inhalation to sized aerosols of $^{239}PuO_2$ to produce a range of initial alveolar depositions (IADs) and effects studied at 1 year (lung tumours) and at the end of their lifespan (general pathology). Lung tumour incidence and some preliminary survival data were reported at the end of the earlier contract (1981).

Complete survival curves have now been obtained for all groups of mice and the median age of death, deduced from these, are shown as a function of IAD plotted on a log scale for convenience only) in Fig. 1. It can be

Fig. 1



seen that there is no really significant variation in the median time of death until an IAD of about 10 nCi is exceeded; there is also no clear difference between the effects of the different sized particles inhaled. However, as the median age uses only one point on the survival curve a better analysis for comparison uses some form of linear transformation (see Gompertzian plots in 1981 report). Another simple method of analysis of the data is shown in Table 1. As can be seen for early occurring deaths the mean death rate increased by a factor of at least 10 in the highest IAD groups compared with the controls whereas for late occurring deaths the increase was much less. There also appears to be no clear differences attributable to inhaled particle size.

Translocation of ^{239}Pu to other organs has been small and, therefore, as expected, most of the increases in long term pathology have been in the

Table 1

MEAN DEATH RATE PER 1000 MOUSE DAYS AT RISK

IAD nCi	0 - 450 DAYS POST EXPOSURE	451 - 820 DAYS POST EXPOSURE	821 - DEATH DAYS POST EXPOSURE
0.8 μm AMAD			
0.12 ± 0.01	0.10	2.40	12.07
2.60 ± 0.15	0.21	3.09	20.91
4.94 ± 0.35	0.23	4.43	-
11.71 ± 0.82	0.56	4.67	56.69
23.93 ± 1.83	1.65	8.07	-
1.5 μm AMAD			
0.13 ± 0.01	0.16	2.36	11.19
0.86 ± 0.04	0.29	2.24	7.67
3.10 ± 0.70	0.38	2.95	8.47
4.03 ± 0.78	0.34	2.50	13.28
25.22 ± 3.58	0.73	4.23	8.93
2.2 μm AMAD			
0.10 ± 0.02	0.08	2.15	13.75
1.56 ± 0.34	0.08	2.85	9.84
12.01 ± 0.32	0.21	2.48	16.95
26.34 ± 5.89	0.87	3.87	-
40.35 ± 16.00	2.52	8.15	10.00
CONTROL	0.08	2.29	6.63

lung. In the higher IAD groups there has been considerable life-shortening mostly due to respiratory insufficiency (caused by obstruction of the alveoli septae by giant macrophages, hyperplasia of the cells of the alveolar wall and fibrosis. At lower IADs the incidence of lung tumours was far greater with a peak incidence at death following IADs of about 2.5 - 3 nCi - and (as at 1 year) more uniform irradiation of the lung parenchyma from smaller particles (0.8 μm AMAD) resulted in more tumours, e.g. following an IAD of 2.6 nCi the overall incidence of lung tumours was 85.0 ± 5.5% compared with a spontaneous incidence in controls of 31.6 ± 6.2%, The histological assessment of these tumours is not yet complete but they are more mixed in cellular character - there being a majority of alveogenic and bronchogenic carcinomas but also a significant number of squamous cell tumours and haemangiosarcomas (which did not occur spontaneously in control mice. Only at later stages did these lung tumours contribute significantly to death of the animals.

Conclusions: It may be concluded from these data that lung tumour incidence in mice was very markedly dependent on inhaled particle size (and therefore dose distribution) with a peak incidence following a deposition of about 2.5 - 3 nCi in the lungs. The tumours appeared, in general, where the energy was absorbed in the lung. Because of the greatly increased incidence of tumours and the ratio of their occurrence at 1 year and over a life span it is evident that the carcinogenic action of $^{239}PuO_2$ is more than shortening of the latent period. Life shortening, because of its link with early occurring diseases, was not obviously correlated with dose distribution and was not significant below an IAD of 10 nCi.

List of Publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

B. E. Lambert, M. L. Phipps, P. J. Lindop, A. Black and S. R. Moores. Induction of Lung Tumours in Mice Following the Inhalation of $^{239}PuO_2$. In Proceedings of the Third International Symposium of the Society for Radiological Protection - 'Radiation Protection Advances in Theory and Practice', Inverness, July 1982, Vol. I, pp 370-375.

B. E. Lambert, M. L. Waller and A. Black. Life Shortening in Mice Following Inhalation of Sized Aerosols of $^{239}PuO_2$. Colloquium on the Toxicity of Radionuclides, Liege, November 1982. (To be published in Journal Belge de Radiologie).

Progress Report
1982

Contractor: Contract no.: BIO-D-378-81-B

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General subject of the contract:

Late somatic and genetic effects of radiation in mammals.

List of projects:

1. Morphological, biochemical and physiological late effects of irradiation in the adult and developing rat brain.
2. Effects of whole body irradiation of the developing organism on the hematopoetic system and on life span and disease incidence.
3. Mechanisms of radiation induced fibrosis in lung.
4. Influence of dose rate and fractionation on life shortening and causes of death of mice irradiated with 50 MeV neutrons and gamma rays.
5. Factors influencing genetic radiosensibility and their influence on the extrapolation of genetic data from animal to man.
6. Influence of dose and inhomogeneous exposure on the aberration yield in radiotherapy patients.

Title of project nr 1 : MORPHOLOGICAL, BIOCHEMICAL AND PHYSIOLOGICAL LATE EFFECTS OF IRRADIATION IN THE ADULT AND DEVELOPING RAT BRAIN

Head of project and scientific staff : G.B. GERBER, H. REYNERS

A. Morphological studies

Different morphometrical measurements were performed on the main cell populations of the rat brain using samples from the grey and white matter of young (3 months old) and old adult (15 months old) animals.

All cellular densities were found increased, particularly in the older group where the changes in alpha astrocytes, neurons and blood capillaries reached significant levels. These increases were found to be closely associated with cellular volume involutions in each of the cell populations where these measurements were available and also correlated with a much thinner cortical thickness (-16.7 %, $P = 0.0003$ in the 15 month-old individuals).

Consequences of the preceding changes have been observed at the level of the associations between the various cells which were found altered ; the percentage of close contacts (satellitism between neurons and alpha astrocytes significantly increased as well as the dyadism - amount of twin cells - of this group in old adult rats. Alternatively, using cortex thickness and cellular volume as correction factors for the computation of the absolute total cell number in the cerebral cortex reveals that such amount is not significantly modified with respect to controls ; in other words, the brain has been quite noticeably "miniaturized" after fetal 0.5 Gy irradiation but remains with the same cellular content.

With respect to the present data, it can be concluded that the maturation (but not the growth) of the central nervous system endures a drastic reversal in ageing rats treated with a dose as low as 0.5 Gy RX on the 15th gestational day.

B. Biochemical studies

These studies aim to elucidate the changes in function of the central nervous system after irradiation in utero. The development of biogenic amines during postnatal life was studied in rats whose mothers had been irradiated with 0.5 or 1 Gy of X-rays at day 10 or 15 of pregnancy. The concentration of serotonin, noradrenaline and dopamine increases markedly during the initial 2 weeks of life and slightly more until the end of the first month. Rats which had been irradiated in utero at day 15 display an increased concentration of serotonin, dopamine and noradrenaline at an age of 3 months compared to controls. Brain weight and, somewhat less, body weight are however reduced in these animals. Better techniques for determining biogenic amines in defined areas of the brain and for measuring the corresponding receptors are needed to explain and extend these observations. Experiments are now under way to measure the entire spectrum of biogenic amines and their metabolites by high performance liquid chromatography using electrochemical detection.

No changes in different parameters of endothelial cells were observed after irradiation in utero with 0.5 or 1 Gy at day 10 or 15. The parameters studied in capillary fragments at an age of 1-4 months are uptake of methyldeoxyglucose, glutaminate transpeptidase and alkaline phosphatase. Less capillary fragments (as determined from DNA content) were however isolated after irradiation, an observation which can be explained by the lower brain weight of the animals. These results suggest that vascular changes are not of primary importance following irradiation in utero as seems to be the case in adults. This assumption is supported by the results on regional blood flow measured by means of trapping injected 15 u large radioactive microspheres which did not show any alterations in relative blood flow in different brain areas unless the entire structure of the brain had undergone gross alterations as for example in animals exposed to high doses at an intrauterine age of 15 days. These experiments are not yet entirely terminated and unfortunately, the determinations using iodoantipyrine and pertechnetate for determination of blood flow in vivo could not be carried out since the supplier could not provide the iodoantipyrine normally coming from Poland.

Title of project nr 2 : EFFECTS OF WHOLE BODY IRRADIATION OF THE DEVELOPING ORGANISM ON THE HEMATOPOETIC SYSTEM AND ON LIFE SPAN AND DISEASE INCIDENCE

Head of project and scientific staff : G. GERBER

Whereas in newborn mice a fractionated irradiation is more efficient than the same dose this seems not to be the case in rats. We have demonstrated that this sensibilisation is due to the replacement of radioresistant newborn hemopoietic stem cells by more radiosensitive adult stem cells. Radiation is not the only agent which can affect this transformation. Various cytostatic drugs (adriamycine, bleomycine, actinomycine-D) which destroy hemopoietic stem cells to different degrees also bring about an increased sensitivity towards radiation in direct relation to their destructive capacity. It appears therefore that newborn stem cells have only limited capacity for repopulation and, when lost, are replaced by adult stem cells. It is still unknown whether stem cells of newborn mice represent a type or clone of cells different from those of adult mice or are only in a different physiological state. Studies on cells obtained from adults whose marrow which had been restored with stem cells from newborn mice may give an answer to this question and are now under way.

Rats and hamsters do not display a sensibilisation when an irradiation is fractionated as do mice. Indeed, the stem cells of adult rats have the same Do as those of 6 day old rats if irradiated in vitro and then transplanted in irradiated host C57BL mice. The seeding efficiency of rat stem cells in mouse spleen is smaller than that of mouse stem cells and this is particularly marked for stem cells from fetal rat liver. It will be necessary therefore to establish the Do for fetal stem cells also by other means in order to exclude the possibility that a difference in seeding efficiency had obscured the difference in radiosensitivity.

Title of project nr 3 : MECHANISMS OF RADIATION INDUCED FIBROSIS IN LUNG

Head of project and scientific staff : G.B. GERBER

The isolation of different collagen fractions and proteoglycanes in rats exposed to a dose of 1.5 kR of X-rays was continued. The increase in the collagen/DNA ratio found earlier was confirmed, but since the experiment was continued over a longer period of 20 months, it appeared that from one year collagen levels tend to decrease. No significant change in collagen composition (collagen III vs I) could be observed but the error of the data is still large. There appears to be a temporary (maximal at one year) relative increase of chondroitin sulfate at the expense of heparin sulfate and hyaluronic acid.

Ultrastructural studies with transmission and scanning electron microscopy (SEM) were carried out in BALB/c male mice exposed to graded doses from 5-50 Gy of X-rays and sacrificed at different time intervals ranging from 15 minutes to 24 months. SEM complements well the observations from TEM since it reveals the changes on the surface of the cells and within the alveoli. The results confirm that the endothelium of the large vessels and the epithelium of the airways is less susceptible to radiation damage than the epithelium of the lung parenchyma. During the period of radiopneumonitis (2-7 months after irradiation) and after the highest X-ray doses, the principal lesions are seen on epithelial cells, mainly those of type II whereas capillary damage appears to play a secondary role at this time. Increased phospholipid secretion by type II cells in conjunction with an impaired synthesis of the protein component of the surfactant resulting in altered surface tension as well as damage to alveolar macrophages are the principal mechanisms in radiation pneumonitis. Later when fibrosis ensues after doses of 10 Gy or more, vascular alterations predominate over those from the epithelium.

Publications

H. Reyners, E. Gianfelici de Reyners, J.R. Maisin. The beta astrocytes a newly recognized radiosensitive glial cell type in the cerebral cortex. Journal of Neurocytology, 11 (1982) 967-983.

G.B. Gerber, J. Maes. Radiosensitivity of hemopoietic stem cells during fetal and early postnatal development, Proc. Intern. Symp. Developmental effects of prenatal irradiation, H. Kriegel, W. Schmahl, G. Kistner, F.E. Stieve, eds., p. 175-180, G. Fischer, Stuttgart, 1982.

G.B. Gerber, J. Maes, B. Eykens. Transfer of antimony and arsenic to the developing organisms. Arch. Toxicology, 49 (1982) 159-168.

G.B. Gerber, Interactions : dose effect relationships and isoeffect curves. Radiat. Environm. Biophys., 20 (1982) 235-243.

Title of project nr 4 : INFLUENCE OF DOSE RATE AND FRACTIONATION ON LIFE SHORTENING AND CAUSES OF DEATH OF MICE IRRADIATED WITH 50 MeV NEUTRONS AND GAMMA RAYS

Head of Project and Scientific Staff : J.R. MAISIN

- The effects of a fractionated gamma irradiation on life shortening and disease incidence in BALB/c mice.

Male mice of the BALB/c strain, 12 weeks of age, were exposed to a single or fractionated exposure of ^{137}Cs gamma rays. The fractionated dose was split into 10 equal doses delivered at an interval of 1 day. The causes and possible causes of spontaneous death were ascertained by autopsy and histological examination and the data were treated by competing risk analysis. Life shortening followed a linear dose dependency and was about the same for fractionated (38 ± 3.1 days/Gy) as for single (46.2 ± 4.3 days/Gy) exposure.

The total incidence of malignant tumors increases with dose after fractionated and decreases after single exposure. Although no single value is significantly different from the control, the entire groups differ as shown by a G-test, and at a dose level of 6 Gy, the difference between single and fractionated exposure is also significant, although both incidences do not differ significantly from controls. Thymoma incidence rises at 6 Gy to a peak which is attained more rapidly after single than after fractionated exposure. On the other hand, significantly fewer carcinomas and sarcomas are seen after single than after fractionated exposure. Death from non stochastic lung and kidney diseases was reduced after fractionated compared to single exposure.

- Life shortening and disease incidence in BALB/c mice following a single d(50)-Be neutron or gamma exposure.

Male BALB/c mice, 12 weeks old, were given a single exposure of either ^{137}Cs gamma rays or d(50)-Be neutrons at a dose rate of 3 Gy/minute.

The animals were kept until dying and causes of death or possible causes of death were ascertained by autopsy and histology. The data were evaluated by competing risk methods. The survival time dose effect curve for both types of exposure was linear and did not differ significantly from another (slopes : 55.8 $\pm$ 4 days/Gy for neutrons and 46.2 $\pm$ 4.3 days/Gy for gamma rays). The incidence of different diseases also was similar for both groups except that more carcinomas and sarcomas and myeloid leukemias seemed to occur after neutron exposure and that non stochastic lung and kidney diseases seemed to arise at lower doses.

Publications

J.R. Maisin
Chemical protection and late effects after whole body supralethal X-irradiation in mice.
- in "Late Effects after therapeutic whole-body irradiation", Report EUR 8070 en, p. 117-132 (1982).

J.R. Maisin, A. Wambersie, G.B. Gerber, G. Mattelin, M. Lambiet-Collier, J. Gueulette
The effects of a fractionated gamma irradiation on life shortening and disease incidence in BALB/c mice.
- Radiation Research, in press.

J.R. Maisin
Protection chimique contre les effets à long terme survenant chez les souris irradiées par des doses supralétales de rayons X.
C.R. Soc. Biol.

J.R. Maisin
Reduction of short- and long-term radiation lethality by mixttures of chemical protectors after single or fractionated whole-body exposure of mice to ionizing radiation
- in "Problems of Natural and Modified Radiosensitivity", in press.

J.R. Maisin, A. Wambersie, G.B. Gerber, J. Gueulette, G. Mattelin, M. Lambiet-Collier
Late effects in mice following whole-body exposure to d(50)-Be neutrons and gamma rays.
- in "Radiation Protection - Neutron Carcinogenesis" Report EUR 8084 en. p. 187-190 (1982).

J.R. Maisin, A. Wambersie, G.B. Gerber, J. Gueulette, G. Mattelin, M. Lambiet-Collier
Life shortening and disease incidence in BALB/c mice following a single d(50)-Be neutron or gamma exposure.
- Radiation Research, in press.

Title of project nr 5 : FACTORS INFLUENCING GENETIC RADIOSENSIBILITY AND THEIR INFLUENCE ON THE EXTRAPOLATION OF GENETIC DATA FROM ANIMALS TO MAN

Head of project and scientific staff : A. LEONARD, G. DECAT, Gh. DEKNUDT, L. FABRY

Previous experiments on different mammalian species demonstrated that, provided the harlequin-staining method is used to estimate the yield of cells in first or subsequent mitosis, it seems appropriate to use animals for studies on the induction of aberrations by acute exposure and to extrapolate the results to man by using appropriate extrapolation factors. Additional experiments were, therefore, performed in order to verify to what extent lymphocytes of the experimental mammals can serve as a model for long-term experiments.

Since dogs are widely used to study biological effects of protracted whole-body irradiation, blood lymphocytes from 2 young male beagle dogs were sampled before irradiation and then after 1, 14, 35 and 50 days following whole-body exposure to 150 rad γ-rays from ^{60}Co. To compare the effectiveness of exposure to ionizing radiation in vitro and in vivo, blood samples were irradiated at the same time. The variations in the white blood cells were followed by counting leucocytes in an aliquot of each sample in an electronic cell counter ; lymphocytes were identified by the Pappenheim stain technique.

Exposure to 150 rad γ-irradiation resulted in a sharp decrease of lymphocytes from 3 156 $\pm$ 160 per μl, in the samples taken before irradiation, to 526 $\pm$ 16 per μl, in the samples taken 1 day after exposure. The number of lymphocytes remained low for up to 35 days, with a return to a normal level after 50 days. One day after whole-body exposure the number of dividing lymphocytes was too low to allow an estimate of induced chromosome aberrations. There was, however, no statistical difference, with

respect to the incidence of dicentrics ($X^2 = 3.5$; $P < 0.005$), between the blood samples given 150 rad γ-irradiation in vitro and the lymphocytes of the dogs examined 14 days after whole-body exposure. Since the observations of Clemenger and Scott (1973) on rabbits showed that no difference exists between the effectiveness of irradiation in vitro and in vivo, we may infer from our results that the proportion of lymphocytes carrying chromosome aberrations remained stable during the first weeks after exposure in vivo and fall rapidly thereafter. No observations were performed between the 14th and the 35th days after whole-body exposure but one can estimate roughly that the half-life time of dog lymphocytes carrying chromosome aberrations is less than 30 days.

These observations as well as previous results obtained on rabbit demonstrate that owing to the very short survival time of lymphocytes carrying chromosome aberrations, experimental mammals cannot be used as a model to study the cytogenetic effects resulting from chronic exposure to ionizing radiation on the persistence of anomalies after acute irradiation.

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Radiosensitivity and lifespan of dog peripheral blood lymphocytes.
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C. Raymakers, Gh. Deknudt and A. Léonard
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- C.R. Soc. Biol., 176, 87-89, 1982.

C. Modave, L. Fabry and A. Léonard
Cinétique cellulaire et radiosensibilité des lymphocytes de vache en culture.
- C.R. Soc. Biol., 176, 90-94, 1982.

B. Debuyst, M. Rosenthal and A. Léonard
Cinétique cellulaire et radiosensibilité des lymphocytes de chèvre stimulés par la phytohémagglutine.
- C.R. Soc. Biol., 176, 82-86, 1982.

A. Léonard, G. Decat and L. Fabry
The lymphocytes of small mammals. A model for research in cytogenetics.
- Mutation Research, 95, 31-44, 1982.

G. Decat, A. Léonard and W. de Meurichy
Cinétique cellulaire et radiosensibilité chromosomique des lymphocytes de quatre espèces de cercapithèques.
- C.R. Soc. Biol., 176, 373-377, 1982.

A. Léonard, L. Fabry, Gh. Deknudt and G. Decat
Chromosome aberrations as a measure of mutagenesis : cytogenetic extrapolation from animal to man.
- Cytogenetics Cell Genetics, 33, 107-113, 1982.

M. Bianchi, L. Fabry and A. Léonard
Evaluation of radiation-induced chromosomal aberrations in human peripheral blood lymphocytes in vitro. Result of an IAEA coordinated programme.
- Mutation Research, 96, 233-242, 1982.

Title of project nr 6 : INFLUENCE OF DOSE AND INHOMOGENEOUS EXPOSURE ON THE ABERRATION YIELD IN RADIOTHERAPY PATIENTS

Head of project and scientific staff : A. LEONARD, L. FABRY and M. LEMAIRE

To obtain information concerning the aberration yields induced in circulating lymphocytes in man after partial-body irradiation additional observations have been performed on a second set of female patients treated with telecobalt therapy for mammary carcinoma.

The mammary area was irradiated from two opposed tangential fields and doses were calculated from the isodose covering the internal face of costal area. The axillary and supraclavicular lymphatic areas were also irradiated from two opposite directions. Blood samples were taken prior to the first radiotherapy session and after absorbed doses of 10-20-30 Gy to each target volume. 200 cells from each sample were analysed from the presence of dicentric and ring chromosomes. The first results which have still to be completed and compared with previous ones obtained on patients from a different medical unit are summarized in Table I

Table I

Frequency of dicentric and ring chromosomes after irradiation of patients treated for mammary carcinoma

Case	Control samples		10 Gy		20 Gy		30 Gy	
	Total cells	Number of dicentrics + rings	Total cells	Number of dicentrics + rings	Total cells	Number of dicentrics + rings	Total cells	Number of dicentrics + rings
I	200	1	200	28	200	37	200	63
II	200	1	200		200	16	200	36
III	200	0	200		200	33	200	27
IV	200	1	200	12	200	30	200	36
V	200	0	200	8	200	20	200	35
VI	200	2	200	4	200	10	200	16

Publications

A. Léonard and G.B. Gerber
RBE and dose effect relationships in mammalian somatic and germ cells.
- in J.J. Broerse and G.B. Gerber(Eds.), Neutron carcinogenesis,
Commission of the European Communities, EUR 8084 EN, pp. 361-363.

Progress Report
1982

Contractor:

Centre d'Etude de l'Energie
Nucléaire, CEN/SCK
Avenue Plasky 144
B-1040 Bruxelles

Contract no.: BIO-D-379-81-B

Head(s) of research team(s):

Dr. J. R. Maisin
Dép. Radiobiologie
CEN/SCK
Boeretang 200
B-2400 Mol

General subject of the contract:

Genetic recombination as a mechanism of induction of leukemia upon irradiation and its impairment.

List of projects:

1. Genetic recombination as a mechanism of induction of leukemia upon irradiation and its impairment.

Title of project nr 1 : GENETIC RECOMBINATION AS A MECHANISM OF INDUCTION OF LEUKEMIA UPON IRRADIATION AND ITS IMPAIRMENT

Head of project and scientific staff : J.R. MAISIN, M. JANOWSKI

We conducted experiments in order to investigate whether the induction of thymic lymphomas in C57BL/Ka mice by irradiation proceeds or not by a mechanism similar to that exerted by the radiation leukemia virus (RadLV).

In a first step, we performed a restriction enzyme analysis of DNA from several cellular clones derived from BL/VL_3, a cell line which was derived from a RadLV-induced thymic lymphoma and which is a permanent producer of thymotropic and leukemogenic $RadLV/VL_3$ virions. All the investigated clones showed, as compared to control cells, novel provirus at a same site of their DNA. This result was quite unexpected in view of the hypothesis that RadLV might carry the information for cell transformation in its own genome. Indeed, the leukemogenic action of a transforming virus would not require proviral integration at a unique site of the host cell DNA.

In a second step, we examined if the observed phenomenon could be generalized. Three thymic lymphomas were induced in C57BL/Ka mice with the in vivo propagated RadLV, three others with the cell culture propagated $RadLV/VL_3$. Each of the six tumors was established in two distinct culture cell lines, from the primary thymic tumor and from the invaded bone marrow, respectively. Although the cell lines thus obtained were not identical as to the electrophoretic profile of their surface antigens, they were indistinguishable from each other - and from BL/VL_3 - where restriction analysis of their DNA was concerned.

The same novel proviral sequences were observed at the same DNA site, common to all the investigated cell lines. It seems thus plausible that RadLV exerts its leukemogenic action by inducing proliferation of cells in which provirus integration occurred - probably by chance - at a given site of the DNA. In other terms, it may be postulated that a cellular oncogene might undergo activation under the control of the proviral promotor happening to become inserted in its neighbourhood. This hypothesis predicts the presence, in the investigated tumor cells, of a new mRNA species whose nucleotide sequence starts with the 5' end sequence of the viral genome. Testing this prediction is under way.

The third step of our studies was to test the hypothesis that RadLV might be the causative agent of radiation-induced leukemogenesis in C57BL/Ka mice. Sixteen individual radiogenic thymic lymphomas - although neither cloned nor even established in culture - did not contain detectable novel proviral sequences. In addition, series of clones derived from a radiogenic tumor cell line (BL/RL_12-NP) also yielded results which proved negative in that respect, even when clones were derived after that the cell line had become spontaneously virus producer (BL/RL_12-P). This lead us to the conclusion that radiation induces malignant proliferation by a mechanism other than RadLV, such as oncogene activation by mutation or sequence rearrangement either in its neighbourhood (appearance of an active promotor) or in its own sequences (conversion of a protooncogene into oncogene).

Publications

M. Janowski, J. Boniver & J.R. Maisin. Sites communs d'intégration de provirus dans les lymphomes induits chez des souris C57BL par le virus des radioleucoses murines (RadLV) et absence de nouvelles séquences provirales dans l'ADN des lymphomes radio-induits. C.R. Soc. Biol. 176, 411-419, 1982.

M. Janowski, J. Boniver & J.R. Maisin. Common proviral integration sites in C57BL mouse lymphomas induced by radiation leukemia virus and absence of novel virus-related sequences in radiogenic lymphoma DNA. Leukemia Res. 6, 285-297, 1982.

R. Hooghe, D.C. Hoessli & M. Janowski. Surface proteins of radiation-induced and radiation leukemia virus induced lymphosarcoma in mice. Leukemia Res., in press.

Progress Report
1982

Contractor:

Centre d'Etude de l'Energie
Nucléaire, CEN/SCK
Avenue Plasky 144
B-1040 Bruxelles

Contract no.: BIO-492-D-B

Head(s) of research team(s):

Dr. J.R. Maisin
Dép. Radiobiologie
CEN/SCK
Boeretang 200
B-2400 Mol

General subject of the contract:

"In-vivo" radiation-activation of cellular oncogenes.

List of projects:

1. "In-vivo" radiation-activation of cellular oncogenes.

Title of project nr 1 :"IN VIVO" RADIATION-ACTIVATION OF CELLULAR ONCOGENES

Head of project and scientific staff : J. MERREGAERT
L. MICHIELS
M. JANOWSKI
J.R. MAISIN

In order to develop specific probes to detect the expression of cellular oncogenes in spontaneous and radiation-induced osteosarcomas, we studied the structural organisation of two bone tumor viruses : FBJ and FBR-murine virus complex. The two components of each osteosarcoma inducing virus complex have been isolated separately in tissue-cultures FBJ-, FBR-MuLV by endpoint dilution and FBJ-MuSV, FBR-MuSV by the establishment of nonproducer cell lines. The nonproducer cell lines of FBJ-MuSV in NRK and hamster cells as well as FBR-MuSV nonproductively transformed NIH/3T3 and REF cells do contain complete copies of FBJ and FBR-MuSV. The molecular cloning of a 13.5 kbp Hind III fragment containing the FBJ-MuSV provirus and a 5.2 kbp Hind III fragment containing the FBR-provirus is in progress.
In addition, the FBJ-MuLV helper virus was molecularly cloned as a 8.2 kbp Pst I fragment in PBR 322 plasmid DNA. The restriction map indicates that this virus is related to Akv, ecotropic retrovirus endogenous to the AKR mouse. These findings were confirmed by competition radioimmunoassays developped for FBJ-MuLV structural proteins. Further, we demonstrated that FBJ-MuLV is endogenous to the CF1 mouse and most likely involved in the generation of the naturally occuring transforming virus (FBJ-MuSV).

List of publications in 1982

1. L. Michiels and J. Merregaert :
   Molecular cloning of infectious Finkel-Biskis-Jinkins murine leukemia proviral DNA.
   - Arch. Int. Phys. Biochem., in press.
2. J. Merregaert, L. Michiels and M. Janowski :
   FBJ murine osteosarcoma virus complex : immunological and biochemical characterisation of FBJ-murine leukemia virus.
   - J. Gen. Virol., in preparation.

Short communications

1. Genomic characterisation of FBJ- and FBR-murine bone tumor viruses.
   J. Merregaert, L. Michiels and M. Janowski
   - EULEP Newsletter, 32 (1983) 12.
2. Characterisation of osteosarcoma cell lines.
   J.R. Maisin, J. Merregaert, L. de Saint-Georges and M. Janowski
   - EULEP Newsletter, 32 (1983) 14.

Progress Report
1982

Contractor:

United Kingdom Atomic Energy
Authority, UKAEA
Charles II Street 11
GB-London SW1Y 4QP

Contract no.: BIO-D-386-81-UK

Head(s) of research team(s):
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Env. and Med. Sciences Div.
AERE
Harwell, Didcot,
GB-Oxon OX11 ORA

General subject of the contract:
The effect of firing temperature on the solubility in lung of Pu-238 dioxide and Pu-239 dioxide; implications in biological monitoring.

List of projects:
1. The effect of firing temperature on the solubility in lung of Pu-238 dioxide and Pu-239 dioxide; implications in biological monitoring.

Title of project nr 1: The effect of firing temperature on the solubility in lung of Pu.238 dioxide and Pu.239 dioxide; implications in biological monitoring.

Head of project and scientific staff: A. Morgan, A. Black, S.R. Moores, J.N. Pritchard, J. Wilkins

It has been suggested that the solubility of plutonium oxide in vivo may depend upon the temperature at which it was fired during its preparation. If this is the case then urine analysis, which has barely adequate sensitivity for the routine monitoring of workers exposed to conventional materials, may be invalid for the surveillance of workers exposed to high-fired oxides. The purpose of the present investigation is to study the effect of firing temperature on the solubility in vivo of a) $^{238}PuO_2$ b) $^{239}PuO_2$ and c) mixed U/Pu oxide.

To this end samples of these materials, fired at various temperatures, have been prepared by water sedimentation to give particles with similar AMADs (~1.5 μm). They have been administered to CBA/H mice by inhalation to give an initial alveolar deposition (IAD) of 100-250 Bq. This value was chosen because it permits the relatively small amounts of plutonium translocated to organs such as lymph nodes, liver and bone to be determined with adequate sensitivity. At the same time, it avoids the pathological changes which occur in mice with IADs exceeding 800 Bq (Morgan et al., 1982). Details of the materials used in this study and the IADs achieved are given in the following table:-

Material	Firing temperature (°C)	Mean IAD (Bq)
$^{238}PuO_2$	550	231
"	750	222
"	1000	174
"	1250	178
$^{239}PuO_2$	550	226
"	750	124
"	1000	129
"	1250	183
U/Pu oxide	1400	156
"	1600	107

As explained in the progress report for 1981, an infection of the SAS 4 mouse colony set the programme back and necessitated a change to CBA/H mice which, being an inbred strain, have certain advantages. Out of 57 mice exposed to each material, 6 were killed at 1 d to establish the IAD and

groups of 5 at predetermined times thereafter. Samples taken for analysis include lungs, thoracic and cervical lymph nodes, liver, bone and the residual carcass. Before each group of mice are killed they are transferred to cages with suspended mesh floors and faeces collected over a predetermined period. Comparison with the plutonium content of the animals' lungs enables daily excretion to be expressed as a percentage of the contemporary lung content.

It is too early to attempt a detailed assessment of the results of this study. However, lung retention data for mice exposed to $^{239}PuO_2$ are available up to 6 months post exposure (see Fig. 1). The retentions at 3 and 6 m were about 20 and 8% respectively which are very similar to the results obtained with SAS 4 mice using $^{239}PuO_2$ fired at 550°C (Morgan et al., 1982). Firing temperatures did not appear to have affected retention over this period. However, the analysis of liver and bone will provide a more sensitive index of solubility in vivo.

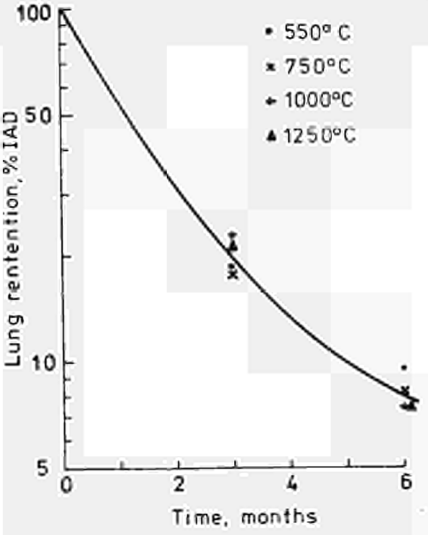

Fig. 1. Lung retention of ^{239}Pu following administration of $^{239}PuO_2$ fired at temperatures from 550 to 1250°C.

Publication

Morgan, A., Black, A., Belcher, D.R., Moores, S.R., Lambert, B.E., Hall, W.S. and Scott, D.A. Retention of ^{239}Pu in the mouse lung following inhalation of sized $^{239}PuO_2$. UKAEA Unclassified Report AERE-R 10718 (1982).

Progress Report
1982

Contractor:

United Kingdom Atomic Energy
Authority, UKAEA
Charles II Street 11
GB-London SW1Y 4QP

Contract no.: BIO-D-448-81-UK

Head(s) of research team(s):

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General subject of the contract:

The induction by actinides of non-neoplastic changes in rodent lung.

List of projects:

1. The induction by actinides of non-neoplastic changes in rodent lung.

Title of project nr 1: The induction by actinides of non-neoplastic changes in rodent lung.
Head of project and scientific staff: A Morgan, S R Moores, A Black, S E Sykes, J N Pritchard, M Walsh and R J Talbot

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Studies on the alveolar macrophage

Results derived from the previous contract, involving inhalation exposure of mice, have been prepared for publication in 1983 (Moores et al., in press). The studies are being continued by using intratracheal instillation (IT) to administer actinides to rats, as an alternative in vivo model of damage to the alveolar macrophage (AM) population of the lung.

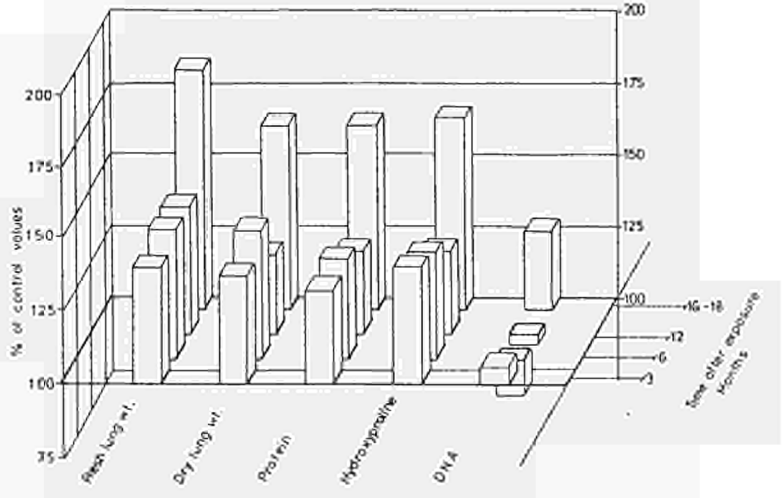
Using rats, it has been found necessary to increase the concentration of $^{239}PuO_2$ in the lung to over 2.5 kBq g^{-1} fresh weight in order to produce a reduction in the total number of AM. This effect developed within a week and was progressive for at least one month. It was accompanied by changes in the AM population, including increases in their mean size, in their lysosomal enzyme activity and in the occurrence of multinucleate cells.

Studies on the fibrogenicity of inhaled actinides

Following IADs of 6 or 42 Bq of $^{239}PuO_2$ in SAS/4 mice there were no significant biochemical changes in the lungs over the ensuing 18 months. At the highest IAD (925 Bq) biochemical changes were evident throughout the period as summarised in the figure (below). The biochemical changes could only be associated with structural disturbances during the second, more massive phase of damage between 12 and 18 months after exposure. The results from all the biochemical and histological investigations are currently being compiled for publication. The data from these experiments could form the norm against which the effects of other α-emitting materials might be compared. However, due to disease in the SAS/4 mouse colony, CBA/H mice have been used for the continuation contract. A dose/response study has been set up using one, sized fraction of $^{239}PuO_2$ particles (1.5 μm AMAD). Against this series, the effects of particle size are being assessed as well as the long-term toxicity of $^{241}Am(NO_3)_3$, which will produce a more diffuse irradiation of the lung.

The rate of clearance of ^{239}Pu from the lungs of CBA is similar to that of the previous SAS/4 strain, over the first 6 months. It was

independent of IAD over the first 3 weeks, but thereafter some dose-dependent inhibition was apparent. This was accompanied by an increase in fresh lung weight, which by 98 days was significant following IADs in excess of 350 Bq.



Changes to mouse lung following an I.A.D. of 925 Bq $^{239}PuO_2$ (% of control)

Metabolism of ^{239}Pu

Reports on a) the deposition of sized $^{239}PuO_2$ particles in the SAS/4 strain of mice and of b) the effect of clearance on their long-retention in the lung have been prepared for publication (Morgan et al., 1982; Morgan et al., in press). Some low-level analyses are currently being undertaken to determine the extent of ^{239}Pu translocation to the skeleton. When these results become available a report on translocation of ^{239}Pu to extra-pulmonary tissues will also be prepared.

Publications

Moores, A., Evans, N., Talbot, R.J., Sykes, S.E., Black, A. and Coggle, J.E. (in press). The responses of pulmonary alveolar macrophages to inhaled plutonium dioxide particles. UKAEA Unclassified Report AERE-R 10635.

Morgan, A., Black, A., Moores, S.R., Pritchard, J.N., Walsh, M. and Lambert, B.E. (1983). Alveolar deposition of sized particles of $^{239}PuO_2$ in the mouse. Radiat. Res., 93, 85-92.

Morgan, A., Black, A., Belcher, D.R., Moores, S.R., Lambert, B.E., Hall, W.S. and Scott, D.A. (1982). Retention of ^{239}Pu in the mouse lung following inhalation of sized $^{239}PuO_2$. UKAEA Unclassified Report AERE-R 10718.

Progress Report
1982

Contractor:

Contract no.: BIO-D-380-81-UK

United Kingdom Atomic Energy
Authority, UKAEA
Atomic Energy Establishment Winfrith
Dorchester
GB-Dorset DT2 8DH

Head(s) of research team(s):

Dr. G. H. Palmer
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Dr. D. Ramsden
Radiological and Safety Div.
Atomic Energy Est. Winfrith
Dorchester
GB-Dorset DT2 8DH

General subject of the contract:

Plutonium exposures in man : direct monitoring of the lung, re-assessment of the ICRP lung model, and solubility studies.

List of projects:

1. Distribution of plutonium activity within the lung.
2. Lung models.
3. Solubility studies on industrial plutonium dusts.

Title Nr I : THE DISTRIBUTION OF PLUTONIUM ACTIVITY WITHIN THE LUNG

Head of Project and Scientific Staff: D Ramsden, P P Foster, K Kingman

This project, by means of detector arrays surrounding the thorax aims at identifying the probable locations of plutonium contaminants within the lung and hence allocating the appropriate calibration factors. Present systems for determining plutonium in lung either assume a homogeneous distribution within the lung (phantom calibrations) or take an unknown distribution (human volunteer inhalation techniques). This project also aims at studying the changes of distribution within the lung as a function of time following small accidental intakes. It is hoped that the techniques can be applied at or around the present limits of detection for plutonium in the lung and considerable effort is being allocated to the interpretation of data of poor statistical quality.

The 1982 programme consisted of:

1 Recalibration measurements were carried out using the recently delivered realistic chest phantom. The results for two of the detectors in the array were used to investigate the effects on calibration of varying the chest wall thickness. Several alternative calibration algorithms were derived to calculate the plutonium contents for each of the subjects' lungs. This approach has been shown to give a marginally better estimation of total lung contents and can give a good indication of the distribution between the two lungs (Publication 1).

2 The derived calibrations were applied to a set of measurements on a group of plutonium workers with known chest wall thicknesses. There was a general improvement in the agreement of results from the two detectors viewing the front left and front right chest when measured partial chest wall thicknesses were used. In some subjects with small but detectable levels of plutonium in their lungs this improvement was not observed. In these cases a non uniform distribution of

activity between the two lungs was suspected. The statistics of measurement are poor and these effects are being investigated further.

3 The existing data bank of subjects measured (see 1981 progress report) has been extended throughout 1982 and been used in two ways.

(a) An investigation into the use of background counting for plutonium in lung assessment:- This work concludes that the application of a range of background measurements to groups of normal 'clean' subjects does not reduce the error on an estimation of a 'zero result', indeed the overall error on any particular measurement is often made worse by employing background subtraction in some circumstances.

(b) Predicting the response of low energy X-ray detectors from 'clean' subjects:- This work has been concluded during 1982 and is reported in Publication 2(ii).

4 Attempts at developing a flexible computer model of the human chest have continued. A development code now exists which enables 'standard man' chest data to be scaled to fit the external dimensions of any individual subject. In 1983 this code will be further refined and will incorporate subject dependent data (arising from ultrasonic scans and X-rays).

5 Initial experiments have been undertaken in the construction of lungs with a non-homogeneous distribution of activity for use in the realistic chest phantom. This work will continue during 1983 in order to proceed with studies of the variations of response with activity distribution along the length of the chest.

List of publications in 1982

I. AEEW M-1997 Calibrations of Plutoniun in Lung Detectors a Progress Report. P P Foster, K Kingman

II(i) RSD Tech Memo 1/83 Some Observations on the Effects of Variation of Chest Wall Thickness on the Ability to Estimate Plutonium in lung. P P Foster

(ii) RSD Tech Memo 2/83 Predicting the Responses of Low Energy X-ray Detectors for Subject Backgrounds. K Kingman

Title nr II : LUNG MODELS

Head of Project and Scientific Staff: D Ramsden

This project was not funded by CEC during 1982. It aimed at modifying the ICRP Lung Model so as to explain observed clearance patterns from accidental inhaled industrial plutonium oxide dusts and to extend the models so as to predict urine excretion rates and hence to compare such predictions with observed data.

The model and some results were presented at the Special Symposium on lung dosimetry. Radiation Research Society - Salt Lake City April 1982.

Complete publication of the methods and data has been postponed because some late observations on 'lymph-node' retained plutonium did not satisfactorily match this model and there were similar observations of a few cases of long term retained cobalt 60 oxide. Slight modifications to the model have been made but the resulting statistical tests on observed and predicted urinary excretion levels have not yet been done.

List of publications in 1982

I Modifications to the ICRP Lung Model for Inhaled Plutonium Oxide based on the Observed Lung Clearance and Urinary Excretion.
- 'Current Concepts in Lung Dosimetry' US Government Printing Office 1983

Title nr III : SOLUBILITY STUDIES ON INDUSTRIAL PLUTONIUM DUSTS

Head of Project and Scientific Staff: D Ramsden, P P Foster,
C C R Hunt, M E D Bains

This project aims at determining the long term transportability of industrial plutonium oxide dusts in the lung by means of 'in vitro' studies. It is hoped that the conclusions of the studies can be used in conjunction with Projects I and II to predict effective dose equivalents from accidental intakes based on early lung and urine measurements and on metabolic models.

Work in 1982 has seen the completion of the basic experimental facility, ie the glove box containing parallel mock lung exchange tanks as described in the 1981 progress report (Publication 1). The original design of each exchange tank system required the recirculation of plasma simulant, however a number of problems were encountered with this concept leading to the adoption of a 'once through' plasma system. There has been a delay in introducing industrial plutonium dust to these facilities.

These problems were:-

(1) Sampling and concentration control of transported material in a re-circulating system.

Experiments to date have illustrated that the net transport of material from mock lung volume to circulating plasma is dependent on the concentration of ions in that plasma. Two methods of preventing concentration build-up and back diffusion of transported ions were investigated and rejected.

(a) The use of ion exchange resins in circulating plasma was rejected because such resins affect the composition of the plasma. It also proved impossible to trap more than 90% of the plutonium ions present.

(b) Complexing 'transported' plutonium by the addition of transferrin to the circulating plasma was also rejected when using an 'in-line' ultra filtration system. This filter system, using high pressure gas, was controlled by electromagnetic valves and timing circuits. This control system proved too

unreliable for continual use over the time period envisaged (∿2 years).
Because of these problems the experimental procedure was changed from a re-circulating plasma, to a 'once through' plasma concept. This removed the problems of sampling and concentration, together with the plasma stability problem (see below) but introduced the need to handle bulk quantities of plasma.

(2) Circulating plasma stability.

Long term tests (∿2 months) have shown that the circulating plasma becomes cloudy and some precipitation occurs. This was attributed to dissociation of amino acids in the fluid (cysteine) and liberation of sulphur.

The problem was solved by

(a) Keeping a nitrogen atmosphere in the glove box.

(b) Adopting a once through flow system as proposed in (1) above.

(c) Introducing new plasma into the glove box via an ultra filtration assembly external to the box.

(d) The addition of a small amount of thymol to the plasma.

A series of experiments using ^{239}Pu, ^{59}Fe, (1-5 ^{14}C) citric acid and ^{14}C cysteine to investigate the stability of the plasma and membrane characteristics were completed (Publication 2).

(3) Determination of 'effective pore size' for the membrane. Problems were encountered with the chemical stability of radioactive tracer solutions used in experiments. Soluble ^{198}Au, colloidal ^{198}Au, and ^{111}In (oxine) tests were all repeated in the glove box apparatus. The results of these latest experiments agree well with tests using other materials described in (2) above - ie transport rate through the membrane decreases rapidly to zero as molecular weight beyond 650. This gives an indication that the effective pore size of the memberane is ∿1nM. The tracer tests have now proved the integrity of the actual membranes that will be used to study industrial plutonium dusts.

Samples of plutonium dust from the fuel fabrication facilities at AEEW has been obtained and preliminary particle sizing has been undertaken by the National Radiological Protection Board. Methods of re-suspending the dust in plasma fluid have been tested and a suitable technique devised. Experiments using this material will start at the end of January 1983.

List of publications in 1982

I (i) Solubility Studies on Industrial Plutonium Dusts - A Progress Report. AEEW - M2011. P P Foster, C C R Hunt

(ii) Experiments to Investigate the Behaviour of the Constituents of Serum Simulant with Respect to the Dialysis Membrane in a Static System. RSD Tech Memo 3/83. M E D Bains

Progress Report

1982

Contractor:

Polytechnic of Central London
Regent Street 309
GB-London W1R 8AL

Contract no.: BIO-D-381-81-UK

Head(s) of research team(s):

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New Cavendish Street 115
GB-London W1M 8JS

General subject of the contract:

Microdosimetry of lung.

List of projects:

1. Image analysis of alpha tracks through lung tissue.
2. Radiosensitivity of lung cells to alpha particles.

Title of project nr 1: IMAGE ANALYSIS OF ALPHA TRACKS THROUGH LUNG TISSUE

Head of project and scientific staff: DR J A SIMMONS
MR S R RICHARDS

Using samples of alveolar lung from rat, beagle and human prepared as previously described (Radiation Protection Report 1981) image analysis measurements have been carried out to improve our knowledge both of the morphology of the material and of the distributions of the energy depositions from the alpha particles. From these basic measurements various distributions of interest have been calculated.

The first distribution is the fraction of total area traversed that is tissue. This is obtained by examining each track in turn and calculating the thickness of tissue in each micrometre increment of distance. For each distance increment the thickness from all of the two hundred tracks generated were added together and divided by two hundred to give the fraction of tissue. Four seperate sites in rat and human lung were investigated.

Histograms showing this fraction as a function of distance from an imaginary plutonium dioxide particle embedded in the lung were drawn for all the sites. Although there were naturally variations from one site to another, all showed a common feature of an initial fraction of unity for the first few micrometres followed by a rapid drop to the mean fraction for the whole site. For the rat lung this mean value turned out to be about 0.15 for all four sites, and for the human lung about 0.10.

The second distribution is that of the fraction of alphas in tissue as a function of distance. This was

calculated in a similar manner to the tissue fraction, but the length of the track was only counted if the alpha - particle was still continuing along the track. The initial parts of the two histograms are the same since up to a distance of 37μm no alpha-particle will have completed its track. From this point onwards the fraction of alpha-particles in tissue becomes progressively lower than the tissue fraction as an increasing number complete their tracks.

Another distribution is the fraction of continuing alpha-particles. This was found by recording the distance at which each one terminates and decreasing the remaining fraction by $\frac{1}{200}$. Half the tracks continued out to between 300μm and 400μm, and the final tracks terminated at between 800μm and 1200μm from the plutonium dioxide particle.

Finally, using the methods described previously (Radiation Protection Report 1980) preliminary histograms of the volume of tissue receiving a particular specific energy have been calculated. This has been done for particle activities of .003, .03 and .3 pCi and on the assumption that the period of irradiation is 100 days. Peaks of the distribution occur at about 1, 10 and 100 rad respectively, with a spread of about a factor of 10 about this peak. The corresponding volumes lie within the range $3 - 7 \times 10^6 \mu m^3$.

The results of the morphometric analysis of lung cells and their nuclei are now being used to calculate the distributions of specific energy deposited in these cells and nuclei. These measurements are still at a preliminary stage and the results are not yet considered sufficiently reliable for publication.

The mathematical model of the alveolar lung referred to in last year's report has been developed further. This is mainly to improve its approximation to real lung by increasing the randomness of its construction. The earlier version was constructed from identical polyhedra, each polyhedron representing an alveolus. Now each polyhedron has a different shape, giving a better approximation to the observed variations in alveolar shape and size.

Title of project nr 2: RADIOSENSITIVITY OF LUNG CELLS TO ALPHA PARTICLES

Head of project and scientific staff: DR J A SIMMONS
DR P COHN
MR T MIN

Alpha-particles from curium-242 have been used to irradiate Chinese hamster V-79 cells. These were grown on Mylar base Petri dishes as a monolayer to allow track-segment techniques to be used. The work previously carried out to establish a protocol for the routine culture and maintenance of these cells, together with regular karyotyping, was described in last year's report.

The alpha-source (details of whose construction were also given in last year's report) was calibrated using a nuclear track emulsion placed at the position of the cell monolayer. This gave a measurement of the fluence rate. The dose rate to the cells was then calculated from the fluence rate, the energy deposited per particle and the mass of the cell monolayer. The half-life of the source was taken to be 162.5 days, and the initial energy of the alpha-particles 6.1MeV. From a knowledge of the stopping power values of air and water the energy of the alpha-particles incident on the cells was determined as 5.0MeV.

Results of measurements of survival following irradiation indicate an exponential curve with a D_o value of about 75 rad. This corresponds to a fluence of 5.3×10^6 alpha-particles per cm^2. From the determinations of the nuclear area of the V-79 cells on Mylar (average value about $95\mu m^2$) it was calculated that the passage of about five particles is required in order to result in inactivation. These results are consistent with those found by other workers.

Cells irradiated over a similar range of doses (up to 150 rad) were also examined for chromosome aberrations. About

1300 cells have been studied so far, and a linear increase in the number of fragments and dicentrics has been found with increasing dose.

The preliminary studies with V-79 cells described above demonstrate that the arrangement is capable of giving reproducible results consistent with the findings of other workers. This leads us to believe that there are no serious unsuspected sources of error and the work is now being extended to human lung cells.

Progress Report
1982

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB-Chilton, Oxon OX11 ORQ

Contract no.: BIO-D-388-81-UK

Head(s) of research team(s):

Dr. H. Smith
Biology Department
NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

General subject of the contract:

The transport in and clearance from the body of radionuclides.

List of projects:

1. Studies of the uptake of radionuclides from the gut of rodents and rabbits.
2. Studies of mechanisms influencing the transport of actinides within the body.
3. Optimisation of treatment regimes for removing radionuclides from the body.

Title of project nr 1

Studies of the uptake of radionuclides from the gut of rodents and rabbits

Head of project and scientific staff :

Dr. D. S. Popplewell

Measurements have been made of the distribution and excretion of plutonium and americium in the rat after the intravenous injection of the citrate complexes or oral administration of the nitrates. For each actinide, the tissue distribution after absorption from the gut was the same as after direct injection into the bloodstream. Approximately 20% of the plutonium entering the circulation was retained in the liver after 3 days and urinary excretion over this period was about 5%.

Livers containing plutonium-239 and americium-241 in a "biologically incorporated form" were fed to rats. The fractional uptake of plutonium and americium was shown to be 0.03% and 0.02% respectively. These values are similar to those obtained for the fractional absorption of the citrate complexes but are consistently higher than those obtained for the hamster by a factor of about 3.

The gut uptake of neptunium-239 has been measured in rats and hamsters. Using this isotope of neptunium ($T_{\frac{1}{2}}$ = 2.36 days), the concentration administered was less than 10^{-5} g l^{-1}. The ICRP 30 value of 1% absorption for neptunium (Ann. ICRP, 4, 3/4 (1980)) was based on measurements of uptake for neptunium-237 nitrate in the rat in which the concentrations were over 10 g l^{-1}. The values obtained for neptunium-239 administered as the nitrate were 0.03% uptake in the rat and 0.02% in the hamster (Table). Administration in bicarbonate solution increased absorption in the rat but not in the hamster. The enhanced uptake of neptunium in newborn hamsters was similar to that observed previously for plutonium and americium.

Table The gut uptake of neptunium-239

Chemical form	% absorbed by Adult rat	Adult hamster	2 d old hamster	4 d old hamster
Nitrate	0.03 ± 0.01*	0.02 ± 0.003	2.5 ± 0.3	1.7 ± 0.3
Bicarbonate	0.15 ± 0.02	0.02 ± 0.002	5.5 ± 1.7	2.1 ± 0.4

*$\bar{X}$ ± SE (n = 6)

Publications

HARRISON, J. D. and STATHER, J. W.
The tissue distribution and excretion of actinides absorbed from the gastrointestinal tract of rodents.
Health Phys. 43, 283 (1982).

HARRISON, J. D.
Gut uptake factors for plutonium, americium and curium.
NRPB Report R 129, HMSO, London (1982).

COOPER, J. R. and HARRISON, J. D.
Phytate may influence the absorption of plutonium from food materials.
Health Phys. (in press).

Title of project nr 2

Studies of mechanisms influencing the transport of actinides within the body

Head of project and scientific staff :

Dr. G. N. Stradling

Studies are continuing on the mechanisms involved in the translocation of actinides after their deposition in the lungs of rodents.

After exposing hamsters to an aerosol of plutonium nitrate, more than 90% of the plutonium deposited in the alveoli was removed by repeated weekly treatment with Zn DTPA. Such a high efficiency of removal suggests that the chelating agent was associating with a monomeric form of plutonium. Even the plutonium inside macrophages was amenable to removal by DTPA. In vitro studies showed that the plutonium present in macrophages is mobilised by Zn DTPA after a delay of a few hours.

In contrast the plutonium, absorbed as the nitate compound onto corroded material occurring typically in an industrial glove box in which actinides are handled, was much less mobile. DTPA treatment of animals exposed to this type of dust was ineffective. The reasons for this observation are being investigated further.

Previous workers have shown that uranium associated with tetrafluoride is poorly translocated from the lungs. Experiments in this laboratory have not confirmed this observation. Two forms of uranium tetrafluoride prepared by different industrial processes were administered to rats. The uranium readily translocated to blood and its tissue distribution and urinary excretion were consistent with that of U (VI). If this occurs similarly in man, then estimation of body content based upon urine analysis and the current ICRP model for Class Y material, will be widely in error.

Studies have begun to investigate the kinetics of uptake and removal of radioactive particles from cultured macrophages. The techniques for optimising their culture and their examination by scanning electron microscopy have been established. The first materials to be studied will be those that are moderately transportable in the body (e.g. $^{241}AmO_2$ UF_4).

These studies have demonstrated that lung deposits of transportable forms of both americium and plutonium can be effectively treated with either aerosol or systemic administration of the zinc salt of DTPA. The main difficulty lies in giving treatment before much of the transportable component has moved to the blood as this fraction is much less effectively removed from the body. The effectiveness of prompt treatment will be examined in forthcoming studies.

Publications

STATHER, J. W., STRADLING, G. N., SMITH, H. et al.
Decorporation of Pu-238 oxide from the hamster by inhalation of chelating agents.
Health Phys. 42, 520 (1982).

STRADLING, G. N., STATHER, J. W. and SMITH, H.
Decorporation of actinides deposited in the lungs with DTPA - a rational approach to optimisation.
EULEP Newsletter 30 Sept 1982, pp 22-24.

SMITH, H., STATHER, J. W. and STRADLING, G. N.
Toxicological data on Puchel.
EULEP Newsletter 30 Sept 1982, pp 25-27.

STRADLING, G. N., STATHER, J. W., HAM, S. E., SUMNER, S. A. and COOPER, J. R.
The efficiency of Zn-DTPA for the decorporation of Pu-238 TBP injected into the lungs of hamsters.
(Accepted by Health Phys.).

SMITH, H., STATHER, J. W., STRADLING, G. N., TAYLOR, D. M. and VOLF, V.
Recommendations for testing new chelating agents for removal of incorporated radionuclides from the body.
J. Rad. Environ. Biophys. 21, 45 (1982).

Title of project nr 3

Optimisation of treatment regimes for removing radionuclides from the body

Head of project and scientific staff :

Dr. J. W. Stather

Zn DTPA was administered to hamsters on a weekly basis either by inhalation (2 μmole kg^{-1}) or by intraperitoneal injection (30 μmole kg^{-1}) between 4 and 74 days after the animals had been exposed to an aerosol of plutonium-238 nitrate. The average lung content in each treated group was reduced to 0.3% of the initial lung deposit, compared to 35% in the control group, despite the fact that the dose of Zn DTPA administered in aerosol form was 15 times less than that injected. Tissue deposits other than lung in the treated groups were reduced to 56% of the control group. Larger amounts of injected Zn DTPA (200 μmole kg^{-1}) did not reduce the extrapulmonary tissue deposit further.

When larger masses of plutonium were administered as plutonium-239 nitrate in aerosol form (i.e. ≃ 2000 ALIs in man), much less plutonium was removed by weekly Zn DTPA treatment. For example, the lung content was reduced to 4.7% compared to 48% in the control group. This study demonstrated the importance of assessing the effectiveness of methods of treatment at exposure levels in animals comparable to those likely to be encountered in man.

A further series of studies have compared the effect of Zn DTPA given in aerosol form (2 μmole kg^{-1}) or by intraperitoneal injection (200 μmole kg^{-1}) in animals previously exposed to americium nitrate or oxide. After weekly treatment with aerosolised Zn DTPA over the period 4-74 days after exposure, the average lung content of americium in the americium nitrate exposed group was reduced to about 2% of the control group. The reduction in the americium oxide exposed group was about 15% of the control group. Intraperitoneal injections of Zn DTPA were more effective than aerosolised Zn DTPA in accelerating the removal of americium from tissues other than the lungs.

Progress Report
1982

Contractor:

National Radiological
Protection Board,
Chilton, Didcot,
Oxfordshire,
GB-OX11 ORQ

Contract no.: BIO-D-489-UK

Head(s) of research team(s):

Dr. H. Smith
NRPB - Biology Dept.
Chilton, Didcot
Oxfordshire,
GB-OX11 ORQ

General subject of the contract:

The deposition and clearance from the lungs and nasal passages of inhaled radioactivity.

List of projects:

1. Long term alveolar clearance of relatively insoluble particles.
2. Deposition and retention of particles and radionuclides in mucosal tissue in the respiratory tract.

Title of project nr 1

Long term alveolar clearance of relatively insoluble particles

Head of project and scientific staff :

M. R. Bailey

Measurements of lung retention of 1 μm and 4 μm diameter fused aluminosilicate particles (FAP) have been completed in a second group of healthy non-smoking volunteers, bringing the number now studied up to 12. Retention of the initial alveolar deposit after 350 days was 46 ± 11% ($\bar{x}$ ± SD) for the 1 μm particles and 54 ± 11% for the 4 μm particles. The wide inter-subject variation found shows the importance of using, whenever possible, data appropriate to an individual for dose assessment following accidental intakes. It also shows the limitations of interpreting observations on small groups. In each subject retention of the two particle sizes was correlated, as was retention over different periods. Lung dissolution rates were estimated to be 8×10^{-4} d^{-1} and 3×10^{-4} d^{-1} for the 1 μm and 4 μm particles respectively. It was calculated that in the absence of dissolution, with clearance resulting only from particle (mechanical) transport to the GI tract, mean retention would have followed a two-component exponential function (half-times about 30 days and 700 days) with 10% of 1 μm particles and 23% of 4 μm particles associated with the faster phase.

Alveolar clearance of FAP in man was similar to that reported by other workers for dogs, but was much slower than we have observed in rodents. The dissolution rate of FAP in the lungs, however, showed no great difference between man, dog or rat. Alveolar clearance in rats was found to be very similar for relatively insoluble materials of very different physical and chemical forms (including FAP, and PuO_2 at levels insufficient to cause lung fibrosis). It is therefore proposed that assessment of doses to man from inhalation of a material for which adequate human data are not available, may be based on its lung dissolution rate measured in animals, and on the mechanical clearance of particles from the human lung as measured in this experiment.

Proposals have been prepared for a complementary study of current cigarette smokers, for consideration by the NRPB Ethics Committee. They

include a review of the literature relating to the effects of smoking on lung clearance. Significant impairment of alveolar clearance has been reported, but the magnitudes of the effects on translocation of material from lungs to blood, and on the amount of radioactivity carried in macrophages to the GI tract are not known.

The spatial distribution of inhaled particles in rodent lungs is being studied to account for differences in clearance kinetics observed in different species. A technique is being developed for fixing rodent lungs in a manner which preserves the morphology without displacing the particles. A system for measuring the positions of particles and morphological features in thin sections of lung and quantifying the particle distribution, has been developed using a semi-automatic image analyser linked to a microcomputer. The use of FAP labelled with a suitable electron-capture nuclide such as ^{85}Sr or ^{57}Co enables the particles to be located precisely by Auger or internal conversion electron autoradiography. The procedure also allows a useful number of particles per section to be observed when the lung content in terms of both mass of particles and radiation dose is low enough not to interfere with normal clearance processes.

A study of the effect of alpha-dose on alveolar clearance has begun. Groups of 30 rats inhaled PuO_2/AmO_2 (Am/Pu ratio by alpha activity = 0.26) to give initial lung deposits averaging 8, 1.2 or 0.1 kBq plutonium. After 24 days the high and medium level group animals inhaled ^{57}Co-FAP. Retention of the oxide and the FAP are followed in individual animals by external counting of ^{241}Am and ^{57}Co. Groups of animals were killed at 0, 6 and 30 days to determine the tissue distribution of activity. Preliminary analysis of results to 3 months post-inhalation indicates similar lung retention for the oxide in the low and medium level groups, for FAP in the medium level group, and for FAP in rats not exposed to the oxide. The high level group showed impaired clearance of both materials, but the effect was greater on the oxide than on the FAP.

Title of project nr 2

Deposition and retention of particles in mucosal tissue in the respiratory tract

Head of project and scientific staff :

M. R. Bailey

Proposals for studies of the effects of particle size and breathing pattern on human nasal deposition and clearance, and on the effectiveness of nose-blow sampling for assessing intakes of radionuclides by inhalation, have been prepared for consideration by the NRPB Ethics Committee. A review of the literature relating to nasal deposition and clearance has been carried out, from which it was concluded that:-

In moving air, particles of aerodynamic diameter (AD) up to at least 100 μm may enter the nose. In still air there is likely to be a cut-off at about 35 μm.

Total nasal deposition of particles in the 0.5-9 μm AD range has been studied extensively but there are large variations between subjects and the results of different studies. Inhaled particles >9 μm AD would not be expected to penetrate the nose.

There have been few studies of the clearance of inhaled particles deposited in the nose. These data and observations of nasal mucus flow patterns suggest that particles deposited in the posterior nasal passages are cleared to the GI tract by mucociliary action within a few minutes; some of the anteriorly deposited particles are slowly transported by mucus to the posterior passages, while the rest is more likely to be cleared by nose-blowing. It is the anteriorly deposited particles which are available for sampling.

The ICRP does not treat the nasopharynx separately as a target organ, and there is no standard procedure for assessing the radiation dose the nasal epithelium receives. In the proposed experiments the nasal epithelium will generally be the most highly irradiated tissue. Therefore a dosimetric model has been developed to assess doses to the basal cells of the nasal epithelium. This involved writing computer programs to calculate depth-dose curves in tissue from plane sources of

monoenergetic electrons and photons. Radionuclides were chosen to label the test particles on the basis of minimising the effective dose-equivalent and the dose-equivalent to the nasal epithelium, consistent with the experimental objectives.

In another study a dosimetric model has been developed for calculating doses to the stem cells of the human bronchial epithelium following inhalation of relatively insoluble alpha-emitters, and for estimating risks of radiation-induced lung cancer for miners. For uranium ore dust the mean dose to the bronchial stem cells was calculated to be 0.5 μGy per Bq of inhaled uranium. In the model, up to half the dose is assumed to arise from particles deposited on the bronchial epithelium and retained in it, the rest from particles in transit which have cleared from the alveoli. Using the regional lung dose approach adopted by ICRP to derive limits for intakes of radon daughters by workers, the corresponding lifetime risk for bronchial cancer was estimated to be 10^{-8} Bq^{-1} inhaled uranium compared to 2×10^{-7} Bq^{-1} for the pulmonary region (5×10^{-7} Bq^{-1} if the dose to the tracheobronchial lymph nodes is included). It was estimated that for miners extracting high grade uranium ore, the risk of cancer from pulmonary irradiation approximates to that of bronchial cancer from inhalation of radon daughters, but in non-uranium mines the risk from long term alpha emitters is negligible compared to that from radon daughters.

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

BAILEY, M. R., FRY, F. A. and JAMES, A. C.
The long-term clearance kinetics of insoluble particles from the human lung.
In: Inhaled Particles V (W. H. Walton Ed.), Ann. Occ. Hyg. 26, 273-290.

BAILEY, M. R. and FRY, F. A.
Pulmonary retention of insoluble particles in man.
In: Current concepts in lung dosimetry (D. R. Fisher Ed.), Proceedings of the Radiation Research Society's Special Workshop on Lung Dosimetry, Salt Lake City April 18-22 1982, US Government Printing Office, Washington DC (in press).
Abstract Radiat. Res. 91, 385 (1982).

HODGSON, A., BAILEY, M. R., BIRCHALL, A. and MURFITT, A. J.
Spatial distribution of inhaled particles in rodent lungs, ibid p. 382.

JOHNSON, J. R.,* JAMES, A. C. and BIRCHALL, A.
An estimation of the bronchial cancer risk from the inhalation of long-lived alpha emitting radionuclides, ibid p. 407 *(AECL, Chalk River, Canada).

JAMES, A. C.
Relative hazard of radon daughter exposure in mines and homes; the dosimetric approach.
In: Radiological Protection - Advances in theory and practice. Proceedings of the Third International Symposium of the Society for Radiological Protection, Inverness June 6-11 1982, pp 411-417.

GREENHALGH, J. R., BIRCHALL, A., JAMES, A. C., SMITH, H. and HODGSON, A.
Differential retention of ^{212}Pb ions and insoluble particles in nasal mucosa of the rat. Phys. Med. Biol. 27, 837-851.

II. Short Communications, Theses, Internal Reports, Patents ...

BAILEY, M. R., FRY, F. A. and JAMES, A. C.
Pulmonary retention of inhaled particles in man.
International Environment and Safety, Feb. 1982, 18-21.

Progress Report
1982

Contractor:

Kernforschungszentrum
Postfach 3640
D-7500 Karlsruhe 1

Contract no.: BIO-D-367-81-D

Head(s) of research team(s):

Prof. Dr. D. M. Taylor
Inst. für Genetik
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General subject of the contract:

Chelation therapy of actinides : biochemical basis and improvement of treatment procedures.

List of projects:

1. Chelation therapy of actinides : biochemical basis and improvement of treatment procedures.

Chelation Therapy of Actinides: Biochemical Basis and Improvement of Treatment Procedures BIO-D-367-81-D

Head of Project: Prof. Dr. D.M. Taylor
Prof. Dr. V. Volf
Dr. F. Planas-Bohne
Prof. Dr. A. Seidel
Dr. J. Duffield
Dr. M. Lehmann (to 31.8.82)

The work carried out during 1982 has involved an in depth study into the metal binding properties of human serum transferrin. In vitro, transferrin is the iron transport protein in plasma. It has also been implicated in the transport of a number of other metal ions both endogenous and exogenous including plutonium, thorium, gallium and hafnium. It is thus of great importance to have a good understanding of the binding and release mechanisms which govern the transport of metal ions by transferrin particularly with respect to iron.

Transferrin is known to possess 2 independent, and inequivalent metal bindin sites, one at the C-terminal and one of the N-terminal, end of the protein. Thus in any given metal-transferrin solution four different species may exist at any one time. Namely, apo-transferrin (TF) the metal free protein; transferrin with one metal bound at the N-terminal site (M_NTF) and one at the C-terminal site (TFM_C), and the fully saturated protein (M_2TF). These species differ in their electrophoretic mobility and so can be separated using iso-electric-focussing. This has been carried out for iron and hafnium. With iron, under the conditions used (Tris-HCl buffer pH 7.4 and an ionic strength of 150 mmol dm^{-3} NaCl) the TFM_C species forms preferentially up to a percentage saturation of approximately 50% after which the M_2TH species begins to predominate. By using solutions containing different amounts of iron, it has proved possible to build up an iron transferrin species distribution profile so that a quantitative model can be calculated. Similar experiments have been performed using hafnium (N), a plutonium analog, instead of iron. Unfortunately because of its complex chemistry in aqueous solution, hafnium has proved to be a rather intractable. It does appear to behave similarly to iron in that each protein molecule will bind 2 metal ions (proven by difference ultraviolet spectroscopy) and that it shows similar site preferences.

The strength of binding of iron and hafnium to transferrin has also been investigated. The method used was an equilibrium gel filtration technique. This method allowed a determination of the percentage saturation of transferrin to be made under known concentration conditions, following which the species distribution could be determined using the profile constructed by iso-electric-focusing and the metal transferrin formation constants calculated.

This yielded the constants given in Table I.

Table I: Metal-Transferrin formation Constants.

	log ß		
Species	Fe^{3+}	Hf^{4+*}	
$M_N TF$	5.79 (21.29)	$Hf_N TF$	5.72
$TF_C M$	7.68 (23.18)	TF Hf	7.07
M_2 TF	14.19 (45.19)	$Hf_2 TF$	12.98

* Assumes a similar species distribution to that calculated for transferrin.

It should be noted, that these are given in terms of the complexing agent used to prevent hydrolysis of the metal ion, the values in parentheses for ion take this into account. For hafnium the literature constants for interaction with the complexing agent used are uncertain so no correction is made. However, it can be seen that the affinity of transferrin for iron is less than that for iron.

In vivo studies of the effectiveness of various treatment regimes for the removal of plutonium and americium have continued with special reference to oral therapy using Na_3 Zn DTPA. This agent shows a remarkable effectiveness in rats with doses of about 30 µMoles/kg for both prompt and late, 28 days post plutonium intake, treatment.

Publications -

H. Smith, J.Stather, G.N.Stradling, D.M.Taylor and V.Volf - Radiat.Environ.Biophys. 21, 45-50 1982.

E. Peter, V.Volf, F.Planas-Bohne, D.M.Taylor, EULEP Newsletter, 30, 39-49, 1982.

J. Duffield - "Metal Binding Properties of Transferrin" Poster Presentation at Symposium on Metal Toxicity and Chelation Therapy, London Dec. 1982.

F. Planas-Bohne, J.R. Duffield, D.M. Taylor, G. Darai - "The Role of Transferrin in the uptake metals by human lymphoblasts (Wil-2) in vitro" ibid

J. Duffield, F. Planas-Bohne, D.M. Taylor, Inorg. Chem. Acta in the press.

Progress Report
1982

Contractor:

Studiecentrum voor Kernenergie, SCK/CEN
Plaskylaan 144
B-1040 Brussel

Contract no.: BIO-D-377-81-B

Head(s) of research team(s):

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General subject of the contract:

Decorporation of heavy alkaline - earth metals and transuranics.

List of projects:

1. Decorporation of heavy alkaline - earth metals and transuranics.

Title of project nr 1 : DECORPORATION OF HEAVY ALKALINE - EARTH METALS AND TRANSURANICS

Head of project and scientific staff : O.VANDERBORGHT, G.SCHOETERS

Decorporation experiments in mice with ^{226}Ra and ^{241}Am showed that a decrease in radionuclide content in the skeleton did not result necessarily in a reduction of radiation damage. In these experiments weekly Zn-DTPA injections were used to eliminate ^{241}Am from the body, daily oral alginate administration was used to reduce the ^{226}Ra burden. The decorporative treatments started a few days after i.p. injection of the radionuclides. The lack of accordance between radionuclide removal, resulting in a reduced radionuclide content in bone, and changes in radiotoxicity in bone marrow has been further investigated in 1982.

Different marrow cavities in mice were compared with respect to the observed radiation damage to blood-forming stem cells between 4 hours and 300 days after i.p. injection with ^{226}Ra and ^{241}Am. Changes in total number of stem cells and in the concentration of stem cells per 10^5 bone marrow cells was investigated separately for multipotential stem cells, via the CFU-s technique, and for granulocyte-macrophage stem cells via the CFU-c assay. Differences in radiation response were examined in relation to : 1. the injected radioactivities, 2. morphometric characteristics of the surrounding bone such as the amount of inner and outer bone surfaces and the volume of bone and bone marrow, 3. the radionuclide content of the surrounding bone, 4. the calculated radiation dose to bone marrow.

The results show that the study of haemopoietic stem cells in various bone marrow sites can be used as a biological indicator of α-radiation damage to radiosensitive cells within bone marrow. Compared to other parameters such as the number of bone marrow cells or the number of peripheral blood cells, the blood-forming stem cells in bone marrow are more radiosensitive to radiation damage of the α-emitting bone-seekers ^{226}Ra and ^{241}Am.

The importance of anatomical and physiological factors on radiotoxicity of α-emitting bone-seekers is demonstrated. Radiation damage to blood-forming stem cells is examined in marrow of lumbar vertebrae, of sternum, of the distal end of the femur and in the femoral diaphysis. Marrow cavities with a large surrounding bone surface versus their bone marrow volume, show the highest radiation damage of CFU-s and CFU-c. Spatial differences in the fraction of mitotic CFU-s seem important for the rate at which radiation damage occurs after radionuclide injection.

Haemopoietic stem cell studies yield also information about α-radiotoxicity to bone marrow on various moments after contamination and about the reaction of radiosensitive cells on a continuous α-bombardment. After injection of the lower radioactivities (230 kBq ^{226}Ra/kg mouse and 185 kBq ^{241}Am/kg mouse), the induced changes are often increases in stem cell concentrations above the control level in non-radiocontaminated animals. After the higher radioactivities, initial decreases in stem cell concentrations are often followed by a re-establishment to the control level.

The lack of an obvious relationship between the calculated radiation doses in bone marrow and the observed damage at haemopoietic stem cells indicates that calculated radiation doses need to be further tested on their adequacy to predict radiotoxicity in a given bone marrow site. For example, blood-forming stem cells in large marrow cavities show after ^{226}Ra injection more radiation damage than expected from the calculated radiation doses. After ^{241}Am contamination marrow of the lumbar vertebrae is damaged to a larger extent than marrow of the distal end of the femur, but the calculated radiation doses are very similar in both locations.

The study demonstrates that dosimetric studies are not able to explain completely the observed radiation damage and to predict radiotoxicity from α-emitting bone-seekers. Further studies are thus needed to elucidate the relationship between the physical aspects of radiation and its biological effects.

Publications

G.E.R. Schoeters :
Dose delivered to various bone and marrow sites of ^{226}Ra contaminated mice related to the heterogeneity in ^{226}Ra radiation harm at haemopoietic marrow cells.
- Brit. J. Radiol. 55 : 520-529 (1982).

G.E.R. Schoeters, O.L.J. Vanderborght :
Relative effectiveness of ^{241}Am versus ^{226}Ra approached by haemopoietic stem cell studies in various bone marrow sites of contaminated mice.
- Health Physics, accepted 31/3/82.

G.E.R. Schoeters :
Evaluation of long-term treatment with alginate of ^{226}Ra contaminated mice and Zn-DTPA treatment of ^{241}Am contaminated mice.
- Eulep Newsletter 30 : 50-51 (1982).

G.E.R. Schoeters, A. Luz, O.L.J. Vanderborght :
^{226}Ra induced bone-cancers : the effects of a delayed Na-alginate treatment.
- Int. J. Radiat. Biol., accepted 2/12/82.

Short communications

19-20/11/82
G.E.R. Schoeters
Colloquium on the toxicity of radionuclides, Liège (Belgium).
Communication : Haemopoietic stem cell studies to assess the adequacy of Zn-DTPA treatment in protecting the health of ^{241}Am contaminated mice.

Progress Report
1982

Contractor:

REP-Institutes of the
Organisation for Health Research
TNO
P.O. Box 5815
NL-2280 HV Rijswijk

Contract no.: BIO-D-374-81-NL

Head(s) of research team(s):

Prof. Dr. L. M. van Putten
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NL-2280 HV Rijswijk

General subject of the contract:

Carcinogenicity of iodine-123, -125 and -131 in the mouse

List of projects:

1. Carcinogenicity of iodine-123, -125 and -131 in the mouse.

Title of the project:
CARCINOGENICITY OF IODINE-123, -125, AND -131 IN THE MOUSE.

Head of project and scientific staff:
Dr. L.M. van Putten, Dr. C. Zuur and Dr. C. Zurcher.

In the 1978-1980 we made a pilot study to induce thyroid tumours in mice. Groups of eight female mice were injected with various doses of either I-123, I-125 or I-131. We made the choice to take the BALB/c mouse for this project because of the availability of this strain, so there would be a possibility to reproduce the project.

However, after two years, when almost all the mice had died, we had found only one (parafollicular) thyroid tumour and some salivary gland tumours. Therefore, for the continuation of the project in 1981, we have used CBA mice since this strain was reported to have a high probability to develop thyroid tumours. We injected the mice intraperitoneally with the following doses:

I - 123 : 1500, 500, 150, 50 and 15 uCi
I - 125 : 500, 150, 50, 15 and 5 uCi
I - 131: 15, 5, 1.5, .5 and.15 uCi

We have chosen these doses on the basis of previous research in our laboratory. The intention is to give doses low enough that there is not too much killing of thyroid cells and, on the other hand, high enough that thyroid tumours will be induced. We have started early in 1981 to inject the mice and we continue until we have about 45 male and 45 female for each dose of each isotope and a group of 100 male and 100 female controls. Because of difficulties we have in breeding this special strain, we are planning to be ready half 1983. The groups are relatively big because the expected incidence of thyroid tumours in mice is low. Up to now we have let them live till they die a "natural" death or become moribund. We also try to investigate the influence of propyl-thiouracil (PTU) together with the administration of an isotope on the thyroid gland, since it is reported to be a stimulator for the induction of thyroid tumours. Therefore, we give during six months from the age of about seven months the half of each group a diet with 0.8% PTU in

normal food. We give this diet also to about 40 additional controls who will be sacrificed after three, six and nine months to see the influence of PTU without radiation. The retention of the iodine is determined by whole body counting during several days, weeks and months after injection, for I-123, I-131 and I-125, respectively. The late measurement is usually only possible for the long-lived isotope I-125.

Because of the difference in retention between the pilot study and this project we compare also the iodine-retention in BALB/c and CBA/T6 mice after injection with I-125. Probably this will give us an explanation of the above mentioned fact that it seems to be only in CBA/T6 mice possible to induce thyroid tumours. Up to now, we investigated this for the lowest dose group (5 uCi) and found a significantly higher retention for the CBA/T6 mice, both for the female and the male mice. So, we are going to extend this also to the higher dose groups. The next thing we are going to look at is an eventual difference in retention between female and male. On this moment we are not yet able to report about the results.

Progress Report
1982

Contractor:

Medical Research Council
MRC
Park Crescent 20
GB-London W1N 4AL

Contract no.: BIO-D-384-81-UK

Head(s) of research team(s):

* Dr. J. Vennart
Radiobiology Unit
MRC
Harwell - Didcot
GB-Oxon OX11 ORD

Dr. L.M. Cobb
Radiobiology Unit
MRC
Harwell - Didcot
GB-Oxon OX11 ORD

General subject of the contract:

Distribution and long-term retention of insoluble radioactive particles in the lungs of man and rat.

List of projects:

1. Long-term retention of insoluble particles in the lungs of man.
2. Long-term retention of insoluble particles in the lungs of rats.
3. Interaction in vitro of human and rat lung macrophages with insoluble actinide particles.

*From 1 November 1982: Professor G E Adams

Title of project nr 1. Long-term retention of insoluble particles in the lungs of man.

Head of project and scientific staff: Dr L.M. Cobb
Dr A.L. Batchelor
Mr D. Papworth

The search has continued for a suitable 'marker' element to identify insoluble dust retained long-term in human lung. Samples of tissue were taken from the fixed lungs of 3 deceased Cornish tin miners and thin sections cut from the samples after processing and embedding them in plastic. The sections have been examined for particulates containing uranium which were expected to be present in the rock dust inhaled from the mines. Neutron induced autoradiography was used to reveal any uranium in such inhaled particulates but so far no uranium above background level has been seen in the sections. The amounts of uranium present appeared to be no greater than would be expected in people not exposed to uranium or its compounds. However, the neutron induced autoradiographs did show boron or lithium, but most likely boron, associated with the rock dust deposits retained in the lung. Boron is present in the insoluble mineral compound tourmaline which is likely to form a small fraction of the rock dust inhaled in the mines. This makes boron a possible 'marker' element.

Title of project nr 2. Long-term retention of insoluble particles in the lungs of rats.

Head of project and scientific staff: Dr L.M. Cobb
Dr G. Patrick
Mr D. Papworth

A technique has been further developed by which particles may be injected directly through the chest wall into subpleural alveoli of rat lung. Colloidal gold particles have been prepared which are suitable for this technique. They have a count median diameter of 10-30 nm. The particles can be identified in ultra-thin sections of rat alveolar macrophages, which have ingested them in vitro. Identification is by electron microscopy and X-ray microanalysis. The injection procedure uses glass micropipettes which are made with a bevelled tip of outside diameter around 12 µm. Early trials were on exposed lung lobes (open thorax): vital stains and gold particles were injected at a known depth (100-200 µm) beneath the pleura. Histological examination by light microscopy revealed almost no damage to the pleura or alveolar septa from inserting the micropipette. The first injections have now been made without opening the thorax, by passing the micropipette through a small 'window' of pleural membrane after removing the overlying intercostal muscles. At the present phase in this work the lungs are fixed shortly after the time of injection. In subsequent phases the time from injection to fixation will be extended and the path(s) taken by the particles mapped out.

Title of project nr 3. Interaction *in vitro* of human and rat pulmonary macrophages with insoluble radioactive actinide particles.

Head of project and scientific staff: Dr L.M. Cobb
Mr S.J. Ladyman
Mr D. Papworth

Internalised radioactive particles have radiological, chemical and physiological effects on macrophages. In order to isolate the effects of *radiation*, rat macrophages, obtained by lung lavage, have been X-irradiated *in vitro* following various stimuli. The viability of macrophages which had been stimulated by the presence of antibody-sensitised-erythrocytes was found to be unaffected by absorbed doses of 150 Gy. On the other hand, the viability of quiescent (unstimulated) macrophages was reduced by absorbed doses in excess of 60 Gy. Stimulated macrophages irradiated in the presence of a metabolic inhibitor responded in a similar way to quiescent cells.

Experiments intended to determine the effect of X-rays on the macrophage cytoskeleton have also been initiated. Preliminary results indicate that X-irradiation primarily disrupts microtubules rather than microfilaments.

Progress Report
1982

Contractor:

Medical Research Council
MRC
Park Crescent 20
GB-London W1N 4AL

Contract no.: BIO-D-385-81-UK

Head(s) of research team(s):

* Dr. J. Vennart
Radiobiology Unit
MRC
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Dr. E.V. Hulse
Radiobiology Unit
MRC
Harwell-Didcot
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General subject of the contract:

Studies in radiation - carcinogenesis and the interaction of radiation and chemical carcinogens.

List of projects:

1. Radiation induced skin tumours in mice.
2. Effects of radiation on ENU induced tumours in rats.
3. Radiation carcinogenesis in rabbits.

*From 1 November 1982: Professor G E Adams

Title of project nr 1: Radiation-induced skin tumours in mice

Head of project and scientific staff : Dr E.V. Hulse
Dr S.J. Lewkowicz
Dr S. Cole

This long-term experiment on skin carcinogenesis continues. Mice aged 3 months are irradiated with 5000 rad low-energy beta-particles over about 35 per cent of their body surface. This dose produces a definite but sub-maximal incidence of skin tumours with latent periods ranging from 15-36 months. The localised dose of ionizing radiation is combined with high sub-lethal doses of whole-body X-irradiation either at 3 months or 12 months after exposure to beta-particles, i.e. either when the initial skin reaction has subsided or a month or two before tumours would be expected to begin appearing. It is still too early to quantify the effects of the whole-body irradiation on the carcinogenic effects of the localised irradiation.

As completed studies (recently submitted for publication) led to the conclusion that some relatively radio-resistant factor normally restrains radiation-induced cancer cells in the skin from becoming tumours, various aspects of radiation skin biology are being investigated. These are:

i) effects of whole-body irradiation on the healing of localised radiation damage to the skin;
ii) effects of localised irradiation on skin hyper-sensitivity (to 2-4 dinitroflurobenzene);
iii) effects of ionizing radiation on Langerhan's cells.

Title of project nr 2: Effects of radiation on ENU induced tumours in rats

Head of project and scientific staff: Dr J.F. Knowles

Nervous system tumours are produced when our HMT strain of inbred rats is given ENU shortly after birth and the effect of X-irradiation 24 hours after ENU on tumour induction has been examined.

(i) Results from this experiment for the period up to 2 years post-treatment have now been published (Knowles, 1982a). A statistically significant reduction in the incidence of tumours occurred when neonate rats injected with 10 mg/kg body wt ENU were then given 1.25 Gy of X-rays. A selective reduction in malignant schwannomas of peripheral nerves and nerve roots accounted for most of the difference. Brain and spinal cord tumour-incidence (all gliomas), was unaffected by radiation. Neonate rats injected with 4 mg/kg body wt ENU and then given an absorbed dose of 0.2 Gy or 1.25 Gy also had reduced incidences of nervous system tumours though these were not statistically significant (Knowles, 1982a). The most likely explanation for this reduction in ENU-induced nervous system tumours after X-irradiation is that the radiation kills the ENU-transformed potential tumour cells.

As part of this experiment control neonate rats were given X-irradiation alone. They were irradiated when their nervous system development was incomplete and thus they resembled the human fetus given ante-natal irradiation, after which an excess of nervous system tumours have been reported. No rat given 0.2 Gy in these experiments developed a nervous system tumour but 3 (9 per cent) of those given 1.25 Gy did develop brain tumours. This incidence is far too high for all of these tumours to be accounted as spontaneous occurrences and it is virtually certain that they were radiation-induced. A description and discussion of the occurrence of all these radiation-induced nervous system tumours has now been published (Knowles, 1982b).

All rats in this experiment have now completed their life spans and, although several nervous system tumours occurred later than 2 years, the previous conclusions are not affected. A large number of non-nervous system tumours occurred in rats from all experimental groups, especially in rats more than 2 years old. The results for these tumours are at present being analysed for inclusion in a final report.

(ii) Further experiments investigating the possibility that the radiosensitivity of ENU-transformed cells change with time are now well underway. Neonate rats have been injected with 10 mg/kg body wt ENU and given 125 rad of X-irradiation 5, or 30 days later. In order to determine the most sensitive period for radiation-induction of nervous system tumours groups of rats have been given radiation only at 1, 5, 15 or 30 days.

List of publications in 1982

I. Knowles, J.F., 1982a. The effects of X-radiation given after neonatal administration of ethyl nitrosourea on incidence of nervous system tumours. Neuropathology and Applied Neurobiology, 8, 265-276.

Knowles, J.F., 1982b. Radiation-induced nervous system tumours in the rat. International Journal of Radiation Biology, 41, 79-84.

Title of project nr. 3: Radiation carcinogenesis in rabbits.

Head of project and scientific staff: Dr. E.V. Hulse

A total of 338 year-old rabbits have been irradiated or are acting as unirradiated controls, the first irradiations were done in 1970 and the last in 1981. Three different dose-rates have been used. As rabbits live 9-10 years the experiment is likely to last for another 7-8 years. Up to the present a total of 213 rabbits have died or been killed for humane reasons, 18 of them during the last year. Thorough autopsies and histopathological studies are done on all decedents. The animals still alive, 125 at the moment, need specialised care. Basal-cell tumours of the skin are one of the most interesting radiation-induced tumours which are occurring in this experiment. They are found after remarkably low whole-body doses (100-250 rad) and not infrequently have to be surgically removed because of size, ulceration or a tendency to bleed: 14 such operations have been done during the last year. Elderly rabbits, whether irradiated or not, develop degenerative changes of the teeth, occasionally with abscess formation and 4 such abscesses have needed drainage during the last year. Various other minor veterinary problems have arisen and been treated.

Progress Report
1982

Contractor:

The European Late Effects
Project Group (EULEP)
Avenue E. Plasky 144
B-1040 Bruxelles

Contract no.: BIO-D-390-81-D

Head(s) of research team(s):

Dr. J. F. Duplan
Chairman of EULEP
Rue de Saint-Genès 180
F-33076 Bordeaux Cedex

General subject of the contract:

Late somatic effect of ionizing radiation in the mammalian organism.

List of projects:

1. Late somatic effects of ionizing radiation in the mammalian organism.

Contractor :
EUROPEAN LATE EFFECTS PROJECT GROUP (EULEP)

Contract nr. : BIO-390-D (D)

Head of research team : J.F. DUPLAN

General subject of the contract : LATE SOMATIC EFFECTS OF IONIZING RADIATION IN THE MAMMALIAN ORGANISM

EULEP has pursued its efforts during the year 1982 on

a) standardization of experimental conditions in the participating institutions
b) coordination of the planning and performance of ongoing research projects in the areas of radiation late effects, and
c) on execution of specific cooperative projects on carcinogenesis, on dysplastic and dystrophic lesions and on the toxicity of internal emitters.

The activities of EULEP are subdivided in five committees :

1. Committee of Pathogenesis of Neoplastic Diseases
2. Committee of Pathogenesis of Nonneoplastic Diseases
3. Committee of Internal Emitters
4. Committee of Pathology
5. Committee of Dosimetry for External Radiation.

Committee on Neoplastic Diseases H. SEIDEL

The work within the committee has made considerable progress during 1982, focussing on the field of leukemogenesis - as in the years before - and on the role of viruses in radiation carcinogenesis. Activities in these areas have been reviewed in two EULEP workshops, one in Bordeaux (October 4-5, 1982) and reported in the EULEP Newsletter of January 1983 in detail, the other on "bone marrow to thymus interactions in murine leukemogenesis" at Schloss Reisensburg (December 3-5, 1982). The other areas represent activities in solid tumor induction in dependence of radiation dose and quality and, to a more limited extent, studies on the function of the immune system in radiation-induced oncogenesis.

1. Leukemia studies

The work on the induction of myeloid leukemias in CBA mice has been continued. The induction seems to depend, after brief exposure to low LET radiation, on the square of the radiation dose. After protracted exposure the leukemia frequency is much reduced and the variation of frequency with variation in dose is small. An outstanding high spontaneous mutation rate has been assumed as a basic property of the leukemia cells in transplantation experiments after comparing serial clonal passage of small versus large numbers of cells (Oxford). Dose-effect relationships for various endpoints (longevity, leukemia and tumor incidence and rates) are also studied with neutrons and attenuated fission spectrum neutrons in another laboratory (Casaccia). The combined effect of chemical leukemogens and external whole body irradiation has been studied with butylnitrosourea and methylnitrosourea. In most experiments the leukemogenesis was dominated by the chemical,effects of radiation are expected in protocols with reduced doses of the chemicals (Ulm).

Pathophysiological studies in various laboratories concentrated on prothymocytes, thymic nurse cells and the protective effect of hemopoietic cell suspensions for radiation leukemogenesis and were discussed at Schloss Reisensburg. The T cell repopulating ability of the bone marrow

and the spleen was tested after leukemogenic exposure to radiation, to chemical leukemogens and in viral leukemia. The analysis of radiation chimeras used specific antigens on T cells as well as biochemical markers. The development of extrathymic lymphomas was studied after thymectomy in AKR mice and varied by irradiation and/or bone marrow or spleen cell restoration. Finally leukemic cell membrane constituents were analysed and compared to normal T cells. A considerable heterogeneity of the radiation or RadLV induced thymic lymphosarcomas was found (Bordeaux, Mol, Rijswijk, Ulm).

2. Solid tumor induction

Studies on lung tumors in mice have been continued with variations in the radiation exposure devices (London). In 1980/81, a total of 1024, 3 month old SAS/4 mice were given thoracic doses of 0, 0.1, 0.25, 0.5, 0.75, 1, 2 and 3 Gy of cyclotron neutrons (64 males and 64 females per dose point. In males there is a definite broad plateau to the curve, whilst in females there is no significant LT induction at any dose level used. So the "unexpected sex difference" reported in our 1980 EULEP report is a real finding. Ovarian cancers have been induced in whole-body irradiated mice in dependenc of radiation dose and quality. Transplantation studies early after radiation exposure suggested very little, if any, influence of the general conditions for tumor expression (Casaccia).

3. Function of the immune system

In one laboratory three lines of mice have selected for high and low antibod production activities and the longevity and tumor induction rate is presentl recorded (Casaccia). In these and in leukemia induction experiments with chemicals, eventually combined with radiation, the role of NK (natural kille cells is under study (Ulm).

Committee of Non-Neoplastic Diseases J.W. HOPEWELL

The investigations undertaken by members of this Committee would indicate that vascular damage and late tissue fibrosis are still of major importance in the study of radiation induced non-neoplastic changes.

Studies in the adult rat brain, which are mainly directed to the evaluation of vascular damage, continue to involve the collaboration of five EULEP laboratories : Louvain (Keyeux), Oxford (Hopewell, Wilkinson), Ulm (Calvo), Rijswijk (Reinhold) and Mol (Gerber, Maisin, Reyners). The results of this multidisciplinary study indicate a broad degree of agreement as to the timing of changes after irradiation.

Quantitative histological studies indicate changes in the vascular architecture in the cerebral cortex after brain irradiation with doses in the range 17.7 to 30 Gy. These were characterised by a significant decline in the total vessel density, blood volume index and vessel wall surface area index during the first six months after exposure. Later (12-18 months) the effect was reversed. The biphasic time related change in the radiation effect was confirmed by the results of isotope studies, from which the cerebral blood flow velocity and the nutritive cerebral blood flow were estimated. However, the close correlation between morphometric and dynamic evaluations of the blood circulation in the irradiated brain could be based on a misinterpretation of blood flow data. The effect of radiation on the permeability of the "blood brain barrier" has not yet been investigated in depth under the present experimental conditions. Preliminary measurements of the brain/blood partition co-efficient of the radioactive tracer (antipyrine) used in the determination of blood flow would indicate a biphasic modification of the capillary permeability. This was characterised by a 15 % increase in the partition co-efficient six months after 20 Gy. This parameter was reduced by 10 % after eight months.

Additional semi-quantitative histological studies have also been undertaken in the choroid plexus. These demonstrate that vessel changes appear within five months of irradiation. This is followed, shortly afterwards, by lesions in the secretory epithelial cells, vascularisation being followed by atrophy.

In the connective tissue oedema and phagocytes were seen after 5-6 months. Evidence of regeneration was seen from the tenth month up until the end of the period of investigation (18 months).

After 20 Gy ultrastructural studies would suggest a greater radiosensitivity of the grey than of the white matter. As far as the glial cell population was concerned the oligodendrocyte density was reduced by > 50 % after 2 years, although the number of alpha astrocytes was increased by about 10 %. A highly radiosensitive glial cell type has also been identified which was rapidly (<24 hour) and completely eliminated from the brain after a dose of $\geq$ 15 Gy. Autoradiographic studies are in progress which will, it is hoped, provide kinetic data for this cell type which is thought to represent a multi-potential glial stem cell.

Studies of late radiation fibrosis in the lung involve the active collaboration of laboratories at Mol (Gerber, Maisin) and Warsaw (Dancewicz). Biochemical estimations as to the composition of collagen and proteoglycanes in the rat lung after doses of 15-18 Gy have continued. Long term follow up, for periods up to 20 months, have confirmed earlier reports of an increase in the collagen/DNA ratio. However, no significant change could be determined in the composition of collagen as isolated by the pepsin procedure, although collagen not dissolved by pepsin contained an increased proportion of hexoses and dityrosine but not aldehydes.

Ultrastructural studies with transmission (TEM) and scanning electron microscopy (SEM) were carried out in BALB/c male mice exposed to doses of 5-50 Gy. These were sacrificed at different time intervals ranging from 15 minutes to 24 months after irradiation. SEM complemented observations from TEM since it reveals the changes on the surface of cells and those within the alveoli. The results confirmed that the endothelium of the large vessels and the epithelium of the major airways were less susceptible to radiation damage than the epithelium of the lung parenchyma. During the period of radiation pneumonitis (2-7 months after irradiation) the principal lesions were seen in epithelial cells, mainly type II cells, whereas capillary damage appeared to play a secondary role at this time. Increased phospholipid secretion by type II cells, in conjunction with an impaired synthesis of the protein component of the surfactant, resulted in an altered

surface tension. This and damage to alveolar macrophages was important in the pathogenesis of radiation pneumonitis. Later when fibrosis ensues, after doses of 10 Gy or more, vascular alterations predominate over those in the epithelium.

Committee on Internal Emitters V. VOLF

Research work has been concentrated on the effects of selected radionuclides deposited in the tissues and on the methods suitable for removing incorporated radionuclides from the body. Ten laboratories participated in three cooperative projects.

1. Late effects of bone seeking radionuclides

Again, there was a considerable cooperation among six laboratories. In two cases a consultant's stay was financed by EULEP : Dr Green (MRC-Harwell) adapted at Casaccia his programme for analysis of radionuclide microdistribution in the bone ; Dr Sontag (Karlsruhe) assisted at MRC-Harwell in computer evaluation of bone sections labelled in vivo with a fluorescent dye-calcein.

A successful workshop meeting was held in May at Rome. All papers presented appeared in an abbreviated form in EULEP Newsletter 31. During the workshop suggestions for new work and interlaboratory cooperation were also reviewed. In addition it was decided to proceed with arrangements for a bone symposium to be held in association with the European Calcified Tissue Society in 1984.

Experimental studies on bone used different heavy metal radionuclides and included studies on the metabolism, macrodistribution and microdistribution in the skeleton of Pm-147, Pb-210, Po-320, Ra-226, Th-230, U-233, Pu-230, Am-241 and Cm-244. Further studies investigated the incidence of leukemias and osteosarcoma in mice following administration of Ra-224 and Pu-239 as well as changes of stem cells with an osteogenic potential in bones after deposition of radionuclides.

2. Late effects after inhalation of radionuclides

Six laboratories participated in this project. An interlaboratory consultation was realized in Paris, where Drs Stather and Stradling (NRPB-Harwell) discussed a joint project with Drs Masse and Métivier on the metabolism and decorporation of inhaled plutonium-tributylphosphate. Several joint meetings of MRC- and NRPB-Harwell took place.

Research work concentrated partly on physiological mechanisms of deposition and clearance of inhaled particles, including a variety of industrial radioactive dusts, partly on evaluation of their biological effects, such as tumor dose-response relationships for inhaled PuO_2 in the mouse lung following different dose distribution patterns. Furthermore, methods for homogenization of lung tissue were developed and the behaviour of subcellular organelles was analyzed in different density gradient media. These preparatory steps should enable to study subcellular deposition of radioactive particles introduced into the lung.

3. Decorporation of radionuclides and late effects

Papers presented during a workshop meeting at Mol December 1981 appeared in EULEP Newsletter 30. They give an overview of the current status of research in the field of removal of radioactive materials from the body. It was felt that in spite of effective means such as DTPA and alginates, the search for new compounds has to be continued. In order to make evaluation of effectiveness of such new means most economic and reliable, several drafts of recommendations for testing new chelating agents were discussed and the final version published as a EULEP recommendation.

Research work included studies on the effect of prolonged administration of DTPA in drinking water on long-term retention of Pu-239 and Am-241 in rats as well as studies on beneficial effect of a treatment with alginates after deposition of radioactivity in the bone ; bone stem cells were used as an indicator.

First steps were made to evaluate the effectiveness of a new plutonium specific chelating agent LICAM C. The substance may be synthetized in Paris and Warsaw and tested by several EULEP laboratories. This cooperative project should prove the applicability of the EULEP Recommendation mentioned above.

Committee on Pathology A. LUZ

In 1982, one pathology workshop and two meetings of the Consultation Center took place. The 17th Committee on Pathology workshop was held in Munich in November 1982. The main topic was "Usefulness of electron microscopy on formalin fixed tissues in diagnostic pathology". Twenty-two cases, including lesions from mice, rats and Mastomys were presented. The discussion of these cases was supplemented by presentations given by Dr A.M. de Leeuw (TNO/ Rijswijk), Prof. Dr G. Hübner (University of Munich), Prof. Dr W. Calvo (Ulm), Dr K.H. Marquart and Dr A.B. Murray (Munich/Neuherberg). The presentations clearly demonstrated the usefulness of post-formalin electron microscopy in a number of areas, including the diagnosis of infectious diseases, the differential diagnosis of small cell neoplasms and neoplasms with eosinophilic cells, the diagnosis of pancreatic islet cell components and the influence of the pH of conventional formalin fixation on the ultrastructure in general. Among the cases presented, the value of post-formalin electron microscopy was demonstrated in the following diagnostic fields :

1. Spindle cell sarcomas or pleomorphic sarcomas at the light microscopic level, proved to be of fibroblastic, histiocytic, rhabdomyogenic or leiomyogenic origin or composed of undifferentiated cells when examined electron microscopically. With regard to the diagnosis of neoplasms of Schwann cell origin, poor tissue preservation limited the usefulness of post-formalin electron microscopy in the few cases demonstrated.
2. Evidence for a myeloid or histiocytic origin of neoplasms of hemopoietic and lymphoreticular tissue.
3. Confirmation of epithelial origin of anaplastic carcinomas.
4. Demonstration of neuroendocrine granules in tumors of some tissues, pancreas and adrenal medulla.
5. So-called undifferentiated neoplasms located in the adrenal cortex revealed a higher degree of differentiation ultrastructurally than by light microscopy, and these were compatible with an origin from cells of the adrenal cortex.

During 1982, the sale of copies of the EULEP Pathology Atlas was begun, although initially at a relatively modest level. The chapter on Neoplastic and Nonneoplastic Lesions of the Mammary Gland will be printed by the time of the next general assembly. At the Consultation Center meetings, a total of 18 problem cases was discussed in February 1982, at the Reisensburg and in November 1982, in Munich. The work of the Consultation Center will be intensified at future meetings and more time will be reserved for the discussion of problem cases.

Committee of Dosimetry for External Irradiation J.J. BROERSE

In cooperation with the European Bone Marrow Transplant Group (EBMT) a workshop on physical aspects of total body irradiation was organized at Leiden on May 26 and 27, 1982. The dosimetry methods and irradiation techniques for total body irradiation as practiced in 28 European hospitals were summarized. Special attention was devoted to six aspects notably patient position and beam direction, multiple parallel beams (biological problems - hot spots in critical organs), homogeneity of dose distribution, lung shielding, delivery of dose to the patient (including beam monitoring) and in vivo dosimetry. The proceedings of this meeting will be published in the Journal Européen de Radiothérapie.

The fifth meeting of the EULEP X-ray dosimetry committee was held at Oxford, September 20 to 22, 1982. The meeting was attended by seventeen participants from seven countries. The main topics of this meeting concerned the EULEP X-ray dosimetry protocol plus additional appendices and dosimetry intercomparisons.

During the meeting information was provided on :

- The Scandinavian intercomparison program for electron beam dosimetry in radiotherapy.
- The results of Oxford in dosimetry intercomparisons for 250 kV X-rays, ^{60}Co gamma rays and 4 and 10 MeV electrons.
- Dosimetry intercomparisons at BIPM (Bureau International de Poids et Mesures) Paris and RIV (Rijksinstituut voor de Volksgezondheid),Bilthoven.
- A EULEP dosimetry site visit to Ulm.
- A review of calibration at NPL (National Physical Laboratories), Teddingtom of secondary standard instruments used in British radiotherapy centres.

The EULEP dosimetry protocol has been updated according to suggestions of the committee members. Additional appendices to the protocol were prepared on : examples of X-irradiation procedures at participating institutes ; other dosimetry methods including TLD ; factors affecting X-ray dosimetry for radiobiology and some associated pitfalls ; back- and side scatter influence on the absorbed dose distribution in a mouse phantom : Monte Carlo simulation studies.

An appendix on animal phantoms for radiobiological dosimetry is in progress. The protocol plus appendices will be finalized in the beginning of 1983 ; a concise version will be prepared for publication in a regular radiobiology journal.

EULEP dosimetry intercomparisons on whole body irradiation of mice were organized in 1971, 1973, 1976 and 1980. A EULEP dosimetry intercomparison for partial body X-irradiation of rats was performed in 1978. The fifth EULEP X-ray dosimetry intercomparison on whole body irradiation of mice will be performed in the beginning of 1983. The project will be carried out in two sessions presumably in February and April. The readout and evaluation of the TL dosimeters will be performed at RIV, Bilthoven ; the administration of the project will take place at TNO, Rijswijk.

Publications

EULEP Newsletter : Nrs 27-28-29-30-31
Informal Progress Report on the European Late Effects Project Group

A recommendation for testing new chelating agents appeared in the Journal of Environemntal and Radiation Biophysics as a EULEP recommendation.

EULEP Pathology Atlas

EULEP Symposium on "Late effects after therapeutic whole-body irradiation" Münich, 27-28/8/1981 - Appeared as a Euratom Report EUR 8070 en (1982).

The Proceedings of the EULEP Symposium on "Prenatal irradiation", Bordeaux, July 1982 will be published as a Euratom Report.

Progress Report
1982

Contractor:

The European Late Effects
Project Group - EULEP
Avenue E. Plasky 144
B - 1040 Bruxelles

Contract no.: BIO-D-491-82-D

Head(s) of research team(s):

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F-33076 Bordeaux Cedex

General subject of the contract:

Late somatic effects after prenatal irradiation

List of projects:

1. Late somatic effects after prenatal irradiation

Title of project nr. 1 : LATE SOMATIC EFFECTS AFTER PRENATAL IRRADIATION

Head of project and scientific staff : J.F. DUPLAN

The subcommittee on prenatal irradiation of EULEP which was created in 1982 coordinates the activities on prenatal irradiations of six EULEP laboratories : four laboratories of Germany (Freiburg, Neuherberg, München und Essen), the Radiobiology Department of Mol (Belgium) and the Sundyberg laboratory of Stockholm (Sweden). Two subgroups have been formed : one on the preimplantation period and the other on the fetal period.

An intercomparison study between the different EULEP laboratories on the radiation dose in utero has been initiated with the help of the EULEP dosimetry Committee.

In addition, the subcommittee has organised two meetings on the effects of prenatal irradiation with special emphasis on late effects; one in the Reisensburg (Germany) at the time of the EULEP General Assembly and the other in Bordeaux (France) in connection with the 17th Annual Meeting of the European Society of Radiation Biology. At this last meeting, 16 presentations were made on different aspects of prenatal irradiation including by scientists from oversea : Dr. Yamada (Japan) on changes in radiosensitivity of the in vitro fertilized mouse embryo with the pronuclear and the 2-cell stage and Dr Carsten (Brookhaven, USA) reported on the multiple parameter evaluation of tritium toxicity. The manuscripts of the Bordeaux meeting will be published as a report of the Commission of the European Community.The principal results of these meetings were :

1. A radiation exposure during the preimplantation phase induces chromosomal damage at considerably low doses. As clonal cell proliferation is one of the characteristic features during this and the following developmental stage, stable mutations may become of important relevance for the further development of the organism.
2. During the early fetal period, the cell proliferation of neuroblasts is very rapid, which leads to the development of the forebrain. Newer data from survivors in Hiroshima and Nagasaki have shown that a radiation exposure during the early fetal period causes mental retardation in a very sensitive way. Experimental data from mice and rats, which are obtained with new techniques, underline the sensitivity of these developmental stages.

3. The knowledge about the induction of malignant neoplasia is still very scanty. Experimental data must help us to clarify whether the sensitivity of the embryo/fetus changes during the development. Also the interaction between ionizing radiation and certain chemical substances appears to be very important.
4. The metabolism and effects of radionuclides must be better understood. For many radionuclides, the distribution between the mother and fetus and within the fetus is unknown.

The members of the subcommittee have decided to focus their work and co-operation on these points. It has been agreed that this shall be started with the points 1-3 (see programme for 1983).

III E

GENETISCHE WIRKUNGEN IONISIERENDER STRAHLUNG

GENETIC EFFECTS OF IONIZING RADIATION

EFFETS GENETIQUES DES RAYONNEMENTS IONISANTS

Weitere Forschungsarbeiten zu diesen Themen werden auch in folgenden Tätigkeitsberichten beschrieben :

Further research work on these subjects is also described in the following progress reports :

D'autres travaux sur ces thèmes de recherche sont également décrits dans les rapports suivants :

III A. Barendsen, G.W.	TNO Rijswijk	BIO 300 NL
III A. Broerse, J.J.	TNO Rijswijk	BIO 302 NL
III A. Kellerer, A.M.	Univ. Würzburg	BIO 286 D
III A. Muth, H./Grillmaier, R.E.	Univ. Homburg	BIO 289 D
III A. Peirson, D.H.	UKAEA Harwell	BIO 306 UK
III C. Errera, M.	Univ. Bruxelles	BIO 359 B
III C. Hagen, U.	GSF Neuherberg	BIO 343 D
III C. Kaul	BGA Neuherberg	BIO 518 D
III C. Lafuma, J.	CEA, CEN Fontenay-aux-R.	BIO 346 F
III C. Streffer, C.	Univ. Essen	BIO 290 D
III C. Taaffe, J.K./Malone, J.F.	Coll. Technol. Dublin	BIO 364 EIR
III D. EULEP	Bordeaux	BIO 491 D
III D. Maisin, J.R.	CEN, SCK Mol	BIO 378 B
III D. Maisin, J.R.	CEN, SCK Mol	BIO 379 B

Progress Report
1982

Contractor:
Erasmus University Rotterdam
Postbus 1738
NL-3000 DR Rotterdam

Contract no.: BIO-E-404-81-NL

Head(s) of research team(s):
Prof. Dr. D. Bootsma
Dept. Cell Biology
and Genetics
P.O. Box 1738
NL-3000 DR Rotterdam

General subject of the contract:
The genetic and biochemical basis of radiation sensitivity in human and other cells in culture.

List of projects:

1. Genetic analysis of DNA repair.
2. Biochemical analysis of DNA repair in eukaryotic cells.
3. Consequences of DNA damage and repair.

PREAMBLE

Human cell variants believed to be deficient in DNA repair, continue to constitute a major focus of the work of the contracting laboratories. Further genetic analysis has revealed the existence of three complementation groups for Cockayne syndrome and a further complementation group (H) in xeroderma pigmentosum (XP). Hypermutability of Cockayne syndrome cells following UV has been confirmed in a third cell strain. In the A, D, G and H complementation groups of XP it has been possible to correct the repair defect after microinjection of human cellular extracts. A gene involved in the A group complementation process has been localized on chromosome 1. As a pointer to the future, it has also been possible to obtain transfer and expression of a gene originating in an inactive X-chromosome.

A method has been developed for determining the ability of human cells to effect repair of potentially lethal radiation damage. Among cells shown to be deficient in such repair have been those from a patient with Gorlin's syndrome and those from an apparently normal lymphoma patient who showed a severe erythemal response to radiotherapy.

At the biochemical level, work has progressed in two areas concerned with the more sensitive detection of DNA lesions. Firstly, the UV-endonuclease method for detecting sensitive sites has been developed by incorporating elution techniques and fluorescent dyes, thus eliminating the need for radioisotopic labelling and permitting *in vivo* experimentation. A second approach has involved the development of a procedure for making monoclonal antibodies with a high affinity for specific DNA lesions. The use of monoclonal antibodies has enabled the detection of pyrimidine dimers in human epithelial cells exposed to gamma radiation.

Significant progress has been made in the study of the mechanisms of action of DNA repair inhibitors such as aphidicolin, bringing out the importance of the metabolic state of the cell (whether stationary or exponentially growing). The role of inhibitors of poly ADP-ribose polymerase in repair of damaged DNA, as well as their influence on frequencies of chromosome aberrations, sister chromatid exchanges and point mutations has been studied in detail. Other biochemical studies have concerned the involvement of DNA ligases I and II in DNA repair and a human cell mutant has been characterized with properties consistent with a deficiency in DNA ligase I activity.

The work has been carried out in a joint programme by:

D. Bootsma *et al*, Erasmus University, Rotterdam (BIO 404 NL).
B. A. Bridges *et al*, MRC, Brighton (BIO 414 UK).
P. H. M. Lohman, TNO, Rijswijk (BIO 403 NL).
J. W. I. M. Simons, University of Leiden (BIO 407 NL).

Title of project nr. 1 : Genetic analysis of DNA repair

Head of project and scientific staff: Prof.Dr. D. Bootsma
Dr. A. Westerveld
Dr. J.H.J. Hoeijmakers
Dr. M.P. Mulder
Drs. N.G.J. Jaspers
Dr. W.J. Kleijer
Drs. J. Zwetsloot

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1. Isolation of repair mutants
   a. In 1982 43 skin fibroblast strains were cultured from patients with putative DNA repair defects and from their relatives for biochemical investigation and for storage in the cell bank:
   6 patients with xeroderma pigmentosum (XP), 3 possible (2) or obligate (1) heterozygotes for XP, 2 patients with ataxia telangiectasia (AT), 4 patients with UVL hypersensitivity and/or skin abnormalities (e.g. nevoid basal cell carcinoma), 28 patients and relatives from families with dysplastic nevus syndrome (DNS).
   b. Two prenatal diagnoses were made in pregnancies at risk for XP:
   1 from Finland (not affected) and 1 from France (affected).

2. Genetic complementation analysis
   a. In a collaborative study with Prof. E.G. Jung and Dr. E. Fischer (University of Heidelberg) we have detected a new complementation group in XP (Tentatively coded: group H).
   b. The analysis of complementation kinetics in XP cybrids (fusion of XP cells with cytoplasts of complementing XP cells) was completed in 1982. Evidence was obtained for the existence of an early step in the repair process, occurring immediately after UV exposure, which is followed by additional steps resulting in UDS at a later time period after UV irradiation of the cells. The gene product defective in complementation group A may be involved in this early activity. Inducible repair activities could not be demonstrated in this cybrid system.
   c. Complementation analysis of AT was completed in 1982 and published. Four different complementation groups were found.

3. Localization of repair genes on human chromosomes

a. Last year we reported the assignment of a gene on human chromosome 1 which is involved in the complementation of the XP A defect in our cybridization system. By using a panel of hybrid cell lines, which had retained different pieces of chromosome 1, this gene was mapped in the distal region of the chromosome (1q32→1qter).

Another panel of hybrid cell lines was obtained after fusion of Chinese hamster cells with XP-A fibroblasts. Cytoplasts derived from these hybrids were also able to restore the repair deficiency of XP-A cells with fast kinetics provided that chromosome 1 was present in the hybrid. These results indicate that in proliferating Chinese hamster-XP-A hybrids the A-group complementing factor is restored by interaction of Chinese hamster and XP-cell components.

b. A large number of hybrid cell lines was isolated after fusion of human cells with the repair deficient CHO mutan (CHO12RO). The repair capacity (UDS) of these cells could be correlated with the presence or absence of a gene on human chromosome 1 in these hybrids. Its relationship with the chromosome 1 coded factor identified in our cybridization experiments is unknown.

4. Genetic transformation of mammalian cells and isolation of repair genes

a. Genetic transformation of mammalian cells after transfection with DNA fragments has been investigated in different cell systems.

- Transfection of $HPRT^-$ deficient mouse cells with DNA fragments, originating from inactived human X-chromosomes carrying the normal HPRT allele, resulted in $HPRT^+$ clones. These results suggest that the inactive state of this gene (on a lyonised X-chromosome) is reversible.
- Three dominant vector systems were tested for the transformation of XP cells: vectors containing the prokaryotic dihydrofolate reductase (DHFR), the xanthine-guanine phosphoribosyl transferase (XGPT) and the phosphotransferase (PTR) genes, which provide selection for methotrexate, mycophenolyc acid and G-418 resistence respectively. The XGPT and PRT systems resulted in a relatively high transformation frequency of SV40 transformed XP-A cell lines ($2x10^{-4}$). Lower frequencies were obtained with the DHFR system

(less than 10^{-5}). Experiments are in progress aiming the transfer into XP cells of human DNA fragments ligated to these vectors.

- Similar transfection experiments have been carried out using our CHO repair mutant (CHO12RO) as recipient. The optimal conditions for transfection and selection (including a second UV selection step) have been worked out.
- We have constructed a cosmid library consisting of placenta DNA inserted into a cosmid vector containing the G-418 resistance gene. We hope to use this bank for the isolation of repair genes.

b. We have microinjected purified Escherichia coli uvrA protein, and combined extracts prepared from E.coli cells with a high level of expression of the uvrA, B and C genes into XP-A and XP-G cells to investigated whether these proteins are active in the removal of TT dimers (in collaboration with Dr. P. v.d. Putte, Leiden). No correction of the XP defects was found although in control experiments the prokaryotic enzymes: micrococcal endonuclease and T4 endonuclease V did restore the UDS of these (and the other) XP complementation groups.

Title of project nr. 2 : Biochemical analysis of DNA repair in eukaryotic cells.

Head of project and scientific staff: Prof.Dr. D. Bootsma
Dr. J.H.J. Hoeijmakers
Dr. L.A. Burgoyne
Drs. N.G.J. Jaspers

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1. Characterization of repair pathways

   a. The 6 newly collected cell strains at risk for XP (see project 1) were found to be defective in UV induced excision repair as measured by UDS activity. Reduced inhibition of DNA synthesis after X-irradiation was found in the two new AT cell strains. Normal UDS level was found in cell strains derived from 4 patients with UV hypersensitivity clinically different from XP.

   b. The conformation of chromatin in normal and ataxia telangiectasia (AT) fibroblasts was studied by measuring the effect of sterically modified nucleases on the DNA in isolated nuclei. AT chromatin tended to be slightly more nuclease resistant than chromatin of normal cells, but this was not consistent in AT. Exposure to ionizing radiation did not induce conformational alterations that were detectable by this technique in normal and AT cells.

   The effect of n-butyrate, a drug that affects histone-acetylation and thus DNA-histone interaction, was investigated in X-irradiated AT and normal cells, using the rate of DNA replication as a marker. Both types of cells responded similarly in these experiments.

2. Isolation of repair proteins from mammalian cells

   As reported previously we have detected in extracts from normal cells or tissue (Hela, SV40 transformed fibroblasts, placenta) a protein which upon microinjection into XP-A cells, specifically corrects the XP-A repair defect. A further characterization of this protein gave the following results: its activity can still be detected 8 hrs. after injection; its molecular weight presumably does exceed 100.000 D or it is part of a complex exceeding that size.

By using a 5 - 10x more sensitive modification of the UDS assay we have obtained with the microinjection technique transient correction to a low but significant level of the complementation groups D, G and H. We will further characterize these repair factors with the eventual aim to purify them.

Title of project nr. 3 : Consequences of DNA damage and repair

Head of project and scientific staff: Prof.Dr. D. Bootsma
Dr. A.W.M. van der Kamp
Drs. N.G.J. Jaspers

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1. DNA damage, repair and carcinogenesis

a. In a related project we have recently found that c-abl, the human gene homologous to the oncogene of Abelson murine leukemia virus, is translocated from chromosome 9 to chromosome 22 in the leukocytes of patients with chronic myelocytic leukemia (CML)(see A. de Klein et al., Nature 300, 765, 1982). This finding suggests a role for the c-abl oncogene in the generation of CML. This finding supports the hypothesis that malignant tramsformation of cells, occurring spontaneously or induced by irradiation or chemical agents, is the result of alteration in the expression of cellular oncogenes. These alterations may be the result of point mutations as well as chromosomal translocations.

This new concept of carcinogenesis has stimulated us to start the following experiments:

- in vitro malignant transformation of XP and normal human cells (in collaboration with Dr. A.J. van der Eb, University of Leiden)
- isolation of proliferating hybrids after fusion of rodent cells with human tumor cells, which will enable us to study transposition of oncogenes and cloning of these genes. A panel of hybrid cells was obtained after fusion of rodent cells with human melanoma cells.
- localization of unique human DNA sequences (e.g. cellular oncogenes) by in situ hybridization.

b. DNA repair was studied in a large series of fibroblast cultures established from members of four Dutch families with dysplastic nevus syndrome. This dominant genetic trait confers predisposition to malignant melanoma. In all investigated cell strains the responses to UV-irradiation, measured as unscheduled DNA synthesis, cell killing and inhibition of DNA replication, were normal.

Cytogenetic studies revealed essentially normal levels of spontaneous sister chromatid exchanges, but elevated frequencies of stable chromosomal rearrangements in two patients from one family.

A procedure was set up allowing the establishment of melanocyte cultures from human foreskin, not contaminated with fibroblasts or keratinocytes. The response of these cells to UV exposure is currently under investigation.

List of Publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

1. M. Stefanini, A. Reuser and D. Bootsma
Isolation of Chinese hamster ovary cells with reduced unscheduled DNA synthesis after UV irradiation.
Somatic Cell Genetics 5, 635-642 (1982)

2. W. Keijzer, A. Verkerk and D. Bootsma.
Phenotypic correction of the defect in xeroderma pigmentosum cells after fusion with isolated cytoplasts.
Exp. Cell Res. 140, 119-125 (1982).

3. D. Bootsma, W. Keijzer, E. van der Veer, G. Rainaldi and E.A. de Weerd-Kastelein.
Interaction of human and chick DNA repair functions in UV-irradiated xeroderma pigmentosum-chick erythrocyte heterokaryons.
Exp. Cell Res. 137, 181-189 (1982).

4. E. van der Veer and D. Bootsma
Repair DNA synthesis in heterokaryons during reactivation of chick erythrocytes fused with human diploid fibroblasts or Hela cells.
Exp. Cell Res. 138, 469-474 (1982).

5. N.G.J. Jaspers and D. Bootsma
Genetic heterogeneity in ataxia telangiectasia studied by cell fusion
Proc.Natl.Acad.Sci. USA 79, 2641-2644 (1982).

6. N.G.J. Jaspers, J. de Wit, M.R. Regulski and D. Bootsma.
Abnormal regulation of DNA replication and increased lethality in ataxia telangiectasia cells exposed to carcinogenic agents.
Cancer Research 42, 335-341 (1982).

7. N.G.J. Jaspers and D. Bootsma
Abnormal levels of UV-induced unscheduled DNA synthesis in ataxia telangiectasia cells after exposure to ionizing radiation.
Mut. Res. 92, 439-446 (1982).

8. N.G.J. Jaspers, J. de Wit and D. Bootsma
The rate of DNA synthesis in ataxia-telangiectasia fibroblasts after exposure to DNA damaging agents.
In: Ataxia-telangiectasia - A Cellular and Molecular Link Between Cancer, Neuropathology, and Immune Deficiency. Edited by B.A. Bridges and D.G. Harnden, p. 339-345 (1982).

9. N.G.J. Jaspers, J. de Wit and D. Bootsma
The inhibition of DNA replication in ataxia telangiectasia cells after exposure to a variety of clastogenic agents.
In: Progress in Mutation Research vol. 4, edited by A.T. Natarajan et al. p. 193-202 (1982).

10. N.G.J. Jaspers and D. Bootsma
Genetic complementation analysis of ataxia telangiectasia by somatic cell fusion.

In: Host and Factors in Human Carcinogenesis, IARC Scientific Publications no. 39, p. 127-136 (1982).

11. A.J.R. de Jonge, P.J. Abrahams, A. Westerveld and D. Bootsma
Expression of human hprt gene on the inactive X chromosome after DNA-mediated gene transfer.
Nature 295, 624-626 (1982).

II. Short Communications, Theses, Internal Reports, Patents.

E. van der Veer.
Microanalysis of gene expression in cultured cells.
Thesis, Erasmus University Rotterdam, 1982.

Progress Report
1982

Contractor:
Medical Research Council
MRC
Park Crescent 20
GB-London W1N 4AL

Contract no.: BIO-E-414-81-UK

Head(s) of research team(s):
Prof. B.A. Bridges
Cell Mutation Unit, MRC
University of Sussex
GB-Falmer, Brighton BN1 9QC

General subject of the contract:
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J. W. I. M. Simons, University of Leiden (BIO 407 NL).

Title of Project No. 1: GENETIC ANALYSIS OF DNA REPAIR

Head of Project: Dr. C. F. Arlett

We continue to collect new material into our stocks either as cell strains from skin biopsies or as cultures established in other laboratories. We have now obtained a set of fibroblast cell strains from individuals suffering from autoimmune diseases, notably Behcets syndrome, where there is already some evidence for defects in the repair of chemically induced DNA damage. Other skin fibroblast cultures have been established from individuals who exhibited a brisk radiation response following radiotherapy. We have expanded our collection of fibroblast cultures from Bloom's syndrome, Cockayne syndrome (CS) and xeroderma pigmentosum (plus heterozygotes). The collection now includes some lines from normal (MRC5 CV1), ataxia-telangiectasia (AT5BIVA) and xeroderma pigmentosum (XP12RO and XP20S) individuals that have been transformed with SV40 virus, and lymphoblastoid lines from normal and autoimmune individuals and from patients suffering from A-T, CS and XP. Some lines or cell strains of interest are being marked with 6-thioguanine or ouabain resistance.

Our efforts to clone the genes corresponding to the defect in different ataxia-telangiectasia (A-T) complementation groups are dependent on the feasibility of transfer of radiation resistance to A-T recipients. During the year we have made some progress, and also ruled out some approaches.

Transfer of the plasmid pSV2, which contains the *E.coli gpt* gene linked to the SV40 promoter, followed by xanthine/mycophenolic acid selection has proved successful with a number of cell lines and we are able in some cases to obtain frequencies of transfer better than 10^{-3}. This gene would appear to be extremely suitable for co-transfer as a dominant selectable marker in our experiments. We have also found that those SV40 transformed human fibroblast lines that we have tested (MRC5 CV1, AT5B1VA and XP12RO) are particularly good recipients in DNA mediated gene transfer.

On the negative side, in our attempts to improve discrimination between radiation resistance and sensitivity, it appears from our results that low dose irradiation of non-growing cells may only provide a large enhancement of survival with certain cell lines (see Project 3). Moreover, it is not possible to achieve prolonged non-growth conditions for SV40 transformed fibroblasts.

We have therefore adopted selection strategies that should be effective in isolating transformants with only modest increases in radiation resistance. We have also been unsuccessful in using lymphoblastoid cell lines as recipients in DNA mediated gene transfer and have not persisted with this approach in view of the excellent results obtainable with AT5BIVA. The main disadvantages of SV40 transformed fibroblasts are that SV40 transformation itself may affect DNA repair and that only a limited number of repair-deficient SV40 transformed cell lines are available.

We have now reached the stage that experiments are in progress to obtain co-transfer to AT5BIVA or radiation resistance (using wild-type human genome DNA) and gpt (using the plasmid pSV2).

Title of Project No. 2: BIOCHEMICAL ANALYSIS OF DNA REPAIR

Project Leader: Dr. A. R. Lehmann

1. Studies using cells from individuals with Cockayne Syndrome (CS).

Work described in our previous reports has shown that in UV-sensitive CS cells, DNA and RNA synthesis following UV-irradiation fail to recover to the levels seen in unirradiated cells, even though CS cells appear to have normal levels of both excision- and daughter-strand repair. The failure of RNA synthesis to recover after UV-irradiation has been used in a genetic complementation test for CS, preliminary results of which were described last year. We have now extended these complementation assays, and we have assigned eleven CS strains to three groups, two to group A, eight to group B and one (from a patient who also had xeroderma pigmentosum) to group C.

A recent publication from Fujiwara and co-workers (Expl. Cell Res., 139, 207-215, 1982) suggested that CS cells had reduced levels of NAD, and that many of the cellular defects of CS could be reversed by addition of NAD. We have failed to reproduce these observations. In our hands two CS strains had normal levels of NAD, and addition of NAD to the growth medium had little effect on the recovery of DNA and RNA synthesis in CS cells.

Studies on RNA synthesis in CS showed a lack of correlation between excision repair and the recovery of RNA synthesis. In addition, in normal cells the recovery of RNA synthesis occurred much more rapidly than excision-repair. Nevertheless we have found that, in normal cells, aphidicolin or cytosine arabinoside, which inhibit the repair synthesis step in excision-repair, also inhibit the recovery of RNA synthesis. Since this inhibition was observed even in non-dividing cells, it could not be mediated by effects of these inhibitors on replicative DNA synthesis.

2. Poly (ADP-ribose) and DNA repair.

We previously showed that synthesis of poly (ADP-ribose) was involved in the ligation step of excision-repair after alkylation damage in human fibroblasts, although it did not appear to play a significant role in the repair of radiation damage. Creissen and Shall (Nature, 296, 271-272, 1982) have now shown that DNA strand-breaks give rise to a stimulation of the activity of DNA ligase II. This stimulation can be inhibited by 3-aminobenzamide (3AB), a specific inhibitor of the synthesis of poly (ADP-ribose). This suggests that DNA breaks synthesis of poly(ADP-ribose) activation of DNA ligase II. We have postulated that the function of poly (ADP-ribose) in DNA repair is to reduce the steady-state level of breaks in cells after treatment with mutagens (such as alkylating agents) which result in the continuous production of DNA breaks. The stimulation of DNA ligase II mediated by poly (ADP-ribose) synthesis results in breaks being sealed more rapidly and in a decreased steady-state level of breaks.

3AB prevents the stimulation of ligase activity, and the steady-state level of breaks remains high. We have confirmed our hypothesis by first allowing the synthesis of poly (ADP-ribose) after alkylation damage, so that the level of breaks is reduced. Subsequent addition of 3AB some time later leads once more to an increase in the steady state level, presumably because the activity of DNA ligase II is again reduced.

3. Studies on cell strain 46BR.

The cellular hypersensitivity of this cell strain (derived from an immunodeficient individual) to a broad range of DNA-damaging agents was described in Project 3 last year. 46BR cells are particularly hypersensitive to alkylating agents, but removal of alkylated bases appears to occur at normal rates. DNA strand-breaks after alkylation treatment, however, disappear more slowly in 46BR than in normal cells. Repair synthesis is somewh at elevated. In addition to the hypersensitivity to radiation and alkylating agents, these cells are also very sensitive to the lethal effects of 3-aminobenzamide, the inhibitor of poly (ADP-ribose) synthesis described above. Taken together, these observations have led us to speculate that 46BR cells may be defective in DNA ligase I. This would make the cells more dependent on DNA ligase II, whose activity can be depressed by 3-aminobenzamide via its inhibitory action on poly(ADP-ribose) synthesis. The activities of DNA ligases in 46BR are at present under investigation.

Title of Project No. 3: THE CONSEQUENCES OF DNA DAMAGE AND REPAIR

Project Leaders: Dr. C. F. Arlett and Dr. M. H. L. Green

In the previous report we indicated that the techniques which reveal a lack of repair of potentially lethal damage (PLD) from ionizing radiation in A-T cell strains may allow a better discrimination between cell strains of intermediate hypersensitivity. This study has been extended and the repair of PLD in serum deprived cells investigated in a total of 24 cell strains. A number of important observations have emerged:-

(i) there is variation in the extent to which normal cell strains may repair PLD;

(ii) in one family segregating the A-T gene, the heterozygote (parents) have a limited capacity to repair PLD;

(iii) in Huntington's disease, two cell strains which we believe to be hypersensitive under standard assay conditions show limited capacity to repair PLD while a third cell strain (HD2BR) which has normal sensitivity has a normal capacity to repair PLD;

(iv) a cell strain from an individual with Gorlin's syndrome shows no capacity to repair PLD. This is a particularly significant observation since such individuals are believed to be radiosensitive but their cells are not hypersensitive under standard assay conditions;

(v) two cell strains from Fanconi's anaemia show limited repair of PLD;

(vi) a cell strain (47BR) established from an individual with an extreme erythemal response to radiotherapy and whose cells showed no significant hypersensitivity under standard assay conditions has no capacity to repair PLD. We are now establishing a collection of cell strains from such individuals (see Project No. 1) to determine the extent of this important defect.

Our studies on the repair of PLD in SV40 transformed material have shown that the cells do not cease division in medium with 0.5 per cent foetal calf serum and rapidly lose their ability to clone in the alternative arginine depleted medium. This limitation on the utility of SV40 transformed cells places some constrainsts on the procedures for selecting wild-type transformants amongst A-T cells (see Project no. 1).

A major effort has been devoted to the measurement of induced micronuclei in fibroblast cultures following treatment with ionising radiation. A data base has been established with three normal and one A-T cell strain using a fluorescent technique which has been validated against a standard Giemsa staining method. We have examined the influence of non-cycling conditions on the repair of lesions which give rise to micro-

nuclei and find that cells with limited capacity for the repair of PLD in survival experiments are also limited in their capacity to reduce the frequency of micro-nuclei. This reduced ability has been confirmed in AT5BI, 46BR and 47BR.

Studies of gamma-radiation induced mutation to 6-thioguanine resistance have been extended to the SV-40 transformed cells and confirm the hypomutability of A-T cells. Further attempts have been made to mutate Cockayne syndrome cells with UV and the results are consistent with our earlier observations that such cells are hypermutable when compared with normals.

A preliminary assessment of the ouabain resistance system has been made with SV40 transformed cells and has made it possible to select spontaneous mutants resistant to 4×10^{-7}M ouabain as marker.

PUBLICATIONS

Arlett, C.F., Harcourt, S.A., Teo, I.A. and Broughton, B.C. (1982) The response of ataxia-telangiectasia fibroblasts to the lethal effects of an array of DNA-damaging agents, in Ataxia-telangiectasia - A Cellular and Molecular Link between Cancer, Neuropathology and Immune Deficiency (eds. B.A. Bridges and D.G. Harnden) pp. 169-176, John Wiley and Sons Limited.

Harnden, D.G. and Bridges, B.A. (1982) Ataxia-telangectasia (A-T) - a model of cancer susceptibility, in Ataxia-telangiectasia, a Cellular and Molecular Link between Cancer, Neuropathology, and Immune Deficiency (ed. B.A. Bridges and D.B. Harnden) pp. 3-10, John Wiley and Sons, Chichester.

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Progress Report
1982

Contractor:
Aarhus University
Department of Chemistry
Langelandsgade 140
DK-8000 Aarhus C

Contract no.: BIO-E-416-81-DK

Head(s) of research team(s):
Dr. J.E. Celis
Chemistry Institute
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General subject of the contract:
Changes in gene expression resulting from primary mutational events accompanying X-ray irradiation induced transformation in human cells.

List of projects:

1. Use of X-ray irradiated cells in a search for differences at the polypeptide synthesis level between normal and malignant cells.

Title of project nr. BIO-E-416-81-DK

Use of X-ray irradiated cells in a search for differences at the polypeptide synthesis level between normal and malignant cells

Head of project and scientific staff: *J.E. Celis (Head of Staff)*
P.Mose Larsen
S.J. Fey
A. Celis

Fifty three transformation sensitive polypeptides common to various cell types have been identified in cultured human cell lines. The synthesis of many of these polypeptides is also sensitive to changes in growth rate and a significant number have been identified in other species. Even though the normal role of these proteins is unknown, it is possible that some may be components of the cellular pathway that control cell proliferation. Such a fundamental pathway would be expected to be strongly conserved during evolution showing similar changed upon transformation irrespective of the species origin of the cell. None of these proteins, however, is similar to any of the known viral *oncogenes* or the cellular *oncogenes* identified by transfection experiments.

During the past year we have invested a great deal of effort attempting to prepare antibodies against most of the transformation sensitive polypeptides recovered from two dimensional gels. Mouse polyclonal antibodies have been raised against very few of these polypeptides and their specificity is now being determined. These antibodies should be of value for purifying these proteins for functional studies and will provide a rapid probe to screen large numbers of normal and tumour tissue using indirect immunofluorescence.

To validitate the results obtained with cultured human cells, it has been essential to carry out similar comparative studies of tumours and normal tissue. With this in mind we have concentrated our studies on the human skin and their tumours. Due to the heterogeneity of the tissues, however, it has not been possible to compare directly the polypeptide patterns and therefore it has been necessary to search for techniques that may

allow us to dissect the various cell types present. Two lines of experimentation have been used to achieve this goal:

1. preparation of monoclonal antibodies against surface markers for various cell types in the human skin. The basic idea of this approach is to use specific antibodies against surface markers in conjunction with the cell sorter to isolate specific cell types. A battery of monoclonal antibodies has now been prepared and their specificity is being determined.

2. Microsurgery: A microsurgery technique has been developed for isolating related groups of cells from skin sections 10 μ in thickness. It is expected that microsurgery of [^{35}S]-methionine labelled samples may allow us to compare small numbers of normal and tumour cells.

List of publications

Architecture and polypeptide composition of HeLa cell cytoskeletons. Modification of cytoarchitectural proteins during mitosis. Bravo, R., Small, J.V., Fey, S.J., Mose Larsen, P. and Celis, J.E. *J. Mol. Biol.*, 154, 121 (1982).

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Progress Report
1982

Contractor:
The Polytechnic of Central London
PCL
Regent Street 309
GB-London W1R 8AL

Contract no.: BIO-E-479-81-UK

Head(s) of research team(s):
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Prof. G. Holt
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General subject of the contract:
Studies of radio-induced recombination in eukaryotic cells.

List of projects:

1. Radio-induced recombination in eukaryotic cells.

Title of project: Radio-induced recombination in eukaryotic cells

Head of project and scientific staff: Dr. P. Cohn
Dr. D. Kundu
Mr. C. Igwe
Prof. G. Holt

In order to establish a system capable of detecting mitotic recombination induced by ionizing radiations the characterization of hybrid phenotypes has continued. Several different autosomal markers were investigated for their suitability for insertion into the parental genome. These included resistance to ouabain (1mM), hydroxyurea (1mM) and to vinblastine (1μg ml^{-1}). Attempts to isolate temperature-sensitive variants at the HGPRT locus yielded clones that have not been fully tested. Physical markers such as polystyrene microspheres and particles of inactivated carbon were also used; they were added to cells from a clone which took them up by phagocytosis into the cytoplasm where they remained visible for several generations.

At the HGPRT locus variants possessing a range of enzyme activities arose either spontaneously or after mutagenesis following treatment with ethyl methane sulphonate or ICR-191. These agents would be expected to produce point and frameshift mutations respectively. For fusion in the presence of polyethylene glycol variants expressing less than ten percent of the activity of the wild type were chosen. Phenotypically hybrid clones were then isolated and subjected to a second round of selection for autosomal markers. Cell survival was compared in non-selective (HAT) medium and selective medium which contained 6-thioguanine. Two phenotypes were initially displayed with respect to the HGPRT locus among the 44 clones isolated.

Table 1. Phenotypes of Hybrid Clones of HGPRT Variants

Type	Number of Clones	6-TG	HAT
		Medium	
I	2	sens.	res.
II	7	res.	sens.
III	4	res.	res.
IV	31	sens.	res.

Type III and IV clones initially had phenotype II.

In all the four types of clones average chromosome numbers were scored at intervals of seven days. After one month of continuous culture only five percent of cells of phenotype II contained supernumerary chromosomes. By the end of two months all cells had chromosome numbers similar to those found in wild type cells. Clones of phenotype II showed spontaneous reversion frequencies of less than 10^{-7} per cell. For experiments on recombination these cells were treated with radiation and for comparison with chemical recombinogens
Cells were then isolated after growth in HAT medium. This phenotype was now similar to type IV. No deviation from the diploid chromosome number was noted and HGPRT activities were less than ten percent of that of the wild-type.

Progress Report
1982

Contractor:
Institut National de la
Recherche Agronomique
INRA
Rue de Grenelle 149
F-75007 Paris

Contract no.: BIO-E-419-81-F

Head(s) of research team(s):
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General subject of the contract:
Study of irradiation effects on genetic markers.

List of projects:

1. Research on the nature of radio-induced genetic events in the a_1 - a_2 system of tobacco and study of their relative frequencies in terms of varying doses of acute and chronic irradiation - search for repair deficient mutants.
* 2. Study of the genetic effects of low and very low external and internal chronic irradiations (natural and artificial) - comparison and interactions with other environmental factors.

* Project 2 : see Delpoux, M. CNRS Gif-sur-Yvette BIO 430 F

CONTRACTOR :

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Contract N°
BIO-E-419-81-F

HEADS OF THE RESEARCH TEAMS :

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GENERAL SUBJECT OF THE CONTRACT :

Study of irradiation effects on genetic markers.

Research on the nature of radio-induced genetic events on the a_1-a_2 system of tobacco and study of their relative frequencies in terms of varying doses of acute and chronic irradiation - search for repair deficient mutants.

1 REACTIONS CARACTERISTIQUES DES DIFFERENTS GENOTYPES AU RAYONNEMENT

Dans le rapport 81, on a montré que parmi dix génotypes résultant de combinaisons différentes aux loci a_1^+-a_1-a_1^o-a_1^a et a_2^+-a_2^{yg}-a_2, on pouvait distinguer des groupes dans lesquels la proportion de variations vertes est largement prédominante par rapport aux variations claires. La proportion de variations jumelées reste toujours minoritaire.

Parmi les génotypes les plus intéressants, le double hétérozygote a_1^+/a_1 a_2^+/a_2 (J^1) est couramment utilisé dans les expériences précédentes ainsi que dans les expériences réalisées sur le terrain (DELPOUX et al.) ; le génotype a_1^+/a_1^o a_2/a_2 (J^{11}) ne portant plus qu'un seul gène sauvage et ne possédant pas d'allèle a_1 antimorphe possède des propriétés assez différentes avec un phénotype identique.

On a donc cherché à comparer les réactions de ces deux génotypes sur des effectifs cellulaires équivalents et en fonction des doses chroniques pour lesquelles la relation dose-effet est linéaire chez J^1.

ESSAI 1

Trois doses également espacées ; deux génotypes ; trois répétitions.

Le génotype a_1^+/a_1 a_2^+/a_2 est dans sa zone de réponse linéaire avec une augmentation (pente) très significative en ce qui concerne le taux cellulaire de réversions (cellules vertes).
Le génotype a_1^+/a_1° a_2/a_2 est dans une zone de pente nulle (Tableau 1).

Tableau 1

	J^1			J^{11}		
Doses (Rads)	64	128	192	64	128	192
$\hat{p}.10^4$	6.49	12.51	46.02	2.64	1.89	2.80
	3.43	18.21	17.19	1.89	1.62	1.85
	7.51	12.51	44.39	1.50	2.06	1.17
Totaux	17.43	42.23	107.60	6.03	5.57	5.82
$\bar{p}.10^4$	5.81	14.4	35.9	2.01	1.86	1.94
points ajustés	3.66	18.70	33.75	Pas de réponse à l'augmentation de dose dans cette zone ; b=0		

pente : b = 0.235

On constate un taux plus faible qui correspond donc aux possibilités de variation que possèdent l'un et l'autre génotype a_1^+/a_1 a_2^+/a_2 et a_1^+/a_1° a_2/a_2, par ailleurs isogéniques.

ESSAI 2

Les deux groupes de plantes ont été irradiées à des doses croissantes plus élevées. Le nombre de plages (réversions, comprenant donc les plages vertes isolées et les plages vertes jumelées)

a été compté dans des zones foliaires équivalentes au point de vue ontogénique et sur des surfaces (rondelles découpées à l'emporte-pièce) égales. Le nombre d'événements est dans ces conditions proportionnel à p (taux cellulaire de réversion) (cf. DULIEU & DALEBROUX, 1975, Mut. Res. *30*, 63-70).

Tableau 2

	J^1				J^{11}			
Doses (Rads)	100	200	300	400	100	200	300	400
Nombre de plages	26	75	146	270	7	23	39	51
	37	79	148	299	6	29	44	47
	16	113	178	247	5	16	47	94
	34	99	176	224	9	17	63	106
	113	366	648	1040	27	85	193	298
Totaux ajustés	82,3	388,6	694,9	1001,2	12,6	104,7	196,8	288,9
	b_1 = 3.063				b_{11} = 0.921			
	a_1 = -224				a_{11} = -7.95			
	r_1 = 0.994				r_{11} = 0.992			
	Rapport $b_{1/b_{11}}$ = 3.32							

Les résultats traduisent une réponse linéaire dans les deux génotypes avec une pente beaucoup plus faible pour le génotype J^{11}.

ESSAI 3

Les mêmes individus, irradiés aux mêmes doses que précédemment, ont été observées plus tard sur cinq feuilles entièrement développées et de niveau ontogénique équivalent. A ce stade, il est possible de repérer les plages du type vert, plus clair ou jumelées sans aucune ambiguïté. La proportion de ces trois catégories de plages a donc été calculée.

Tableau 3

Nombre de plages		100	200	300	400	Totaux	P
J^1	Vertes	46	149	134	260	589	0.66
	Jaunes	20	35	60	128	243	0,27
	Jumelées	4	11	12	38	65	0.07
		70	195	206	426	897	-
J^{11}	Vertes	5	6	21	35	67	0.20
	Blanches	29	63	76	76	244	0.72
	Jumelées	5	3	12	6	26	0.08
		39	72	109	117	337	-
Rapport $J^1/_J11$							
- pour les réversions		5	17,8	4,42	7,27	7,03	-
- pour les plages claires		0.69	0.55	0.79	1,68	1.00	

Les proportions des trois types de plages sont peu influencées(*) par la dose comme le montre le Tableau 4. On peut donc estimer les taux des différents types d'événements à partir des nombres de plages et de leurs proportions, appliqués aux résultats du Tableau 1

Tableau 4

	$J^1$400R 1981		$J^1$400R 1982		J^1total doses 1982	
Vertes	900	0.61	260	0.61	589	0.66
Jaunes	484	0.33	128	0.30	243	0.27
Jumelées	91	0.06	38	0.09	65	0.07
	1475	-	426	-	897	-
	J^{11}400R 1981		J^{11}400R 1982		J^{11}total doses 1982	
Vertes	50	0.18	35	0.30	67	0.20
Blanches	213	0.76	76	0.65	244	0.72
Jumelées	16	0.06	6	0.05	26	0.08
	279	-	117	-	337	-

(*) Ceci ne serait rigoureusement vrai que dans les modèles de régression $Y_i = \beta x_i$, ce qui n'est pas le cas ici.

Tableau 5

Hypothèse : Rapport N_{tot} réversions $J^1/_{Ntot}\ J^{11} \simeq 7{,}00$							
		J^1			J^{11}		
		Vert	Clair	Jum.	Vert	Clair	Jum.
$p^{-4}\cdot 10$	(64R)	3.31	1.35	0.35	0.37	1.33	0.15
	(128R)	16.91	6.92	1.79	1.91	6.87	0.76
	(192R)	30.51	12.48	3.24	3.44	12.39	1.38

Bien entendu, les chiffres obtenus reflètent la régression linéaire estimée à partir des données obtenues sur les réversions (Tableau 1). Cependant avec la seule valeur du Rapport Ntot rév.$J^1/_{Ntot\ rév.}J^{11}$, on retrouve des rapports pour chaque catégorie de plages entre J^1 et J^{11} très voisins des nombres obtenus au tableau 4.

	Estimation			Total Tableau 4		
Rapport	V.	Clair	Jum.	V.	Clair	Jum.
$J^1/_{J}11$	8.87	1.00	2.35	8.79	1.00	2.5

Le modèle est donc acceptable et peut être utilisé pour discuter de la nature génétique des variations.

Si l'on se réfère théoriquement à la nature des évènements qui conduisent aux trois types de variations chez les deux génotypes, on a le tableau suivant (Tableau 6).

	Vert	Clair	Jumelé
J^1	deletion a_1 conversion $a_1 \rightarrow a_1^+$	deletion a_1^+ " a_2^+ conversion $a_1^+ \rightarrow a_1$	recomb. ou translocation $\frac{a_1^+}{a_1}\ \frac{a_2^+}{a_2}$ ou $\frac{a_1^+}{a_1}\ \frac{a_2^+}{a_2}$ $\frac{a_1^+}{a_1}\ \frac{a_2^+}{a_2}$ ou $\frac{a_1^+}{a_1}\ \frac{a_2^+}{a_2}$
J^{11}	Conversion $a_1^\circ \rightarrow a_1^+$	deletion a_1^+	recomb. ou translocation $\frac{a_1^+}{a_1^\circ}\ \frac{a_2}{a_2}$ ou $\frac{a_1^+}{a_1^\circ}\ \frac{a_2}{a_2}$

On peut en tirer les indications suivantes :

1. Le taux cellulaire égal de plages claires (jaunes chez J^1, blanches chez J^{11}, indique que l'essentiel de celles-ci ne peut être dû qu'à la délétion au locus a_1^+.

2. La fréquence 8,8 X plus élevée de plages vertes chez J^1 indique le faible poids des évènements du type conversion dans ce type de variations qui résultent donc à 88,7 % de délétions du gène a_1. Il y a malgré tout une discordance entre le taux de délétions de a_1^+ et de a_1 : 44,99 pour 20,75 ; ceci reste inexpliqué.

3. La fréquence 2,5 X plus élevé de plages jumelées chez J^1 est approximativement proportionnelle aux nombres de possibilités d'effectuer une recombinaison ou une translocation, théoriquement 2 X plus élevée chez ce génotype.

En conclusion, une majorité des variations que l'on peut estimer à 85,5 %, résultent de délétions simples, 7 % résultent de recombinaisons et/ou translocations réciproques et 7,5 % d'autres évènements non réciproques du type conversion. La majorité des variations induites semblent donc résulter de cassures non réparées.

2 ESSAIS D'IRRADIATIONS DU GENOTYPE J^3 EN CULTURES *IN VITRO*

Le clone J^3, multiplié végétativement *in vitro* par micro-bouturage est capable de répondre à l'irradiation par des variations jaune-vert, vert et albinos sur les tissus foliaires jaune clair.

Le génotype initial de J^3 est hétérozygote pour un seul locus marqueur : a_1^+/a_1 a_2/a_2. La régénération de cellules provenant des taches JAUNE-VERT a permis de réaliser leur analyse génétique qui donne toujours le même résultat : le génotype des plantes jaune-vert issues

des tissus variants est $a_1^+/-\ a_2/a_2$; il y a donc en perte par délétion de tailles indéterminées du gène marqueur antimorphe a_1.

La régénération des plages vertes, plus rares, donne des plantes de génotype $a_1^+/a_1^+\ a_2/a_2$. Elles peuvent résulter de crossing-overs somatiques concernant le chromosome R, porteur du locus $a_1^+-a_1$.

Les variants albinos n'ont pas pu être étudiés en descendance mais il est raisonnable de supposer qu'ils résultent soit de délétion du gène a_1^+ soit de crossing-overs concernant le chromosome R, notamment comme produits réciproques des variants verts.

La feuille est un organe à croissance finie réalisée après un nombre de cycles cellulaires assez invariant. En effet, avant la fin de la croissance en longueur de l'ébauche foliaire, les cellules ne se divisent plus ; la taille définitive, variable selon les conditions de milieu, dépend de l'accroissement du volume cellulaire.

L'irradiation aiguë peut ainsi être réalisée au stade Go de cycle cellulaire et suivie de la réinduction du cycle en culture *in vitro*.

2.1. Effets Dose sur la Croissance

En irradiation chronique, par doses de 4 Gy, on observe une stimulation significative à 1 Gy, suivie par un effet dépressif linéaire. La dose réduisant la croissance de 50 % étant de l'ordre de 16 Gy.

En particulier, si l'irradiation n'est pas suivie immédiatement par la reprise d'activité cellulaire, on observe une diminution plus rapide de la croissance ; la dose 50 étant de 14 Gy. (Huit jours de latence).

L'intensité de morphogenèse (induction de bourgeons sur les explants foliaires) étant maximale pour 1 Gay dans les deux cas, on a considéré que cette dose correspond encore à une activité maximale des biosynthèses de réparation.

2.2. Effet de l'irradiation sur les nombres de bourgeons variants, appliguée en phase de latence ou en phase de multiplication

2.2.1 *Expérience sur fragments de feuilles (cf. Rapport 1981)*

Tableau 7

	Irradiation 400 R avant mise en régénération	400 R après mise en régénération
Nb variants	314	99
Nb bourgeons observés	9767	5786
$\hat{p}$	$3.21 \cdot 10^{-2}$	$1.71 \cdot 10^{-2}$

2.2.2 *Expérience sur colonies cellulaires provenant de protoplastes*

Tableau 8

		Non irradié	T0	T2	T8
Variants	V	5	78	42	47
	α	5	34	16	50
Nombre de colonies observés ($X10^3$)	N	5266	4048	2612	6762
	$\hat{p}V$	0,9	19,3	16,1	7,0
	$\hat{p}\alpha$	0,9	8,4	6,1	7,4
	$\hat{p}tot$	1,8	27,7	22,2	14,4

T0 = irradié avant la mise en culture (400 R)

T2 = irradié 2 jours après la mise en culture (400 R)

T8 = irradié 8 jours après la mise en culture (400 R)

2.2.3 Expérience d'irradiation avant/après mise en régénération suivie d'un temps de latence à l'obscurité (48 H)

Tableau 9

Lot I avant mise en régénération		Lot II après mise en régénération
Xi	fi	fi
0	12	5
1	11	5
2	5	4
3	2	2
4	0	1
	$\overline{X}$ = 0.9	$\overline{X}$ = 1.35

Le tableau 9 présente les distributions du nombre de variants par boite de culture. Le nombre de bourgeons n'a pas été estimé ; il est de loin plus élevé dans le lot II. On ne peut donc retenir ces estimations comme significatives.

CONCLUSION

1. Les types de variations observées peuvent être induites dans des cellules au repos.
2. Lorsque les cellules irradiées sont en activité cyclique, les fréquences tendent à diminuer.
3. Les mécanismes de réparation liés à l'activité cyclique semblent donc plus efficaces que le mécanisme de réparation du type excision.
4. Les variations résultent très vraisemblablement de lésions non réparées par les systèmes sans-erreur, et transmises telles quelles à la lignée cellulaire. Elles sont éventuellement réparées par recombinaison entre chromatides homologues, engendrant alors des plages jumelées sur les feuilles.

2.3. Essai d'irradiation du génotype a_1^+/a_1 a_2^+/a_2 (J[1]), en fonction du cycle journalier.

Afin de vérifier si la radiosensibilité des cellules change dans la plante entière avec le moment de la journée, ce que laissaient prévoir les résultats précédents, on a réalisé quatre irradiations de 400 R sur des individus 1h30 avant l'allumage des lampes et 1h30, 4h30 et 7h30 après l'allumage. Le Tableau 10 donne les estimations des taux cellulaires de réversions et le Tableau 11 l'analyse de variance.

Tableau 10

	- 1,5		+ 1,5		+ 4,5		+ 7,5
$\hat{p}x10^5$	911		955		1572		990
	1309		1583		1233		1439
	1131		1526		1370		1356
	3351		4064		4175		3785
$\hat{p}x10^5$	1117	$<$ **	1355	ns	1392	ns	1262

Tableau 11

		dl	CM
Traitements	19 834 049	3	6611350 **
Erreur	493 034	8	61629
	20 327 083	11	-

Ceci confirme donc les résultats des années antérieures, qui mettaient en évidence un effet important de l'heure d'irradiation sur la sensibilité globale de la population cellulaire manifestant une certaine synchronisation du cycle des cellules en fonction de la photopériode.

3 RECHERCHE DE MARQUEURS UTILISABLES EN CULTURE CELLULAIRE

3.1. Isolement de mutants sub-alléliques au locus a_1^+

Afin de disposer de génotypes hétéroalléliques de phénotype albinos capables de régénérer la fonction chlorophylienne par recombinaison intragénique dans les cellules somatiques, on a réalisé des traitements mutagènes sur des cellules au stade zygote, de génotype hétérozygote a_1^+/a_1 a_2^+/a_2^+.

Parmi la population de plantules normalement vert clair, on a recherché les individus de phénotype jaune. Parmi 42 mutants, une quinzaine manifestent des ségrégations monofactorielles et ne complémentent pas l'allèle a_1. On a alors construit des génotypes double-hétérozygotes pour ces nouveaux allèles capables de ségréger des individus albinos. L'intercroisement permet alors la ségrégation des individus albinos hétéroalléliques ; ceux-ci sont actuellement en cours de test d'aptitude à la réversion *in vitro*. Les souches manifestant cette propriété (une seule a été isolée à ce jour) vont permettre les tests *in vitro* tant de l'effet des rayonnements que des agents chimiques, sur un matériel biologique utilisable aussi sous forme d'individus organisés.

3.2. La recherche de mutants déficients pour le processus permettant la recombinaison somatique sera faite sur ce type de matériel.

Progress Report
1982

Contractor:
Landbouwhogeschool Wageningen
Salverdaplein 10
NL-6701 DB Wageningen

Contract no.: BIO-E-402-81-NL

Head(s) of research team(s):
Dr. Ir. P. de Boer
Lab. Erfelijkheidsleer
Gen. Foulkesweg 53
NL-6703 BM Wageningen

General subject of the contract:
The exploration of methods for evaluating the induction of autosomal meiotic non-disjunction in the mouse.

List of projects:

1. The exploration of methods for evaluating the induction by ionizing irradiation of autosomal meiotic non-disjunction in the mouse.

Title of project nr BIO-E-402-81-NL
The exploration of methods for evaluating the induction of autosomal meiotic non-disjunction in the mouse.

Head of project and scientific staff:
P. de Boer (head)
J.H. Nijhoff
P.J.J. Wauben-Penris.

The incidence of meiotic non-disjunction can nowadays be assessed most accurate by counting the number of chromosomes in secondary meiocytes, i.e. in cells after meiosis I but before meiosis II. However, in large scale studies on the induction of meiotic non-disjunction the time consuming aspect of determining the number of chromosomes per cell to estimate the percentage of aneuploid ones is a serious disadvantage while moreover the true incidence of non-disjunction can only be estimated via cells which have just passed both meiotic divisions. For male mammals, both points of critisism can be overcome with Flow Cytometry (FCM). With this method the DNA content per spermatid can be determined at high velocity - 100 to 1000 cells per second - after quantitative staining of the DNA with fluorescent dyes. The adoption of FCM for meiotic non-disjunction studies in male mice has been a main goal in the past year.

Except, of course, a good quantitative staining of the DNA and a good detection system the requirements we have to meet with regard to the biological aspects of the system are the following:
1) Meistrich et al. (Nature 274, 1978, 821-823) had shown that a difference of 3.5% in the haploid DNA content can be determined via FCM.This implies that non-disjunction must take place for chromosomes which represent each at least 3.5% of the haploid DNA content and in a frequency, sufficiently high to be recognized in a DNA histogram. To facilitate this approach, we used two translocation heterozygous stocks: Rb(11.13)4Bnr/+ with 10-15% non-disjunction products after meiosis I for chromosome no. 11 and an equal percentage for chromosome no.13, and T(1;11.13S)/+ T(1;13)7OH with 15% non-disjunction for chromosome no. 11 and 8% for the long marker chromosome 13^{1}. Based on chromosome

length differences chromosome 11 and 13 represent 4.5%, marker chromosome 13^1 10.6% of the haploid DNA content.

2) Round spermatids, that are the first stages in development after the second meiotic division, represent the only haploid cell stages appropriate for quantitative DNA staining. Contamination with elongating spermatids and spermatozoa is avoided here by pretreating the mice with Hydroxyurea (350 mg/kg b.w.). This chemical kills spermatogonia in the S-phase and so a gap of 16 days in spermatogenesis was introduced via two series of three injections at 12 hours intervals with between the first injections of the two series a period of 7 days. Animals are sacrificied when the front cells have repopulated the round spermatid stage during two days; testes are then found to be free of gametes at later stages of development. Enzymatically, a single cell suspension of spermatogonic cells was produced.

The estimation of the non-disjunction frequencies must be done via the separate peaks in the DNA histograms obtained after flow cytometric analysis of the round spermatids. Fitting of the DNA distributions is done by standard non-linear least squares, using weights according to a Poisson distribution of the observations. An appropiate computer program for such an analysis has now been developed by us.

At present measurements of the DNA content with a coefficient of variation (cv) of 3% for the haploid fraction are achieved after staining with ethidiumbromide (0.02 mg/ml). In practice this appeared to be exactly the border line: a slightly lower cv will make computer aided analysis of the obtained histograms possible.

List of publications in 1982

I. P.P.W. van Buul, P. de Boer. Induction by X-rays of chromosomal aberrations in somatic and germ cells of mice with different karyotypes. Mutation Res. 92 (1982) 229-241.

P.J.J. Wauben-Penris, S.C. van Buul-Offers. Meiotic non-disjunction in male snell dwarf mice. J. Hered. 73 (1982) 365-369.

II. J.H. Nijhoff, W.R.R. ten Broeke, A. Keen, K.J. Puite. Flow cytometry to study meiotic non-disjunction: preliminary results with translocation carrying male mice. Abstract in: The combined international conference on analytical cytology and cytometry IX and VI th international symposium on flow cytometry. 18-22 October, 1982.

Progress Report
1982

Contractor:
Université Paul Sabatier
Route de Narbonne 118
F-31077 Toulouse Cédex

Contract no.: BIO-E-430-81-F

Head(s) of research team(s):
Dr. M. Delpoux
Service de Botanique
et Biogéographie
Allées J. Guesde 39
F-31077 Toulouse Cédex

General subject of the contract:
Study of irradiation effects on genetic markers.

List of projects:

* 1. Research on the nature of radio-induced genetic events in the a_1 - a_2 system of tobacco and study of their relative frequencies in terms of varying doses of acute and chronic irradiation - search for repair deficient mutants.
2. Study of the genetic effects of low and very low, external and internal, chronic irradiations (natural and artificial) - comparison and interactions with other environmental factors.

* Project 1 : see Dalebroux, M. INRA Dijon. BIO 419 F

CONTRACTOR :

Université Paul Sabatier
118 Route de Narbonne
F-31077 TOULOUSE CEDEX

Contract N° :
BIO-E-430-81-F

HEADS OF THE RESEARCH TEAMS :

Dr. M. DELPOUX and Dr. M. DALEBROUX
Laboratoire de Botanique (Cf. Contr. n° BIO-E-419-81-F)
et Biogéographie
39 ALlées Jules Guesde
F-31077 TOULOUSE CEDEX

Centre d'Ecologie des Ressources Renouvelables
Centre National de la Recherche Scientifique
29 rue Jeanne Marvig
F-31400 TOULOUSE

GENERAL SUBJECT OF THE CONTRACT :

Study of irradiation effects on genetic markers.

Study of the genetic effects of low and very low external and internal, chronic irradiations (natural and artificial) - Comparison and interaction with other environmental factors.

1 STUDY OF THE EFFECTS OF LOW DOSES OF NATURAL RADIATIONS

1.1 External irradiation

1.1.1 Continuation of the experiment started in 1981

The experimental design has been given in the 1981 Report, together with the measured reverted leaf areas. In 1982, a first cell density has been calculated, but we ran into severe trouble with the only microscope available in the laboratory. The work will resume in December 1982 - January 1983.

CONTRACTOR :

Université Paul Sabatier
118 Route de Narbonne
F-31077 TOULOUSE CEDEX

Contract N° :

BIO-E-430-81-F

HEADS OF THE RESEARCH TEAMS :

Dr. M. DELPOUX and Dr. M. DALEBROUX
Laboratoire de Botanique (Cf. Contr. n° BIO-E-419-81-F)
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Centre d'Ecologie des Ressources Renouvelables
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29 rue Jeanne Marvig
F-31400 TOULOUSE

GENERAL SUBJECT OF THE CONTRACT :

Study of irradiation effects on genetic markers.

Study of the genetic effects of low and very low external and internal, chronic irradiations (natural and artificial) - Comparison and interactions with other environmental factors.

1 STUDY OF THE EFFECTS OF LOW DOSES OF NATURAL RADIATIONS

1.1 External irradiation

1.1.1 Continuation of the experiment started in 1981

The experimental design has been given in the 1981 Report, together with the measured reverted leaf areas. In 1982, a first cell density has been calculated, but we ran into severe trouble with the only microscope available in the laboratory. The work will resume in December 1982 - January 1983.

1.1.2. Work performed in 1982 on the same site as in point 1.1.1

1.1.2.1 Modification of the design

Gradient 2 (cf. 1981 Report) had its origin at 0.5 mrd/h and increased by equally spaced doses of 1.5 mrd/h up to 8 mrd/h. It has been modified as follows :

0.015 (background ≃ 0), 1, 2, 3, 4, 5, 6, 7, 8 mrd/h,

so that the statistical handling of the data, taking the background level into account, will be easy since the control is included within a complete set of equally spaced doses.

1.1.2.2 Experiment on the a_1^+/a_1 a_2^+/a_2 system of *Xanthi* tobacco

Forty 3 to 4-leaf carrier-plants were placed at the beginning of July 1981 into each of the plots of gradients 1 and 2 ; counting the plot that yields the background level (0.015 mrd/h) as a common control, this made 19 different plots altogether. The plants were left to grow 15 to 20 additional leaves up to October 10, and than brought to the Laboratory where the search for green reversion on the last two leaves started. The data, expressed as usual as reversion rates, will be available by April 1983, approximatively.

1.1.2.3 Search for *waxy* mutants

Barley plants were grown on the device during the Spring of 1982 (30 individuals on each of the 19 plots). The search for *waxy* mutants had started when the Assistant in charge of the research resigned. A solution is sought so that the research can be carried on as soon as possible.

1.2 Internal irradiation

It is planned to assess eventual genetic effects induced by irradiation (especially alpha) emitted by radionuclides incorporated into living organisms. The use of α-radioisotopes has been proposed. In 1982, a preliminary experiment was carried out by growing a_1^+/a_1 a_2^+/a_2 tobacco in substrates having various uranium contents. These substrates had been made by mixing radioactive and nonradioactive soils in adequate proportions to create a 7-dose rate gradient : 0.015 (background control), 0.6, 1.2, 1.8, 2.4, 3.0 and 3.6 mrd/h (cf. 1981 Report). No seed germinated on the two most radioactive substrates, whereas just a few did germinate on the 2.4 mrd/h substrate. Germination was normal on all the other substrates. The real cause for such a situation has not been elucidated. Twenty one plants were studied on each of the first four substrates, and 23 on the 2.4 mrd/h soil. Up to now, the reverted areas have been measured. The total leaf areas and cell densities are still to be evaluated so that the corresponding reversion rates can be calculated. This will be completed at the beginning of 1983. Furthermore, the plants studied have been calcined and soil samples kept in order to

- help interpret the results ;
- prepare, if significant genetic modifications are observed, further experiments with controlled contaminations

2 STUDY OF THE EFFECTS OF OTHER ENVIRONMENTAL FACTORS

2.1 Natural factors (soils developed from geologically different substrates)

In 1981, most of the tobacco plants grown in eight such soils did poorly or even died ; this was most probably due to mineral deficiencies (cf. 1981 Report). Correction have been made accordingly, but the work load of the year has not yet allowed a new experiment. This will be done in the Spring of 1983.

2.2 Artificial factors

Efforts have been focused in 1982 on the study of the effects of atmospheric pollution in order to provide a final answer to the questions asked in the program proposal and to assess the relative importance of the effects, already detected, of low level radioactivity.

2.2.1 *In situ effects of differently polluted atmospheres in the urban area of Toulouse and its Mural surroundings*

As already explained in the 1981 Report, tobacco carrying marker system a_1^+/a_1, a_2^+/a_2 was grown in 18 different stations scattered within 6 urban and suburban zones :

- Zone 1, rural, to which no pollution could be brought by dominants winds ; may be considered as a control zone :
  Station 1 a (XVI in 1981 Report)

- Zone 2, rural, exposed to dominant winds :
  Station 2a, Donneville (XVIII)
  Station 2b, Auzeville (XVII)

- Zone 3, near-outskirts
  Station 3a, Pouvourville (XV)
  Station 3b, Raymond Naves (XIV)

- Zone 4, downtown Toulouse
  Station 4a, Ramier du Parc (X)
  Station 4b, Grand-Rond (XI)
  Station 4c, Chambre du Commerce (XII)
  Station 4d, Bibliothèque Municipale (XIII)

- Zone 5, around the refuse-destructor of Mirail
  Station 5a, Monlon Experimental Farm (VIII)
  Station 5b, Monlon Experimental Farm (IX)
  Station 5c, Larrieu (VII)

- Zone 6, in the vicinity of industrial plants
  Station 6a, Chemin des Etroits (I)
  Station 6b, Pech David (II)
  Station 6c, Chemical Engineering Center (III)
  Station 6d, Bordelongue (IV)
  Station 6e, Creusot-Loire (V)
  Station 6f, Palayre (VI)

Two experiments were carried out, one in August 1981 and one in September 1981. The results, expressed as reversion rates are presented in the following table :

Zones	Stations	Mean reversion rate ($p \times 10^5$)	
		August 1981	September 1981
1	1a	0.505	0.408
2	2a	0.508	0.602
	2b	0.517	0.563
3	3a	0.610	0.706
	3b	0.711	0.688
4	4a	0.853	0.663
	4b	0.671	0.503
	4c	0.872	0.893
	4d	0.763	0.471
5	5a	0.548	0.748
	5b	0.547	0.609
	5c	0.609	0.619
6	6a	0.893	0.525
	6b	0.678	0.773
	6c	0.735	0.772
	6d	0.796	0.608
	6e	0.655	0.616
	6f	0.660	0.540

These results give rise to many comments that will be full developed in a paper in preparation. However, some important preliminary remarks can be made : attention should be drawn on the fact that, when tobacco is exposed to the action of a radioactive source, the material is constantly submitted to ionizing radiations ; this is really chronic exposure. When one deals with *in situ* experiments on exposures to differently polluted atmospheres, it is clear that the material is not continuously submitted to the action of the same factor, due mainly to meteorological fluctuations which may increase or decrease the influence of certain mutagenic pollutants. This will have to be taken into account when comparisons are made with the effects of ionizing radiations.

2.2.2 *Perfecting of a cultivation device liable to improve the assessment of in situ genetic effects of atmospheric pollution*

The experiment above shows an overall increase in reversion rate for a_1^+/a_1 a_2^+/a_2 tobacco exposed to urban and industrial environment as compared to that observed in rural areas. This experiment was necessary in order to grossly assess the genetic impact of atmospheric pollution. Of course, it is to be kept in mind that rural stations differ from urban or industrial areas not only for pollution but also for a wide variety of characteristics, as climatic and microclimatic differences. Furthermore, given its ecological requirements, the a_1^+/a_1 a_2^+/a_2 carried tobacco can be grown in the open in Summer only, whereas pollution usually reaches a maximum in Winter (stagnation of cold air, fog). In order to eliminate ecological differences between stations, and to allow experimentation even in Winter, two cultivation devices, different only for the nature of the airflow forced through them, have been constructed :

- one, equipped with a series of adequate filters that make it dust- and pollution-free ;
- one through which a natural airflow is forced.

Both devices (≈ 1 m^3), air-tight, are otherwise identical (light, temperature, watering). These two little greenhouses will be placed in a station known to possess a heavily polluted atmosphere. Since they are easy to handle, it will be possible, to more them at will, and at low cost, from station to station.

2.2.3 *Analytical study, in the Laboratory, of two atmospheric pollutants : benzopyrene and dimethylnitrosamine*

This preliminary study is meant to prepare the investigation on interactions between radiations and chemical pollutants. After a thorough study of the literature on BP and DMNA (characteristics, presence in the environment, metabolism and biological effects), a preliminary experiment was designed to determine the BP and DMNA contents liable to induce genetic changes in the a_1^+/a_1 a_2^+/a_2 system ; tobacco seeds were soaked in

(i) DMSO containing various quantities of BP : 0.5, 1, 10, 20, 30, 40, 50, 100, 500 ppm ;

(ii) peanut oil containing 0.05, 0.1, 1, 10, 20, 30, 40, 50, 100, 500, 1000, 1500, 2000, 3000, 4000, 5000, 10 000 ppm of DMNA.

Control seeds were soaked in peanut oil, 50µl/l water solution of DMSO, and water. The observations have started, and the final interpretation of the data (germination, somatic variations on the first two true leaves) will be completed in 1983.

PUBLICATIONS

- Due to the duration of experiments, and to the fact that some results require confirmation, the publication of the conclusions will be possible only by the end of 1983.

- Poster entitled "Essai d'évaluation des effets génétiques de la pollution atmosphérique" presented at the "IV^{ème} Symposium sur la recherche en matière de pollution atmosphérique" (Ministère de l'environnement)", Arles, France, by B. Devaud, M. Delpoux and M.A. Dalebroux.

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* *

Progress Report
1982

Contractor:
Centre National de la
Recherche Scientifique
CNRS
Quai Anatole France 15
F-75007 Paris

Contract no.: BIO-E-426-81-F

Head(s) of research team(s):
Dr. R. Devoret
Laboratoire d'Enzymologie
CNRS
F-91190 Gif-sur-Yvette

General subject of the contract:
Genetic effects induced by radiation and chemical carcinogens : mechanisms common to DNA repair, replication, recombination and mutagenesis.

List of projects:

1. Repair, mutagenesis and induction of dormant viruses following DNA damaging treatments in E. coli.

Title of project nr 1:

Repair, mutagenesis and induction of dormant viruses following DNA damaging treatments in E. coli.

Head of project and scientific staff:

R. DEVORET, A. BAILONE, A. LEVINE, A. BRANDENBURGER

RESULTS OBTAINED IN 1982

1- Radioprotective effect of native RecA protein

RecA protein is induced by DNA damage to reach 10 % of the total of cellular proteins (Paoletti et al, 1982; Moreau, Pelico and Devoret, 1982). No other cellular protein is induced by radiations to such an extent.

We have demonstrated that cell survival is roughly proportional to the cellular concentration of native RecA protein.

More important, RecA protein has a radioprotective effect. When we increased artificially the cellular level of RecA protein before irradiation, cell survival increased dramatically in radiation-sensitive mutants and substantially in wild type cells (Quillardet, Moreau, Ginsburg, Mount and Devoret, 1982).

2- Activation of RecA protein to a protease controls induced mutagenesis

Inducible mutagenic repair is controlled by RecA protease whose activation is not proportional to the concentration of native RecA protein. Inducible mutagenesis results from the proteolytic inactivation of the LexA represssor, the basic cellular repressor of SOS genes, including the uvr and umu genes, which govern excision repair and mutagenesis.

We have quantified "damaged-site independent mutagenesis". We monitored mutagenesis occurring on intact viral DNA introduced into a cell whose SOS genes had been induced before the infection. Mutations arising on intact phage occurred at late times during the phage replication cycle. Intact phage did not mutate in hosts carrying the uvm-25 mutation known to prevent the occurrence of UV-reactivation. These findings suggest that damaged-site independent mutagenesis results from inducible error-prone repair as does damaged-site dependent mutagenesis. Damaged-site independent mutagenesis accounts for about half of the mutations that arise upon inducible error-prone repair. This fact entails a limitation of mutagenic specificity of DNA damaging agents.

Mutations of phage DNA constitutes a sensitive assay for the detection of damaged-site independent mutagenesis since it replicates a hundred-fold

and the host chromosome once per half hour, period corresponding to the time of induction of SOS genes (Quillardet and Devoret, 1982).

3- Specificity of cleavage of repressors by RecA protease

There is no correlation between the kinetics of repressor cleavage and of the accumulation of native RecA protein. Repressor inactivation is dependent on the activation to a protease of RecA protein but independent of the induction level of the protein (Moreau et al, 1979).

The recA430 mutation suppresses the proteolytic activity of RecA protein and therefore prophage induction. Yet, prophage 80 is induced in a recA430 lysogen as is prophage 21, though to a lesser degree, and LexA repressor is inactivated as shown by the induction of RecA protein.

The recA430 mutation alters the specificity of RecA protease: the farther from lambda repressor conformation, the more cleavable a repressor is (Devoret, Pierre and Moreau, 1982).

4- Complementation of recA mutations reveal the structure of RecA protease

The phenotypes of lexB and recA proper mutations have revealed the existence of two functional domains in RecA protein, the lexB domain governing the proteolytic action on LexA and phage repressors (Devoret, 1981).

We found that cell radioresistance can be restored by complementation of two recA$^-$ alleles. Proteolytic cleavage of repressors by the composite RecA protein was also recovered, indicating that RecA proteolytic activity is expressed after formation of a multimer (Rebollo, Moreau, Collado, Blanco and Devoret, 1983).

5- Aborted DNA replication triggers the expression of SOS genes

We have found that introduction of a UV-irradiated mini-F plasmid into 80 or lambda lysogens induce those prophages (Devoret, Levine, Pierre, and Couturier, to be published). The mini-F plasmid acts as does a UV-damaged whole F plasmid in activating RecA to a protease in the recipient cell (Moreau, Pelico and Devoret 1982). If plasmidic DNA replication is prevented there is no protease activation. Aborted DNA replication leads to a perturbed DNA initiating structure that constitutes the SOS signal.

List of publications in 1982

I. Scientific Journals

Moreau, P. L., Pélico, J. V., and Devoret, R., 1982, Cleavage of lambda repressor and synthesis of RecA protein induced by transferred UV-damaged F sex factor, Mol. Gen. Genet. 186: 170-179

Quillardet, P., and Devoret, R., 1982, Damaged-site-independent mutagenesis of phage lambda produced by inducible error-prone repair, Biochimie 64: 789-796

Quillardet, P., Moreau, P. L., Ginsburg, H., Mount, D. W., and Devoret, R., 1982, Cell survival, UV-reactivation and induction of prophage lambda in Escherichia coli K12 overproducing RecA protein, Mol. Gen. Genet. 188: 37-43

Lévine, A., 1982, Lysogens for "Laboratory" lambda have the repressor level of lysogens for "natural" lambda, FEMS Microbiol. Letters 15: 219-222

II. Theses

Sagliocco, F., 1982, Induction par les agents mutagènes et cancérigènes d'un changement épigénétique stable et héritable chez E. coli K12: Etude de mutants non inductibles, Diplôme d'Etudes Approfondies, Orsay p. 1-59

Progress Report
1982

Contractor:
Commissariat à l'Energie
Atomique, CEA
CEN de Fontenay-aux-Roses
F-92260 Fontenay-aux-Roses

Contract no.: BIO-E-398-81-F

Head(s) of research team(s):
Dr. B. Dutrillaux
Institut de Progénèse
Rue de l'Ecole de Médecine 15
F-75006 Paris

General subject of the contract:
Radiation induced and transmissible chromosome aberrations : Comparative sensitivity of mammals, risk assessment in man and interference of genetically radiation - sensitive constitutions.

List of projects:

1. Comparison of chromosomes and their mutagenesis in different species of mammals.
2. Risk estimate of chromosome aberration after induction of structural rearrangements in man.
3. Detection of radiosensitive persons by a cytogenic test.

Projet n° 1.
Comparaison des chromosomes et de leur mutagenèse chez les différentes espèces de mammifères.

Chef du projet : B. DUTRILLAUX
Equipe scientifique : J. COUTURIER
M. MULERIS
M. PARAVATOU
E. VIEGAS-PEQUIGNOT

Résultats obtenus.

1) Cytogénétique comparée : parmi les primates, une étude approfondie des cercopithécoïdes a été terminée. Elle porte 42 espèces et sous espèces, appartenant aux différentes familles et sous familles. Les homoeologies entre tous les chromosomes de toutes les espèces ont pu être proposées, ainsi que leur correspondance avec les chromosomes humains. De plus, un schéma phylogénétique précis a été établi. L'étude de ce groupe de primates présente un grand intérêt, puisque 3 des genus les plus utilisés en laboratoire (Macaques, babouins et cercopithèques) s'y rencontrent (ref. 1).

L'étude des gibbons a été également complétée, montrant le grand nombre de remaniements survenus dans ce groupe(ref.2). Chez les rongeurs, l'étude des Gerbillidae a permis de montrer l'existence de remaniements complexes des chromosomes sexuels (ref. 3 et 4). Enfin chez les carnivores, une reconstitution du caryotype ancestral a pu être réalisée, après la comparaison de nombreuses espèces (ref. 5).

2) Mutagenèse chromosomique :

L'étude de 2 espèces a été particulièrement avancée : Chez le chimpanzé Pan troglodytes, une étude qualitative des remaniements induits par les rayons γ est en voie d'achèvement, et a fait l'objet d'un rapport de Diplôme d'Etudes Approfondies (ref. 13).
Chez le macaque Macaca fascicularis, une étude semblable est au stade de la préparation d'une publication.

Enfin, l'analyse d'autres espèces est en cours de développement.

Projet n° 2
Estimation du risque d'aberration chromosomique après induction de remaniements de structure chez l'homme.

Chef du projet : B. DUTRILLAUX
Equipe scientifique : J.L. ANTOINE
A. AURIAS
J. COUTURIER
M. PRIEUR
E. VIEGAS-PEQUIGNOT

Résultats obtenus en 1982.

L'étude des translocations induites dans les fibroblastes a été achevée (ref. 6). Elle a porté sur des remaniements induits "in vivo" (ref. 7, annoncée dans le rapport précédent) et "in vitro". Le résultat obtenu est tout à fait comparable à celui obtenu sur les lymphocytes : 2 translocations sur 5 sont susceptibles de jouer un rôle en pathologie, si elles existent dans les cellules germinales, car elles peuvent entraîner la naissance d'enfants à caryotype déséquilibré. Les 3/5èmes restant entraîneraient des déséquilibres chromosomiques trop grands pour être compatibles avec la vie.

Devant la similitude des résultats obtenus sur les lymphocytes et sur les fibroblastes, il est légitime de penser que les extrapolations de tissu à tissu, sont valables chez une même espèce. Il est donc probable que cette estimation s'applique effectivement aux cellules germinales, seules susceptibles de jouer un rôle dans la pathologie génétique.

L'accumulation des données sur les lymphocytes, dans le but de rechercher la transmission des anomalies chromosomiques de tous ordres a été poursuivie. Leur analyse pourra très probablement se faire en 1983, ainsi que la publication des résultats concernant les inversions péricentriques.

L'étude des remaniements induits en phase S a été très avancée. Elle fait l'objet d'une thèse de doctorat d'Etat, dont le rapport d'épreuve de confirmation a été présenté par J.L. ANTOINE aux Communautés Européennes en octobre 83 (ref. 14). Celui ci montre la très large dépendance des types de remaniements par rapport à l'avancement de la réplication de l'ADN. Une étude détaillée des figures radiales est en cours, afin d'estimer quel type de remaniements elles pourront donner à la génération cellulaire suivante. En addition des analyses faites sur les remaniements, nous avions montré que les positions des éléments ayant échangé des segments n'étaient pas aléatoires : ceux-ci étant trop rapprochés. L'étude des positions chromosomiques a donc été pour les translocations constitutionnelles (ref. 8), pour les translocations t(7;14) (ref. 9) et pour les remaniements affectant les chromosomes X (ref. 10).

Projet n° 3
Detection de sujets radiosensibles par test cytogénétique.

Chef de projet : A. AURIAS
Equipe scientifique : J.L. ANTOINE
B. DUTRILLAUX
M. PRIEUR

Résultats obtenus en 1982

1) Ataxie telangiectasie

L'étude des sujets atteints (A.T.), et de leurs parents (hétérozygotes pour le gène anormal) a été poursuivie à plusieurs niveaux.

- dépistage de nouveaux cas, avec mise en évidence des anomalies spécifiques des chromosomes 7 et 14 .
- surveillance cytogénétique des anciens cas.

Parmi ceux-ci, un sujet a été trouvé porteur d'un clone t(14;14) dans une sous population de lymphocytes stimulé par la phytohémagglutinine. L'utilisation de marquage a haute résolution a montré que l'un des points de cassure est différent de celui retrouvé dans les t(14;14) non clonales. La relation point de cassure-spécificité immunologique-caractère invasif des cellules parait très probable (ref. 11).

- mise au point d'une nouvelle méthode de détection, par utilisation de l'enzyme terminale-transférase, qui accroche des nucléotides préalablement marqués au tritium sur les extrémités 3'OH libre de l'ADN, ce qui permet de détecter les cassures au niveau moléculaire. Chez les sujets normaux, un marquage modéré est observé. Chez les 3 sujets AT étudiés, un marquage autoradiographique intense apparaît, permettant leur reconnaissance immédiate. La mise au point de cette méthode se poursuit en collaboration avec Mr. B. FERTIL du laboratoire de radiobiologie clinique (Institut Gustave ROUSSY). Elle permet les plus grands espoirs pour l'obtention d'un test très discriminant.

2) Anémie de Fanconi (A.F.)

L'analyse du cycle cellulaire de nombreux patients AF, et d'autres atteints de syndromes voisins fait l'objet d'une publication (ref. 12), qui montre le très fort ralentissement du cycle chez les sujets AF seulement.

Chez les parents (hétérozygotes), un ralentissement plus modéré semble exister et les études seront poursuivies pour savoir si cela permettra de développer une méthode de reconnaissance.

Des irradiations de cellules AF en phase S ont été effectuées, montrant leur très grande sensibilité à certains moments du cycle.

PUBLICATION DANS DES REVUES INTERNATIONALES

1- Chromosomal phylogeny of forty-two species or subspecies of cercopithecoids (primates catarrhini). DUTRILLAUX B., COUTURIER J., MULERIS M., LOMBARD M., CHAUVIER G. Ann. Génét. (1982) 25, n°2, 96-109.

2- Comparaisons chromosomiques chez quatre espèces de gibbons . COUTURIER J., DUTRILLAUX B., TURLEAU C., GROUCHY J. de. Ann. Génét. (1982) 25, 5-10

3- Complex evolution of sex chromosomes in Gerbillidae (Rodentia). VIEGAS-PEQUIGNOT E., BENAZZOU T., DUTRILLAUX B., PETTER F. Cyto. Cell. Gent.(1982) 34, 158-167

4- Phylogénie chromosomique des Gerbillidae II. Etude de 6 Meriones, de Taterillus gracilis et de Gerbillurus tytonis. BENAZZOU T., VIEGAS-PEQUIGNOT E., PETTER F., DUTRILLAUX B. Ann. Génét. (1982) 25, 212-217

5- The ancestral karyotype of Carnivora. Comparison with that of Platyrrhini. DUTRILLAUX B., COUTURIER J. Cytogenet. Cell. Genet. (sous presse)

6- Risk of chromosomal disease due to radiation : tentative estimate from the study of radiation-induced translocations in human fibroblasts. DUTRILLAUX B., VIEGAS-PEQUIGNOT E., MOUTHUY M., ANTOINE J.L., PROD'HOMME M., SPORTES M. Accepté pour publication dans Mutation Research.

7- Cytogenetic study of skin fibroblasts in case of accidental acute irradiation. MOUTHUY M. and DUTRILLAUX B. Mutation Research (1982) 95, 19-30

8- Position non aléatoire des chromosomes metaphasiques. III : position des chromosomes dans les translocations constitutionnelles. ANTOINE J.L., AURIAS A., DUTRILLAUX B. Ann. Génét. (1982) 25, 226-228

9- Position non aléatoire des chromosomes métaphasiques. IV : étude des translocations t(7.14)(p14;q12) et t(7.14)(q35;q12). AURIAS A., DUTRILLAUX B., ANTOINE J. L., PRIEUR M. Ann. Génét. (1982) 25, 22 -231

10- Position non aléatoire des chromosomes métaphasiques, II : effet de la condensation sur la position des chromosomes X. ANTOINE J.L., COUTURIER J., DUTRILLAUX B. Ann. Génét. (1982) 25, 223-225

11- Tandem t(14;14) in isolated and clonal cells from ataxia telangiectasia are different. AURIAS A., DUTRILLAUX B., GRISCELLI C. soumis pour publication

12- The cell cycle of the lymphocytes in Fanconi Anemia. DUTRILLAUX B., AURIAS A., DUTRILLAUX A.M., BURIOT D., PRIEUR M. Human Genetics sous presse.

RAPPORTS

13- M. PARAVATOU.
Diplôme d'Etudes Approfondies en Biologie Evolutive
PARIS

14- Sur l'analyse des remaniements induits au cours de la phase S, étude par le marquage aux analogues de bases : ségrégation et position des chromosomes.
J.L. ANTOINE
Rapport de confirmation de thèse doctorat d'Etat.
Université de LOUVAIN (Belgique)

Progress Report
1982

Contractor:

Gesellschaft für Strahlen- und
Umweltforschung mbH, GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

Contract no.: BIO-E-395-81-D

Head(s) of research team(s):

Dr. U. H. Ehling
Institut für Genetik
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

General subject of the contract:

Radiation-induced gene mutations in mice.

List of projects:

1. Radiation-induced dominant cataract mutations in mice.

Title of project no. 1:
RADIATION-INDUCED DOMINANT CATARACT MUTATIONS IN MICE

Head of the project and scientific staff:
U.H. Ehling, J. Favor, J. Graw, J. Kratochvilova

A comparison of radiation-induced dominant cataract (DC) and recessive specific locus (SL) mutations was extended to include 3+3 Gy dose X-irradiation in the present experiment. A total of 20 DC mutations in 45 429 offspring and 70 SL mutations in 47 516 offspring have been recovered. The frequencies of dominant and recessive mutations observed are both statistically different from their respective historical control rates (0/10691 and 8/113909). The relative yield of recessive to dominant mutations of the different doses ranged from 1.5 to 5.44 with an overall weighted mean of 3.35.

Experimental procedures

Fourteen-week old homozygous wild type (101xC3H)F_1 hybrid male mice were exposed to 3+3 Gy, 24 hr fractionation interval, X-ray dose. Immediately after irradiation each male was paired with an untreated test-stock female, homozygous recessive at the seven specific marker loci. Resultant offspring were scored for SL and DC phenotype variants when 3 weeks old. All presumed SL mutations were confirmed genetically by an allelism test. Presumed DC mutants were genetically confirmed by outcrossing to strain 101 mice.

Results

Results are tabulated below according to germ cells stage treatment. For purposes of comparison, similar results for previous dose points tested are included. Of the 31 SL mutations recovered in the present experiment, 20 have so far been confirmed, 1 was sterile, 3 died before testing and 7 remain to be tested. However, the phenotypes were such that one may confidently include the 11 variants which can not or are not yet confirmed. In addition to the 5 genetically confirmed DC mutations in the

present experiment, 55 phenotypic variants remain to be subjected to a genetic confirmation test. Therefore, the reported DC mutation rate is very likely an underestimate.

The wide range of values for mutation yields observed in post-spermatogonial stages and in particular the lack of effect observed in the present results certainly is a reflection of the small sample sizes that were screened. In the treated spermatogonial germ cell stage results, the presently reported SL and DC mutation yields were both greater than the respective single 6 Gy dose mutations rates.

Dose (Gy)	Germ cell stage	Source	Recessive Mutants No./Offspring	Recessive Mutants μ*	Dominant Mutants No./Offspring	Dominant Mutants μ*	μ*Rec/μ*Dom
0	-		6/103218	0.08	-		-
0	-		2/10691	0.27	0/10691	-	-
5.34	pg	Cs^{137}	3/1721	2.49	1/1721	0.29	8.57
6	pg	Cs^{137}	3/865	4.95	1/865	0.58	8.57
3+3	pg	X-ray	1/1127	1.27	0/1120	0	-
4.55+4.55	pg	Cs^{137}	2/272	10.50	1/272	1.84	5.66
5.34	g	Cs^{137}	7/10212	0.98	3/10212	0.15	6.66
6	g	Cs^{137}	14/11095	1.80	3/11095	0.13	13.84
3+3	g	X-ray	31/16993	2.60	5/14913	0.17	15.54
4.55+4.55	g	Cs^{137}	9/5231	2.46	6/5231	0.57	4.29

pg = post-spermatogonia
g = spermatogonia
μ* = (Mutations/Offspring/Loci)x10^4
For the dominant cataract test 20 loci were assumed.

Conclusions

The ratio of per locus mutation rates calculated for the SL and the DC tests at the different dose points ranged from 4.29 to 15.54 with a weighted mean of 9.57. Considering that the per locus DC rates were based on the assumption of a minimum of 20 loci screened, the results emphasize the large difference in the induced per locus mutation rates for SL and DC mutations. Regardless of whether this difference is due to an inherent lower per locus mutation rate to dominant than to recessive alleles or due to the selection of a set of unusually mutable loci in the SL test, the results indicate that in order to more accurately calculate genetic risk estimates for man, calculations should be based upon the corresponding experimental data.

I. Publications in Scientific Journals, Monographs, Proceedings

Ehling, U.H.: Cataracts - indicators for dominant mutations in mice and man. In: Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk. (Eds.: F.J. de Serres and W. Sheridan). Plenum Press, New York (in press)

Ehling, U.H., Favor, J., Kratochvilova, J., Neuhäuser-Klaus, A.: Dominant cataract mutations and specific locus mutations in mice induced by radiation or ethylnitrosourea. Mutation Research 92, 181-192 (1982)

II. Short Communications, Theses, Internal Reports, Patents

Favor, J.: The dominant cataract mutation test in mice. Mutation Reseach 97, 186-187 (1982)

Graw, J., Summer, K.-H.: Glutathione, glutathione-dependent and glycolytic enzymes in a dominant cataract in the mouse. Hoppe Seyler's Z. Physiol. Chem. 363, 965 (1982)

Progress Report
1982

Contractor:
Medical Research Council
Park Crescent 20
GB-London W1N 4AL

Contract no.: BIO-E-493-82-UK

Head(s) of research team(s):
Prof. H.J. Evans
Clin.& Popul.Cytogenetics Unit
Western General Hospital/MRC
Crewe Road
GB-Edinburgh EH4 2XU

General subject of the contract:
Spontaneous and radiation induced chromosome mutation and deletions of specific chromosome regions of the human karyotype which contain genes of known importance.

List of projects:

1. Spontaneous and radiation induced chromosome mutation and deletions of specific chromosome regions of the human karyotype which contain genes of known importance.

Title of project nr B10-E-493-82-UK

Spontaneous and radiation induced chromosome mutation and deletions of specific chromosome regions of the human karyotype which contain genes of known importance.

Head of Project: H J Evans

Scientific Staff: K E Buckton

Since the grant was awarded, we have identified 52 individuals and 8 families with diseases which may result from the person having a small chromosome rearrangement. Cells in prophase/prometaphase are in the process of being studied from these persons. We would hope to identify many more persons of interest in the coming year.

With the techniques that we are using for prophase banding, we do not obtain exactly the same banding patterns as given as standard in ISCN (1981), therefore we are drawing up our own profiles for each chromosome and determining which bands are subject to variation in normal individuals. At the present time this is being done by direct microscopic examination and from photographs, but in the coming year we hope to have the use of a computer aided scanner, which will automatically digitise the grey levels of the chromosomes, give us a banded profile and a variety of measurements.

Since the project is only in its first year, it is too early to present any results. Chromosome analysis of prophase/prometaphase is considerably more time consuming than metaphase chromosome analysis. We now have complete profiles of two chromosomes on which we have been particularly focussing our attention. These are chromosome 8 because of its known involvement in a variety of haematological diseases and the presence of at least two onc genes on the long arm and chromosome 20 which it has been suggested has a small deletion in the short arm in patients with familial medullary carcinoma of the thyroid (Men II).

Reference

ISCN (1981) An International System for Human Cytogenetic Nomenclature - High Resolution Banding (1981). Birth Defects: Original Article Series Vol XVII No5 (March of Dimes Birth Defects Foundation, New York 1981).

Progress Report

1982

Contractor:

Finseninstitutet
Strandboulevarden 49
DK-2100 Kobenhavn 0

Contract no.: BIO-E-455-81-DK

Head(s) of research team(s):

Prof. Dr. M. Faber
Finsenlaboratoriet
Finseninstitutet
Strandboulevarden 49
DK-2100 Kobenhavn 0

General subject of the contract:

DNA - repair capacity in patients with multiple malignant tumours.

List of projects:

1. Measuring methods for DNA repair capacity.
2. Epidemiological studies on groups of patients with multiple tumours.
3. Control studies on DNA repair capacity in other diseases.

Title of project No. 1 Measuring methods for DNA repair capacity

Head of the project and scientific staff:

M. Faber, K. Wallevik, B. Munch-Petersen, B. Squire

The following routine tests for examination of DNA repair capacity in human lymphocytes have been elaborated. All tne investigations are performed with Ficoll-isopaque isolated human lymphocytes.

a. DNA repair synthesis in the lymphocytes is determined as the netto-incorporation over 2 hours of ^{3}HTdR into UV irradiated cells kept under conditions where the spontaneous DNA synthesis is inhibited 18 J/m^2 UVR. The UV dose was monitored with an UVX digital Radiometer from Ultraviolet Productions.

b. In Vitro DNA Synthesis or spontaneous DNA synthesis is determined as the incorporation over 2 hours of ^{3}HTdR at optimal conditions for the lymphocytes.

c. Survival of lymphocytes after UVR or X-ray is determined as the ratio between the cell growth in cultures irradiated with various doses, and the cell growth in unirradiated control cultures. The cells are stimulated to growth with Phytoheamagglutinin added to the cells immediately after irradiation and the cells were incubated for 7 days and counted. The survival capacity of the cells is expressed by the dose, D_{75} (in J/m^2 or rad) where 75% of the cells survives.

d. DNA strand-breaks are determined by a fluorometric method. The principle is described by H.C. Birnboim and J.J. Jevcak, Cancer Res. 41, 1889--1892 (1982). The rate of unwinding of DNA in alkali increases with increased number of strand-breaks. After the alkaline unwinding the amount of single-stranded DNA is determined with Acridin orange, which fluoresces differently, whether bound to single-stranded or double-stranded DNA.

The analyses including the culture procedures have been made with the same media as other cultures in the laboratory where no long term difficulties have been met with.

Title of projects No. 2 and 3: Epidemiological studies on groups of patients with multiple tumours or other diseases

a. Seasonal variations in DNA repair capacity in human lymphocytes from healthy controls

The DNA repair capacity in controls after UVR have been investigated by repeated determinations of the DNA repair synthesis and the survival of the lymphocytes.

The results show a clear seasonal variation. From o1.1o.81 to 15.o5.82 the intraindividual variation in DNA repair synthesis was low, about 1o% (s.d.). The mean of 15 controls DNA repair synthesis was 5552 $\pm$ 56o (s.d.) cpm/2h/1o^6 cells. From medio May, however, extreme fluctuations occur in the DNA repair synthesis. In some controls, the results could fluctuate between 25oo and 8ooo within a few days. These fluctuations were observed until the beginning of October 1982, whereafter they ceased and the previous more constant pattern reoccurred.

Seasonal fluctuations were also observed in the DNA synthesis in the lymphocytes. In the 15 controls, the incorporation of ^{3}HTdR in cpm/2h/1o^6 cells was between 1ooo and 9ooo in the period o1.1o.81 - 3o.o6.82, increasing to between 25oo and 23,ooo in the period o1.o7.82 - o1.1o.82, thereafter decreasing to between 1ooo and 9ooo.

Furthermore, the survival of the lymphocytes after UVR changed dramatically during this experimental period. In the winter, from o1.11.81 - 15.o3.82, the D_{75} values (in J/m^2) were between 13 and 28. From 16.o3.82 to 3o.o6.82 the D_{75} values decreased to between 9 and 12 and a further decrease to between 2 and 6 was observed in the period o1.o7.82 - 3o.o9.82. From o1.1o.82 to the present the D_{75} values are between 4.5 and 9.5. There seems to be a correlation in the seasonal variation of DNA repair synthesis and survival of the lymphocytes, since the drastic decrease in the D_{75} values occurred during the period with the marked fluctuations in the DNA repair synthesis and the increase in the DNA synthesis.

These results raise the question, whether the lymphocytes are the correct cells to obtain the results wanted in this project.

b. DNA repair capacity in lymphocytes from patients with multiple skin tumours

The seasonal variations described above have interfered with these

investigations. When, however, the DNA repair synthesis was measured in the stable winter period, 01.10.81 - 15.03.82, the results gave a mean of 5537 ± 1981 (s.d.) cpm/2h/10^6 cells for 15 patients. 3 of the observations were above 4 below the limits of the control persons.

The survival capacity of the lymphocytes was measured in the winter period 01.11.81 - 15.03.82 in cells from 7 patients. The D_{75} values were between 8 and 13.6 J/m^2 and thus lower compared to the control persons which were between 13 and 28. Only two of the 7 patients had D_{75} values above 13 J/m^2.

After 15.03.82, the survival capacity has been investigated in 8 patients and if the results are corrected for the seasonal variations, 4 of these D_{75} values were below the control values and the other 4 D_{75} values were below the mean control values.

c. DNA strand-break repair after UVR and X-ray

The fluorometric determination of DNA strand-breaks allows investigation of DNA strand rejoining after DNA damage in nondividing cells, since no preincorporation of radioactivity is needed. The assay is simple and well suited for routine investigations.

The strand-rejoining after UVR and X-ray has been studied in lymphocytes from controls and demonstrates the characteristic difference in the repair of the two types of damage. Initially after a dose of 800 rad, only 50-60% of DNA remained doublestranded. After incubation for only 20 min. 85% of DNA was in a doublestranded form, and after 2 hours DNA was completely repaired. When the repair process was examined by ^{3}HTdR incorporation, the incorporation was nearly immeasurable.

In contrast, after damage with UV with a dose of 3 J/m^2 the maximal damage was observed 1-2 hours after irradiation. At this time, only about 40% was in a doublestranded form. After incubation for 5 hours 75% of DNA was in a doublestranded form. After incubation for 5 hours 75% of DNA was in a double stranded form. After a dose of 18 J/m^2 which is the dose, at which maximal DNA repair synthesis is observed, no rejoining is observed within 6 hours after irradiation and only 20-30% of the DNA was in a doublestranded form.

Progress Report
1982

Contractor:
Consiglio Nazionale delle
Ricerche, CNR
Piazzale Aldo Moro 7
I-00185 Roma

Contract no.: BIO-E-428-81-I

Head(s) of research team(s):

Dr. A. Falaschi
Istituto di Genetica Biochimica
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Via S. Epifanio 14
I-27100 Pavia

Dr. U. Bertazzoni
Istituto di Genetica Biochimica
ed Evoluzionistica, CNR
Via S. Epifanio 14
I-27100 Pavia

General subject of the contract:
Genetic effects induced by radiation and chemical carcinogens : mechanisms common to DNA repair, replication, recombination and mutagenesis.

List of projects:

1. Study of enzymes of DNA metabolism and isolation of subcellular DNA repair systems from animal cells.

Title of project no. 1
Study of enzymes of DNA metabolism and isolation of subcellular DNA repair systems from animal cells.

Head of project and scientific staff: F. Falaschi; U. Bertazzoni, G. Ciarrocchi, F. Cobianchi, E. Giulotto, F. Nuzzo, G. Pedrali-Noy, M.A. Pedrini, S. Riva, A.I. Scovassi, S. Spadari and M. Stefanini.

1) Enzymes of DNA metabolism (U. Bertazzoni, F. Cobianchi, A. Falaschi, S. Riva, A.I. Scovassi).

We have isolated and purified to homogeneity two classes of proteins which specifically stimulate DNA polymerase α from HeLa cells on model replication forks in vitro: a DNA-dependent ATPase and a family of SS-DNA binding proteins. These last proteins (purified from calf thymus) are heterogeneous with regard to both molecular weight (23-24 kdal) and stimulation. We have produced antibodies against the SS-DNA binding proteins and shown that the different molecular species are antigenically related.

By using a procedure that allows the renaturation of the DNA polymerase catalytic activity in situ after SDS-polyacrylamide gel electrophoresis, we have compared the active polypeptides present in extracts covering a wide evolutionary range, from prokaryotes to eukaryotes. Our results indicate that the structure of DNA polymerase has been highly conserved during evolution, so that an active fragment of mol. wt. $\geq$ 70 kdal is always found in prokaryotic enzymes and in the replicative species of eukaryotic and mitochondrial DNA polymerases.

Terminal deoxynucleotidyl transferase (TdT) has been used in combination with Adenosine Deaminase as enzymatic markers for the characterization of acute and chronic myeloma leukemia.

2) Subcellular DNA repair systems (G. Ciarrocchi, A.M. Pedrini).

The method for pyrimidine dimer titration in circular, supercoiled DNA has been modified in order to measure apurinic/apyrimidinic (AP) sites. The new assay involves the use of the M. luteus AP endonuclease as a probe for the presence of such damages. AP sites, titrated with this method, have been found to produce structural distortions resulting in a reduction of mobility of single topoisomers of a supercoilded DNA. The unwinding angle has been calculated to be -12°/AP site. In addition we have shown that the unwinding caused by UV radiation is certainly a consequence of the presence of pyrimidine dimers in the irradiated molecule. In fact the unwinding due to UV radiation can be photoreversed by the E. coli photoreactivating enzyme.

3) Study of inhibitors of DNA replication (S. Spadari, G. Pedrali-Noy)
a. Aphidicolin, a specific inhibitor of eukaryotic DNA polymerase α, has been used to control the proliferation of several human and murine neoplastic cells and to study DNA repair synthesis in plant protoplasts.
b. Suitable for the development of antiviral compounds are those nucleoside analogues whose corresponding triphosphates are better substrates or inhibitors to the virus-specified DNA polymerase than to the DNA polymerases of the host cell.
We have shown that several 5-alkylated analogues of thymidylate are incorporated into DNA more readily by DNA polymerases specified by different strains of Herpes Simplex virus I than by human or animal DNA polymerase α

4) Mutant cell lines resistant to Aphidicolin (G. Ciarrocchi, F. Cobianchi, A. Falaschi, G. Pedrali-Noy, S. Spadari)

The characterization of cell lines resistant to aphidicolin has started. One line can now grow in the presence of 4 μg/ml of the inhibitor, for which the parental line has a M.I.C. of about 0.1 μg/ml. We have found that this mutant has also acquired resistance to AraC, hydroxyurea and methotrexate. dCTP and dTTP intracellular levels are increased by about 10-fold, whereas dGTP and dATP are 20 and 25-fold higher than in the parental line, respectively. However both the level and catalytical properties of the ribonucleoside-diphosphate reductase are not altered in our mutant. Also in another independent line resistant to 2 μg/ml of aphidicolin the deoxyribounucleotide triphosphate pool is altered. In this case however the activity of the ribonucleoside-diphosphate reductase is increased by approximately 5-fold.

5) Isolation of in vitro DNA repair mutants (C. Mondello, F. Nuzzo, M. Stefanini)

In our search for mutants with altered sensitivity to mutagens from established mammalian cells lines, we investigated a procedure based on the selection of cells, deficient in DNA repair by killing normal cells after stimulating repair synthesis, in the presence of ^{3}H-thymidine.

Our results indicate that the radioactive thymidine killing method which has already been used to eliminate the S-phase cells, can be used for killing cells in other phases of the cell cycle by radioactivity incorporated during repair synthesis. Since the amount of thymidine incorporated to perform UDS is extremely small, the letal effect due to decay of tritium was reached by keeping the cells in liquid nitrogen. Using this procedure, we isolated from a CHO cell line two mutants whose sensitivity to UV irradiation is higher than that of parental cells.

List of publications in 1982

I. Scientific Journals, Proceedings

1. U. Bertazzoni, E. Brusamolino, P. Isernia, A.I. Scovassi, S. Torsello, M. Lazzarino and C. Bernasconi - Prognostic significance of Terminal Transferase and Adenosine Deaminase in acute and chronic Myeloid Leukemia. Blood 60, 685-692 (1982).

2. V. Bianchi, F. Nuzzo, A. Abbondandolo, S. Bonatti, E. Capelli, R. Fiorio, E. Giulotto, A. Mazzaccaro, M. Stefanini, L. Zaccaro, A. Zantedeschi and A.G. Levis - Scintillometric determination of DNA repair in human cell lines: a critical appraisal. Mutat. Res. 93, 447-464 (1982).

3. E. Capelli, M. Stefanini, E. Giulotto and F. Nuzzo - Validation of a DNA repair synthesis assay on pools of fresh and frozen-thawed lymphocytes. Mutat. Res. 104, 187-191 (1982).

4. G. Ciarrocchi and A.M. Pedrini - Determination of pyrimidine dimer unwinding angle by measurement of DNA electrophoretic mobility. J. Mol. Biol. 155, 177-183 (1982).

5. G. Ciarrocchi, B.M. Sutherland and A.M. Pedrini - Photoreversal of DNA unwinding caused by pyrimidine dimers. Biochimie 64, 665-668 (1982).

6. F. Cobianchi, G. Biamonti, G. Mastromei, A. Falaschi and S. Riva - A DNA dependent ATPase from HeLa cells. Biochem. Biophys. Res. Commun. 104, 402-409.

7. L. De Carli, A. Mottura, F. Nuzzo, G. Zei, M.L. Tenchini, M. Fraccaro, B. Nicoletti, G. Simoni and P. Mocarelli - Cytogenetic investigation of the Seveso population exposed to TCDD. Acad. Press Washington 292-317 (1982).

8. G. Damiani, A.I. Scovassi, S. Romagnoli, E. Palla, U. Bertazzoni and V. Sgaramella - Sequence analysis of heteropolymeric DNA synthesized *in vitro* by the enzyme terminal deoxynucleotidyl transferase and cloned in *E. coli*. Nucleac Acids Research 10, 6401-6410 (1982).

9. L. Kowalzick, K.K. Gauri, S. Spadari, G. Pedrali-Noy, J. Kuhne and G. Koch - Differential incorporation of thymidilate analogs into DNA by DNA polymerase and by DNA polymerases specified by two Herpes Simplex viruses. J. Gen. Virology 62, 29-38 (1982).

10. G. Mazza, M. Perego and S. Riva - A bifunctional plasmid carrying the recA gene of E. coli. Mol. Gen. Genet. 185, 397-403 (1982).

11. G. Mazza and S. Riva - DNA dependent ATPases in B. subtilis: properties and functions. Molecular cloning and Gene regulation in Bacilli. Academic Press. pp. 249-259 (1982).

12. P. Ghelardini, A.M. Pedrini and L. Paolozzi - The topoisomerase activity of TA amG39 mutant is restored in Mu lysogens. Febs letters 137, 49-52 (1982).

13. G. Pedrali-Noy, M. Belvedere, T. Crepaldi, F. Focher, and S. Spadari - Inhibition of DNA replication and growth of several human and murine neoplastic cells by aphidicolin without detectable effect upon the synthesis of immunoglobulins and HLA antigens. Cancer Res. 42, 3810-3813 (1982).

14. A.M. Pedrini - DNA repair and possible relationship to neoplastic transformation. Elettromedicali 2, 9 sept. (1982).

15. S. Spadari, F. Sala and G. Pedrali-Noy - Aphidicolin: a specific inhibitor of nuclear DNA replication in eukaryotes. Trends in Biochemical Sciences 7, 29-32 (1982).

16. F. Sala, E. Magnin, M.G. Galli, X. Dalschaert, G. Pedrali-Noy and S. Spadari - DNA repair synthesis in plant protoplasts is aphidicolin-resistant. FEBS Letters 138, 213-217 (1982).

17. M. Stefanini, A. Reuser and D. Bootsma - Isolation of chinese hamster ovary cells with reduced unscheduled DNA synthesis after UV irradiation. Somatic Cell Genetics. 8, 635-642 (1982).

18. A.I. Scovassi, S. Torsello, P. Plevani, G.F. Badaracco and U. Bertazzoni - Active polypeptide fragments common to prokaryotic, eukaryotic and mitochondrial DNA polymerases. The EMBO Journal 1, 1161-1165 (1982).

Progress Report

1982

Contractor:
Ente Nazionale per l'Energia
Elettrica, ENEL
Via G. B. Martini 3
I-00100 Roma

Contract no.: BIO-E-401-81-I

Head(s) of research team(s):
Prof. A. Farulla
Centro di ricerche biologiche
Via Cuboni 10
I-00197 Roma

General subject of the contract:
The problem of individual radiosensitivity in radioprotection : the use of radiosensitivity-tests for the screening of heterozygotes for the "chromosomal instability" syndromes.

List of projects:

1. Individual radiosensitivity in radioprotection : use of radiosensitivity-tests for screening of heterozygotes for "chromosomal instability" syndromes.

Title of project nr. 1: Individual radiosensitivity in radioprotection: use of radiosensitivity-tests for screening of heterozygotes for "chromosomal instability" syndromes.

Head of project and scientific staff: Prof. A Farulla
Prof. B. Dallapiccola, Prof. F. Mandelli, Prof. F. A. Manzoli, Prof. G. Naro

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Patients affected by genetically determined chromosome instability syndromes and even individuals heterozygous for most of these mutations are highly sensitive to DNA damaging agents and irradiation. In particular, increased clastogenic effects, resulting from cross-linking agents, agents inhibiting rejoining and γ-irradiation are well documented in Fanconi's anemia (FA) cells (Schroeder, Cytogenet. Cell Genet. 33, 119, 1982). Several arguments suggest that the primary defect in FA is to be expected in a very specific DNA repair mechanism. However, it is still unclear if impaired repair mechanisms are due to specific enzyme defects or to abnormalities of the enzyme's passage through the nuclear membrane (Wunder et al., Hum. Genet. 58, 149, 1981). The nuclear membrane itself could be affected, or the structure of the enzyme at a site not relevant for its catalytic activity might be altered. On the other hand, different experiments indicate suppression of chromosome breakage after treatment of FA cells with superoxide dismutase (SOD) (Nordenson, Hereditas 86, 147, 1977) and oxygen-dependence of the rate of chromosomal aberrations (Joenie et al., Nature 290, 142, 1981) suggesting involvement of the free-radical-scavenging-system, which is one of the major biochemical pathways of DNA protection. Furthermore, we have previously shown that butyl-hydroperoxyde (BHP) treatment significantly enhances the chromosomal breakage in FA homozygous and heterozygous lymphocytes (Farulla et al. EUR 1982). Following this reasoning, we have investigated the effects of two peroxydes and of the antioxidant sodium ascorbate in lymphocytes of FA patients and FA heterozygotes.

Materials and methods.

Lymphocyte cultures were set up in four FA homozygotes (FA/FA) and in three heterozygotes (FA/+) using TC 199 containing 15% human AB serum. Six distinct experiments were performed: 1. A series of cultures was grown by the standard short-term (72 h) microtechnique; 2. Cultures from each subject were exposed for 72 h to a non toxic concentration of diepoxibutane (DEB; 0.01 μg/ml); 3. Additional cultures were treated with DEB and sodium ascorbate (200 μg/ml) for 72 h; 4. Lymphocytes were exposed for 72 h to BHP (0.01 μg/ml); 5. BHP and sodium ascorbate were added for 72 h to cultures; 6. Cells were treated during last 24 h of culture with 1 ml of a 1:100 dilution of a 12 v/v % H_2O_2 solution.

Harvesting was performed according to standard technique. 100 Giemsa stained metaphases, with no less than 44 centromeres were selected for analysis in each experiment. On the whole, 4.000 metaphases have been examined. Chromatid and chromosome-type aberrations were classified according to ISCN (1981).

Results and discussion

The frequency of chromosome aberrations in standard lymphocyte cultures and in cultures treated with the cross-linking carcinogen DEB, BHP or H_2O_2 only or in association with the antioxidant sodium ascorbate, is summarized in Table 1.

In agreement with previous observations, the DEB addition dramatically increased the levels of spontaneous chromosome aberrations both in FA/FA (X^2 = 91.94) and in FA/+ (X^2 = 25.83) subjects. A similar although less drastic increase of chromosome breaks was induced by the BHP addition to the cultures of homozygotes (X^2 = 19.48) and heterozygotes (X^2 = 19.04). Comparable results have also been obtained in FA lymphocyte cultures treated with H_2O_2.

Sodium ascorbate was effective in preventing chromosomal breakage induced by DEB and BHP in FA/FA and FA/+ lymphocytes. In fact, this antioxidant was reducing the mean frequency of breaks from 76.5 % to 54.5 % (X^2 = 41.86) in FA/FA DEB treated cells, and from 58 % to 40.25 % (X^2 = 24.26) in BHP treated cells. Thus sodium ascorbate lowered the break rates of lymphocytes grown in the presence of DEB or BHP to levels closely comparable to those found in the standard patients' cultures. Similarly, in the presence of this antioxidant, the DEB and BHP-induced chromosomal breakage of FA/+ subjects was significantly suppressed (X^2 = 8.99 and 10.70), falling in the range of spontaneous levels.

The FA primary defect should involve a very specific DNA repair mechanism and not the free-radical-scavenging system. The high susceptibility of FA cells to cross-linking agents and the proficient response to mono-functional drugs or irradiation are consistent with this idea. However, our and related esperiments also indicate that a defect of the free-radical-scavenging system, possibly resulting as a secondary effect, could be one of the critical parameters for endogenous chromosomal breakage in FA. The enhancement of chromosomal breakage induced by the treatment of cells with agents generating oxygen radicals and the protection against chromosomal breakage produced by sodium ascorbate, which might scavenge more of DNA damaging product and prevent cross-links, well accord with this suggestion.

Publications:

Farulla A., Naro G. e coll.: Ricerche citogenetiche in soggetti professionalmente esposti alle radiazioni ionizzanti. - Securitas, 1982.

Farulla A. e coll.: Esperienze di controllo sanitario dei lavoratori professionalmente esposti a radiazioni. Réunion scientifique "Protection au cours de l'utilisation médicale del rayonnements ionisants et non ionisants" - Soc. Franç. Radioprotection, 1982.

Farulla A. e coll.: Studio dell'effetto dell'esposizione professionale a sostanze mutagene sull'evoluzione clonale del cariotipo nelle leucemie acute. Lav. Umano, 1982.

Dallapiccola B. e coll.: Studio citogenetico familiare delle pancitopenie associate ad instabilità cromosomica. in "Citogenetica oncologica", Edts. Esculapio, Bologna, 1982

Dallapiccola B. e coll.: Retrospective diagnosis of a Fanconi's anemia patient by dyepoxybutane (DEB) test results in parents. Hematologica, 1982.

Table 1. Frequency of chromosome aberrations (%) in standard lymphocyte cultures and in cultures treated with different agents in four FA homozygotes and in three FA heterozygotes.

Subjects	Standard cultures	DEB	DEB + sodium ascorbate	BHP	BHP + sodium ascorbate	H_2O_2
FA/FA 1	56	98	69	70	47	80
FA/FA 2	47	86	71	61	39	81
FA/FA 3	29	44	35	42	34	52
FA/FA 4	40	78	43	59	41	—
FA/+ 1	12	32	18	26	11	25
FA/+ 2	10	31	20	29	19	—
FA/+ 3	8	16	10	16	9	15

Progress Report
1982

Contractor:
Université Catholique
de Louvain, UCL
Halles Universitaires
Place de l'Université 1
B-1348 Louvain-la-Neuve

Contract no.: BIO-E-410-81-B

Head(s) of research team(s):
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Laboratoire d'Enzymologie
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General subject of the contract:
Radiobiology of mitochondrial DNA.

List of projects:

1. Repair of yeast mitochondrial DNA after gamma-irradiation.

Title of project n° 1

Repair of yeast mitochondrial DNA after gamma-irradiation

Head of project and scientific staff :

Françoise FOURY
André GOFFEAU
Livia BIANCHI
Jim BACKER

The respiratory function is essential to cell in most eukaryotes. Mitochondrial DNA is an important target for damages since it codes for several polypeptides of the inner mitochondrial membrane which are strictly required for the respiratory function. Past years we have isolated and characterized mutants which are altered in the mutability and in the repair of the mitochondrial DNA in the yeast *Saccharomyces cerevisiae*.

After ethylmethanesulfonate mutagenesis, the following classes of nuclear mutants have been isolated.

1) 11 antimutators determining at least seven complementation groups exhibit decreased spontaneous mutability of both mitochondrial DNA and nuclear DNA. Three mutants are also gamma-ray sensitive. Gamma-ray sensitivity and antimutability are produced by a unique nuclear gene.

2) 18 mutators determining at least ten complementation groups. Except one, all the mutations enhance the spontaneous mutation rate of mitochondrial DNA exclusively; 13 mutants have the interesting property to produce an increased number of spontaneous point mutations at 25°C and an increased number of large deletions (cytoplasmic petite or ρ^-) at 36°C. Such mutants are good candidates for specific alterations in the fidelity of mtDNA replication or in DNases playing role in repair.

3) About 10 mutants which have the same phenotype as the parental strain at 25°C but which loose their mitochondrial DNA upon incubation at 36°C. In other words at 36°C the mitochondira of these mutants are devoid of DNA. The mutants might be deficient in mtDNA replication at 36°C or in repair enzymes such as DNases.

4) Twelve mutants altered in mitochondrial DNA recombination. Three mutants have been characterized. They are nuclear, allelic and recessive. In these mutants the general recombination between linked and unlinked alleles is normal. However a site-specific recombination system is altered in the mutants. This site-specific involves the recognition of GC sequences and is

used for the integration of tandemly organized cytoplasmic petites into the wild type mtDNA genome. The use of this nuclear mutation has permited to demonstrate the existence of another site-specific recombination system for the mtDNA which involves inverted duplicated sequences and offers analogies with the transposon system in bacteria. These recombination-deficient mutants are also deficient in the repair of the mtDNA damaged by ethidium bromide, UV light and at a less extent by gamma-rays.

Conclusions : The importance of mtDNA in repair is demonstrated by the large number of genes which control this repair. Some genes control also the repair of the nuclear DNA but many genes exist which are involved only in the repair of the mtDNA. Some rare mutations exist which control both the mutability of the mtDNA and repair to gamma-rays. We also have shown that the recombination of mtDNA is required for the repair of damages by ethidium bromide, UV light and possibly gamma-rays.

List of publications in 1982

I. Publications in Scientific Journals

1) F. FOURY - Repair of mitochondria DNA in *Saccharomyces cerevisiae*. Induction of cytoplasmic petites in a nuclear mutant exhibiting a thermosensitive mitochondrial deoxyribonuclease activity.
J. Biol. Chem. (1982) 257, 781-787.
2) F. FOURY - Endonucleases in yeast mitochondria. Apurinic and manganese stimulated deoxyribonuclease activities in the inner mitochondrial membrane of *Saccharomyces cerevisiae*.
Eur. J. Biochem. (1982) 124, 253-259.
3) L. BIANCHI and F. FOURY - Antimutators of mitochondrial and nuclear DNA in *Saccharomyces cerevisiae*. Relationship with gamma-ray sensitivity.
Molec. gen. Genet. (1982) 185, 418-423.

II. Short Communications

1) F. FOURY - Endonucleases in the inner membrane of yeast mitochondria.
Arch. Int. Physiol. Biochim. 90, 29.
2) Nuclear mutants deficient in the transmission of mitochondrial DNA from ρ^- strains in *Saccharomyces cerevisiae*.
Eleventh International Conference on Yeast Genetics and Molecular Biology (Montpellier), p. 230.
3) J. KOLODINSKI and F. FOURY - Antimutators of mitochondrial and nuclear DNA in *Saccharomyces cerevisiae*.
Eleventh International Conference on Yeast Genetics and Molecular Biology (Montpellier), p. 229.

Progress Report
1982

Contractor:
Universität Göttingen
Goslerstrasse 5/7
D-3400 Göttingen

Contract no.: BIO-E-393-81-D

Head(s) of research team(s):
Prof. Dr. I. Hansmann
Institut für Humangenetik
Nikolausberger Weg 5a
D-3400 Göttingen

General subject of the contract:
Mechanisms of non-disjunction.

List of projects:

1. Mechanisms of radiation-induced non-disjunction and genetic effects on germinative cells.

Title of project No. 1:	Mechanisms of radiation-induced non-disjunction
Director of research and project team:	Prof. Dr. I. Hansmann, E. Armbruster, Dipl. agr. I. Taeger, Dr. H.D. Probeck

1. Mechanisms of X-ray induced genome mutations

8 - 12 week-old Djungarian hamster females (Phodopus sungorus) received whole-body irradiation during the sensitive preovulatory phase after gonadotrophin-stimulated follicle maturation. The X-ray doses used are 0, 0.05, 0.20, 0.40 and 0.80 Gy. The ovulated oocytes were cytologically prepared according to our standard-procedure and chromosomes analysed at metaphase II with respect to structural chromosome anomalies (gaps, breaks, translocations) and genome-mutations (hyperploidy and diploidy) as well.
The incidence of oocytes with structural chromosome anomalies increased so far with exposure dose (control: 0,6%; 0.05 Gy: 5.3%; 0.20 Gy: 7.7%; 0.40 Gy: 11.6%; 0.80 Gy: 27.5%). The majority of chromosome anomalies are due to breaks, fragments and deletions, some translocations were, however, detected as well. This observation indicates that preovulatory oocytes from Djungarian hamster do have the capacity for DNA-repair synthesis as well.
The analysis for hyperploidy and diploidy, which is not finished, indicates that preovulatory X-ray exposure decrease the incidence of gonadotrophin-induced aneuploidy at low dose levels.
In a second type of experiment young prepubertal NMRI-females received 0, 0.05 or 0.20 Gy at an age of 7 days p.p. and their oocytes were cytogenetically analysed at an age of 8 - 12 weeks. Oocytes were analysed, additionally, from

8 - 12 week-old females which received an irradiation during their fetal life (15^{th} day of gestation; 0, 0.05 and 0.20 Gy). No significant deviation of chromosome number and structure was noticed so far.

2. Chromosome analysis of human spermatozoa

No significant progress was achieved during the last year with the in-vitro fertilization of hamster-ova with human sperm. Sperm chromosomes reached not the stage of metaphase, which is the only stage appropriate for chromosome analysis.

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings:

1. Genetic risks following exposure of maternal and paternal germ cells to X-rays.
H.D. Probeck, J. Jenderny, I. Hansmann. in: Developmental effects of prenatal irradiation. Fischer, 1982

2. X-ray induced reciprocal translocations and dicentrics in human G_o-lymphocytes.
I. Hansmann, U. Meyding, R.P. Virsik. Radiat. Biol. (1982) in press.

3. Nondisjunction and chromosome breakage in mouse oocytes.
I. Hansmann, J. Jenderny, H.D. Probeck. in: Hum. Genet. 61, 190-192 (1982)

4. Low doses of X-rays decrease the risk for diploidy in mouse oocytes.
I. Hansmann, J. Jenderny, H.D. Probeck. in: Mutat. Res., (1982) in press.

Progress Report
1982

Contractor:
University College of Galway

Contract no.: BIO-E-415-81-EIR

IRL-Galway

Head(s) of research team(s):

Prof. J.A. Houghton
Department of Microbiology
University College of Galway
IRL-Galway

General subject of the contract:
Radiation repair processes in cyanobacteria and effects of radiation on the chromosomes of human gametes.

List of projects:

1. Study of radiation repair processes in cyanobacteria.
2. Study of the effects of radiation on the chromosomes of human gametes.

Title of project nr 1.

Study of radiation repair processes in the cyanobacteria.

Head of project and scientific staff : Professor J.A. Houghton, Dr. D. Nuttall, Mr. P. Lanham, Mr. C. Geoghegan.

In this project, the radiation repair processes of the unicellular cyanobacterium Synechocystis PCC 6308 are being investigated. Our previous studies have shown that Synechocystis PCC 6308 has a dimer-specific excision repair enzyme for the removal of radiation-induced DNA dimers. Recombinant DNA techniques have been used to screen for genes coding for the repair functions in this organism. Restricted Synechocystis DNA has been cloned into the Pst-1 site of plasmid pBR322 and these hybrid plasmids have been transformed into Mod^+ E. coli (HB101). These plasmids were then amplified in HB101, isolated and transformed into a UV radiation sensitive E. coli mutant AB2480 (recA, uvr). Clones are presently being screened for the phenotypic expression of UV radiation repair functions coded by the Synechocystis inserted DNA.

Hybrid plasmid vectors, each containing the uvrA, uvrB and uvrC excision repair genes of E. coli respectively together with ptm-2, a plasmid which contains the recA gene of E. coli, were all nick-translated and used as probes on Southern blot preparations of San3A digests of Synechocystis DNA fragments. The autoradiograms resulting from the DNA-DNA hybridizations indicated a high degree of homology between the E. coli excision repair genes and the cyanobacterial genomic DNA. The recA E. coli probe also showed homology with the Synechocystis DNA preparation. The hybridization studies are now being extended and a gene bank of Synechocystis DNA is being constructed. The phage lambda L47.1 is being used as a

vector and will be packaged using strains BHB2688 and BHB2690. The packaging strains of E. coli have been prepared using a freeze/thaw method in liquid nitrogen and control lambda DNA is packaged with an efficiency of 5×10^6 pfu ug^{-1} of lambda DNA. The gene bank will be probed with the recA and uvr excision repair genes of E. coli and positive plaques will be amplified and the cyanobacterial DNA fragments isolated and cloned in order to establish that the heterologous hybridization is specific to Synechocystis. Work is also underway on the isolation of radiation sensitive mutants of Synechocystis. These will be transformed with recombinant plasmids and examined for the presence of complementation of the cloned radiation repair genes. A suitable cloning vector for cyanobacteria has been constructed and will be used for the transformation of cloned genes into Synechocystis.

The unicellular cyanobacterium Anacystis nidulans 602 has also been examined for the presence of excision repair functions using direct biochemical techniques already established in this laboratory. A radiation sensitive mutant of Anacystis nidulans has been obtained and the complementation of E. coli repair function genes are being investigated by cloning the excision repair genes uvrA, B and C respectively into the mutants of Anacystis nidulans and screening for clones with enhanced survival. Studies are also underway for the presence of an inducible error-prone repair system in Anacystis nidulans by the determination of the presence of w-type reactivation.

Title of project nr 2.

Study of the effects of radiation on the chromosomes of human gametes.

Head of project and scientific staff: Professor J.A. Houghton, Dr. P. Tomkins, Mr. C. Carroll, Dr. S.E. Houghton.

In this project it is intended to investigate directly the effects of radiation on the induction of chromosome aberration in the gametes of man. In order to achieve this, a number of techniques are being studied for the direct visualization of the chromosomes of human spermatozoa. At the present time, these studies have concentrated on the method described by Rudak et al. (1978) for the interspecific fertilization in vitro of zona pellucida-free eggs from golden hamsters. This technique, whilst initially regarded as very promising, has not been generally adopted as many laboratories have found difficulty in using it for sperm chromosome visualization. Over the past year, each step of the procedure has been studied and optimized and a modified procedure has been developed. This modified techniques has proved successful and, in recent months, it has been found that 20-25% of all penetrated eggs have consistently yielded sperm chromosome preparations of analyzable quality. Optimization of the procedure has revealed that several parameters are important to the success of the technique including:

(1) Close attention to hamster maintenance, manipulation and hormone treatment to ensure regular high egg yields from the superovulated animals.

(2) The preparation of human sperm samples by filtration, low speed centrifugation and motile selection to ensure the separation of inhibitory seminal plasma from the active sperm. Flexible conditions are necessary for sperm capacitation. Whilst 5-7 h under mineral oil at 37°C was generally sufficient for most samples, repeat testing revealed that some specimens adequately capacitated in less time whilst others required longer incubation. It was also found that accurate objective monitoring of sperm motility, behaviour and morphology was necessary during the course of each fertilization.

(3) The composition of the culture medium used for egg and sperm preparation was modified. It was also found preferable to add labile energy sources shortly before use of the medium and to buffer with a combination of 20 mM Hepes and HCO_3^-/CO_2. Overnight egg culture was carried out in a Ham's based medium and the composition, pH and osmolarity had to be carefully monitored.

(4) It was essential to achieve sufficient manipulatory skill to ensure that the gamete interaction processes were completed within 30 min.

(5) The fixation of the pronuclear metaphase chromosomes was found to be technically the most difficult stage. However, an acceptable success rate was achieved by a modification of the Tarkowski technique.

At the present time, 110 sets of human sperm chromosomes have been examined using conventional staining and all have revealed apparently normal haploid chromosome constitutions. It was not yet proved possible to develop a suitable technique for G-banding the pronuclear chromosome preparations. The development of a reliable G-banding procedure is a major priority at the moment as this will permit more accurate analysis of the sperm karyotypes.

To enable this technique to be used for sperm samples with low fertility, videomicrographic and biochemical techniques are being used to determine seminal characteristics that influence penetration of zona-free hamster eggs and subsequent development. In particular, the effects of a number of catecholamines, amino acids and co-enzymes on sperm behaviour in culture media are being assessed. The applicability of semen freezing and thawing techniques to this method are also being evaluated to facilitate the transport of semen samples.

Other techniques for sperm chromosome visualization are also being investigated including:

(1) The in vitro decondensation of sperm chromosomes by disulphide cleaving agents.

(2) Fusion of sperm nuclei with cultured somatic cells.

(3) Microinjection of sperm or sperm heads into cultured somatic cells.

(4) Interspecific fertilization by microinjection of sperm or sperm heads into zona pellucida intact eggs from rats, mice or hamsters.

List of publications in 1982

1. Publications in Scientific Journals, Monographs, Proceedings.

Houghton, J.A. (1982). The study of chromosome non-disjunction in man. Irish Journal of Medical Science 150, 357-367.

O'Brien, P.A. and Houghton, J.A. (1982). Photoreactivation and excision repair of UV-induced pyrimidine dimers in the unicellular cyanobacterial Gloeocapsa alpicola (Synechocystis PCC 6308). Photochemistry and Photobiology 35, 359-364.

Houghton, J.A., Geoghegan, C., O'Brien, P.A. and Lanham, P. (1982). Studies on the effects of radiation on the cyanobacteria. Mutation Research 96, 135-136.

Houghton, J.A. and Tomkins, P. (1982). The chromosomes of human gametes. Science Progress 68, 47-62.

Geoghegan, C. and Houghton, J.A. (1982). Isolation of a UV endonuclease from the cyanobacterium Synechocystis PCC 6308. Journal of General Microbiology 128, 1743-1747.

Houghton, J.A. and Lanham, P. (1982). DNA repair mechanisms in unicellular cyanobacteria. Heredity 49, 139.

O'Brien, P. and Houghton, J.A. (1982). UV-induced DNA degradation in the cyanobacterium Synechocystis PCC 6308. Photochemistry and Photobiology 36, 417-422.

Tomkins, P., Carroll, C. and Houghton, J.A. (1982). Studies on the visualization of the chromosomes of human sperm. Irish Journal of Medical Science. (In press).

Tomkins, P.T., Carroll, C. and Houghton, J.A. (1982). Studies on the chromosomes of human spermatozoa. Heredity. (In press).

Progress Report
1982

Contractor:
Universität Hamburg
Krankenhaus Eppendorf
Martinistrasse 52
D-2000 Hamburg 20

Contract no.: BIO-E-391-81-D

Head(s) of research team(s):
Prof. H. Jung
Inst. für Biophysik
und Strahlenbiologie
Martinistrasse 52
D-2000 Hamburg 20

General subject of the contract:
Study of the relationship between DNA-strand breaks, chromosome aberrations and loss of the proliferation capacity of mammalian cells.

List of projects:

1. Study of the relationship between DNA-strand breaks, chromosome aberrations and loss of the proliferation capacity of mammalian cells.

Title of project nr BIO-E-391-81-D
Study of the relationship between DNA-strand breaks,chromosome aberrations and loss of the proliferation capacity of mammalian cells.

Head of project and scientific staff :
Prof.H.Jung
Dr.E.Dikomey

The effect of ionizing radiation on cell survival can be drastically enhanced,when repair of radiation-induced damage is suppressed during exposure,for instence,by keeping the cells at temperatures below $4^{o}C$. For CHO cells,exposed to internal ß-irradiation from DNA-incorporated ^{3}H-thymidine (^{3}H-TdR),it was investigated whether repair inhibition has also an effect on the amount and the repair kinetics of radiation-induced DNA strand breaks.

DNA strand breaks were anallysed by the DNA-unwinding technique and cell survival was determined by colony assay. ^{3}H-TdR was added 24 hrs after seeding 2.5×10^{5} cells in 5 ml medium (spec. activity 5 Ci/mmol). Cells were allowed to incorporate ^{3}H-TdR during an incubation at $37^{o}C$ for 24 hrs Cells were either exposed to internal ß-irradiation at $37^{o}C$ by varying the amount of added ^{3}H-TdR from 0.1 to 2.5 µCi per 5 ml medium or exposed at $4^{o}C$ by storing cells for various time intervals at $4^{o}C$ after labelling with 0.1 µCi per 5 ml medium. For both types of treatment the number of corresponding ^{3}H-decays per cell were calculated. This number could be converted into the number of induced strand breaks per cell since previous experiments had shown that in the mean one ^{3}H-decay results in one strand break.

The effect of internal ß-irradiation on survival is shown in figure 1. When CHO cells were irradiated at $4^{o}C$,where repair is inhibited,D_0 was found to be smaller by a factor of 4.6 compared to irradiation at $37^{o}C$.

The repair kinetics of strand breaks induced by internal ß-irradiation at $4^{o}C$ is shown in figure 2. Labelled cells were stored at $4^{o}C$ for 9 days,leading to about 1000 strand breaks per cell. Then the number of strand breaks was determined after repair incubation at $37^{o}C$ for various time intervals. As was tested by an F-Test,three significantly different components contribute to the observed effect: 50% of all strand breaks

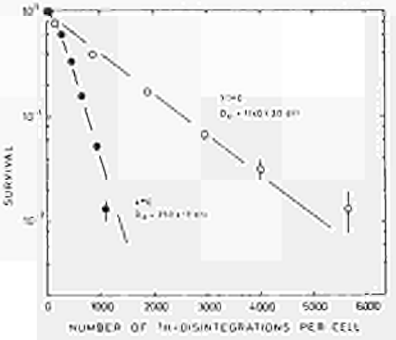

Fig.1: Effect of internal β-irradiation on survival of CHO cells, when exposed at 4° or 37°C.

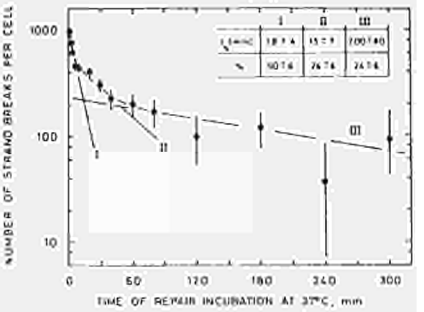

Fig.2: Repair kinetics at 37°C of DNA strand breaks induced by internal β-irradiation at 4°C.

are rejoined with a half time of about 2 mins; 26% with a half-time of about 15 mins and 24% with a half-time of about 200 mins.

Table 1 shows a comparison of the repair kinetics of strand breaks generated by internal β-irradiation at 4° and 37°C, respectively. It should be emphasized that 90% of the remaining strand breaks are associated with the (slow) component III. Thus, the good agreement between the measured and the calculated numbers of strand breaks indicates, that storage at 4°C, where repair is inhibited, jas no effect on the third component of the strand break repair kinetics, whereas a drastic effect is observed on survival.

Table 1: Comparison of strand-break repair kinetics after internal β-irradiation at 4°C and 37°C.

^{3}H-TdR,uCi	N(24 hrs)$_{meas.}$ [a]	N,tot. [b]	N[24 hrs],cal. [c]			
			I	II	III	I+II+III
0.1	0	84	0	0	0	0
0.5	27 ± 17	416	0	2	21	23 ± 6
1.0	48 ± 17	842	1	4	47	52 ± 13
1.5	88 ± 17	1251	1	6	74	81 ± 20
2.0	115 ± 17	1660	2	8	101	111 ± 27
2.5	142 ± 17	2198	2	12	136	150 ± 37

[a] number of DNA strand-breaks per cell measured after exposing the cells to various amounts of ^{3}H-thymidine at 37°C for 24 hrs.

[b] number of DNA strand-breaks per cell induced during the exposure at 37°C for 24 hrs. These numbers were calculated from the total number of ^{3}H-decays and the effectiveness per decay.

[c] number of DNA strand-breaks remaining per cell by the end of exposure at 37°C for 24 hrs. These numbers were calculated from N,tot. by using the coefficients and half-times determined after exposure at 4°C (cf. insert in Fig.2); they represent the contributions of components I,II and III as well as the total number.

List of publications

I.

E. Dikomey, Effect of hyperthermia at 42 and 45°C on repair of radiation-induced DNA strand-breaks in CHO cells, Int. J. Radiat. Biol., 1982, 41, 603-614.

S. Graubamnn and E. Dikomey, Induction and repair of DNA strand-breaks in CHO cells irradiated in various phases of the cycle, Int. J. Radiat. Biol., in press.

S. Graubmann, E. Dikomey and H. Jung, Erzeugung und Reparatur von DNA-Strangbrüchen nach Röntgenbestrahlung von synchronisierten CHO-Zellen, (meeting abstract), Abstract of papers of the "Symposium: Molekulare und zelluläre Mechanismen der Wirkung ionisierender Strahlen", 17-19 march 1982, GSF, München-Neuherberg, Fed. Rep. of Germany.

E. Dikomey, Erzeugung und Reparatur von DNA-Strangbrüchen nach interner ß-Bestrahlung in CHO-Zellen, (meeting abstract), Abstracts of papers of the "Symposium: Molekulare und zelluläre Mechanismen der Wirkung ionisierender Strahlen", 17-19 march 1982, GSF, München-Neuherberg, Fed. Rep. of Germany.

E. Dikomey,Induction and repair of DNA strand-breaks induced by internal ß-irradiation in CHO cells, (meeting abstract), Abstracts of papers of the "XVIIth annual meeting of the European society of radiation biology", 26-28 july, Bordeaux, France.

Progress Report
1982

Contractor:
Justus Liebig-Universität
Ludwigstrasse 23
D-6300 Giessen

Contract no.: BIO-E-392-81-D

Head(s) of research team(s):
Prof. J. Kiefer
Strahlenzentrum
Univ. Giessen
Leihgesterner Weg 217
D-6300 Giessen

General subject of the contract:
Mutation induction and biochemical damages by heavy-ion and low dose-rate gamma-irradiation.

List of projects:

1. Mutation induction in continuous cultures of yeast exposed to low dose-rate gamma-irradiation.

Title of project nr 1:
Mutation induction in continuous culture of yeast exposed to low dose-rate gamma-irradiation.

Head of project and scientific staff:
J. Kiefer, J. Goetzen, S. Rase, F. Zoelzer

The influence of dose-rate on the induction of radiation is of great importance in radiation protection. While there seems to be general agreement that the lethal action is reduced by dose protraction and fractionation the situtation is less clear for cancerogenesis and mutation induction. In order to contribute to the solution of this problem we subjected yeast cells growing in continuous culture under optimal nutrient conditions to low dose-rate γ-irradiation and scored mutant frequencies as a function of total dose. Previous studies were carried out with haploid yeast (see report 1981) but the system gave unreliable results because of diploidisation in the continuous culture. In the present investigations mutant induction was therefore tested in a diploid strain. It was constructed from a haploid strain carrying a deletion at the can-locus and a wild-type of opposite mating type. Because of the recessiveness the diploid is phenotypically sensitive to canavanine. Resistance to the drug can be induced with high frequency presumably caused by recombinational events.

Culture and test conditions have already been described in the 1981 report. Continuous cultures of the heterozygous diploid strain were subjected to various dose-rates which are listed in table 1. The frequencies of induced mutants as a function of total dose are shown in figure 1, the acute dose-response curve is given for comparison. It is clear from these data that decreased dose-rate leads to an increase of mutant frequency. In all cases the curves obtained with continuous exposure rise steeper than the acute one. They were fitted by linear regression analysis yielding the slope parameters listed also in table 1.

With continuous irradiation there was no reduction in cell number or colony forming ability which is in agreement with previous reports from our laboratory (Kiefer, Al-Talibi and Döll, 1977) and demonstrates the recovery potential of diploid yeast. The mutation results are rather unexpected and cannot yet be readily explained although they invite speculations: The increased mutation induction

may simply be done to a rearrangement of cell cycle stages with a relatively higher proportion of sensitive phases (e.g. G_2 which is known to be lengthened by ionizing radiation). It cannot be excluded that irradiation of the medium leads to the formation of mutagenic substances. The most speculative idea is the induction of error-prone repair which could explain the findings. Further experiments are planned to test the hypotheses put forward. Apart from the present lack of conclusive explanation the results obtained -particularly if confirmed in other systems- might provoke new thoughts about the importance of dose-rate in radiation protection.

Table 1: Mutant yields with various dose-rates

Dose-rate	Mutant-yield (mutants/Gy)
33 Gy/min	1.44×10^{-5}
4.84 Gy/h	$(4.3 \pm 0.12) \times 10^{-5}$
6.18×10^{-1} Gy/h	$(6.8 \pm 0.5) \times 10^{-5}$
4.57×10^{-2} Gy/h	$(1.2 \pm 0.14) \times 10^{-4}$

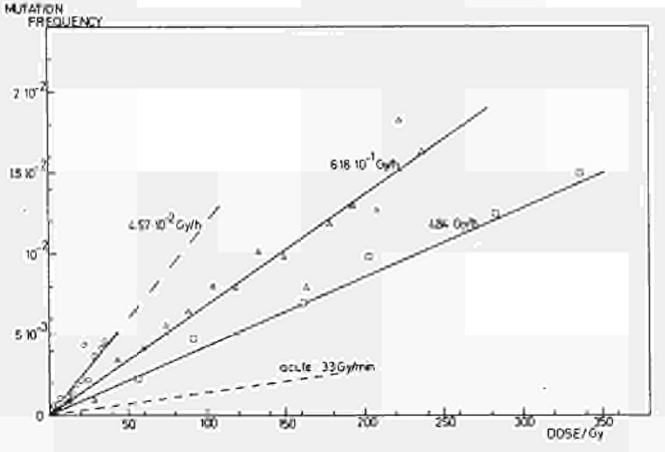

Figure 1: Mutant frequencies with different dose-rates as a function of total dose

Reference:

J.Kiefer, A.A.Al-Talibi and G.Döll (1977): Radiat.Res. 69, 230-240

Progress Report
1982

Contractor:
Stichting ITAL
Postbus 48
NL-6700 AA Wageningen

Contract no.: BIO-E-409-81-NL

Head(s) of research team(s):
Dr. H. P. Leenhouts
Association Euratom - ITAL
Postbus 48
NL-6700 AA Wageningen

General subject of the contract:
An investigation to quantify the effect of synergism between radiation and other mutagenic agents at low radiation doses in eukaryotic cells.

List of projects:

1. Investigation to quantify the effect of synergism between radiation and other mutagenic agents at low radiation doses in eukaryotic cells.

Title of project nr

Investigation to quantify the effect of synergism between radiation and other mutagenic agents at low radiation doses in eukaryotic cells.

Head of project and scientific staff: Dr. H.P. Leenhouts
Dr. K.H. Chadwick
Dr. A. Cebulska-Wasilewska (visiting scientist)

Introduction

The aim of this contract was to investigate whether a synergistic interaction between a mutagen and radiation occurred for the induction of somatic mutations at low radiation doses and low radiation dose rates.

Methods

The induction of pink mutations in stamen hairs of the flowers of *Tradescantia* (clone KU 9) has been used as biological end-point because it permits the detection of a radiation effect down to doses as low as 0.03 Gy (3 rads) with little problem. Short cuttings of *Tradescantia inflorences* were cultivated in a buffered solution of EMS for 2-6 hours before acute irradiation. Rooted cuttings were exposed to gaseous DBE for 6 hours before acute irradiation or for 12 hours simultaneously with a chronic irradiation.

Results and Conclusions

1. Synergism has been demonstrated for combined exposures to either EMS and acute X-rays or DBE and acute X-rays.
2. Synergism with EMS was found to be closely proportional to the product of radiation dose and mutagen exposure (fig. 1).
3. Synergism after acute irradiation increased with radiation dose, at low doses, and also with concentration of DBE (fig. 2).
4. Chronic irradiation with X-rays, when doses between 0.22 and 0.9 Gy (22-90 rads) were given over 6 or 12 hours, reduced the radiation induced mutation effect from a linear-quadratic dose relationship to a strictly linear dose relationship independent of the irradiation time (cf. fig. 2 and 3).

5. Synergism was found at low radiation doses when DBE exposures and chronic X-ray irradiation were combined simultaneously (fig. 3).
6. The frequency of pink mutations exposed to DBE alone was found to be a non-linear function of DBE concentration (fig. 4).

In view of the fact that synergism which increases the low dose effect of radiation induced mutations, has been found at both low doses and low dose rates in simultaneous exposures to mutagen and radiation, it would be prudent to investigate the possible general relevance of this phenomenon to the estimate of radiation risk in more detail.

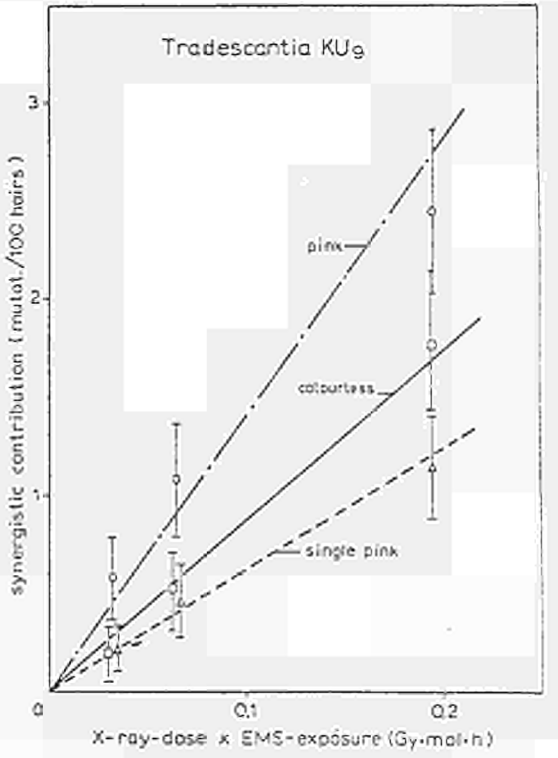

Figure 1: The synergistic contribution to the mutation frequency for total pink, single pink and colourless mutations as a function of the product of radiation dose and EMS exposure.

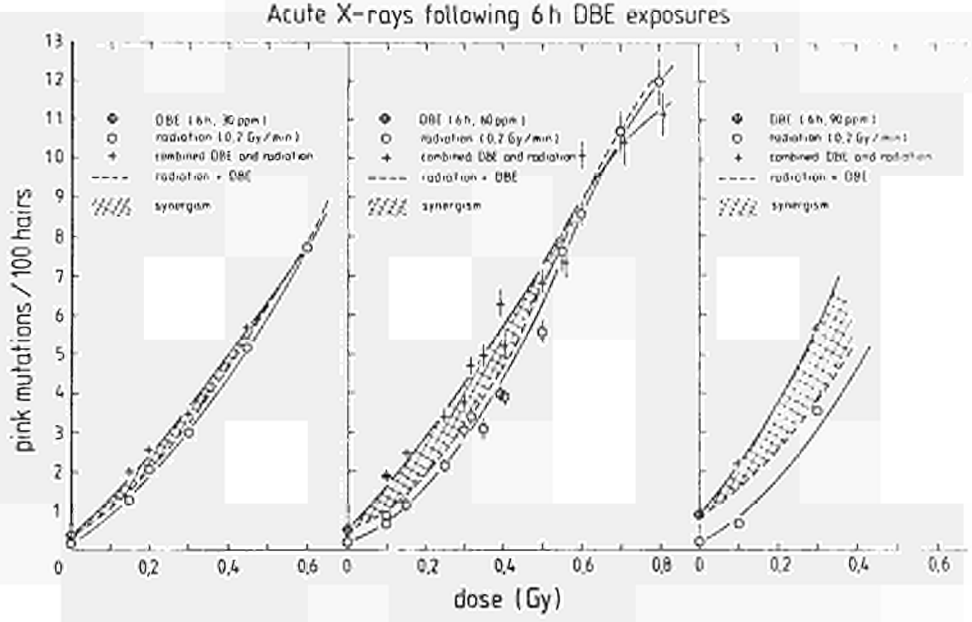

Figure 2: Dose-effect relationships for pink mutations induced by 6 h exposures to three concentrations of DBE followed by acute X-ray irradiation.

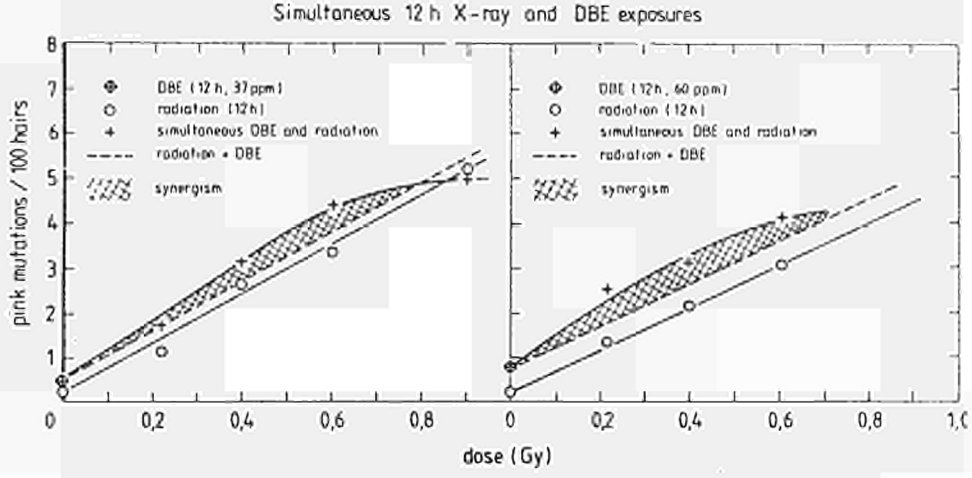

Figure 3: Dose-effect relationships for pink mutations induced by simultaneous 12 h-exposures to two concentrations of DBE and chronic X-ray irradiation.

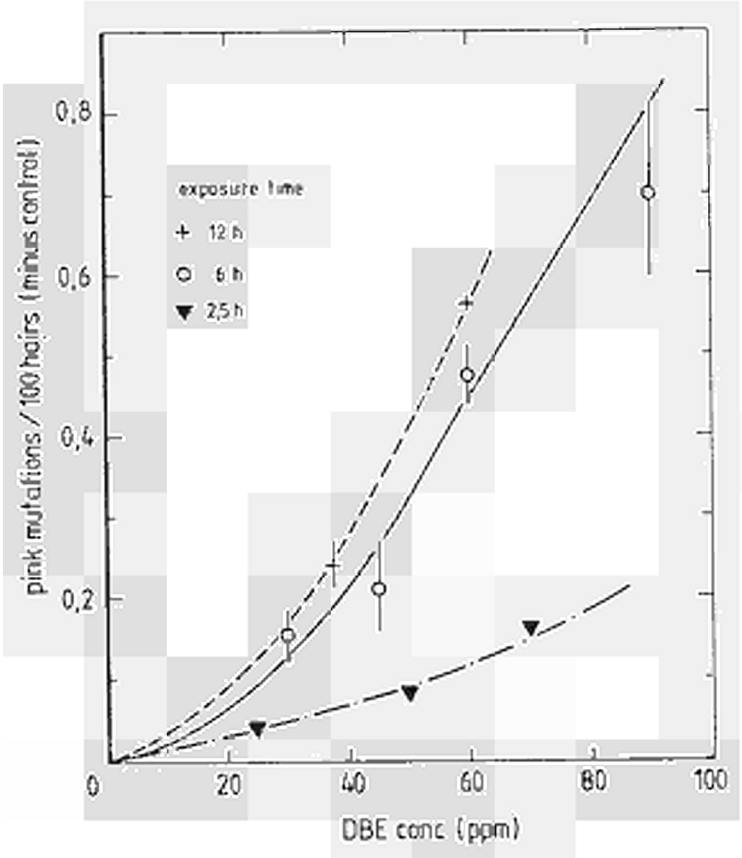

Figure 4: The induction of pink mutations following different exposures to DBE.

List of publications

I Publications in Scientific Journals, Monographs, Proceedings.

1. CEBULSKA-WASILWESKA, A., SIJSMA, M.J., LEENHOUTS, H.P. and K.H. CHADWICK. The influence of time between cutting and irradiation on the sensitivity of *Tradescantia* stamen hairs to mutation induction. Int. J. Radiat. Biol. 41 (1982) 569-574.
2. LEENHOUTS, H.P., BROERTJES, C., SIJSMA, M.J. and K.H. CHADWICK. Radiation stimulated repair in *Saintpaulia*: Its cellular basis and effect on mutation frequency. Envir. Exptl. Botany 22 (1982) 301-306.
3. CHADWICK, K.H. and H.P. LEENHOUTS. A comparison of the cell killing effects of UV and ionizing radiation: implications for low dose effects. pres. 8th Microdosimetry Symp. Julich. Sept. 1982 (in press).
4. CHADWICK, K.H. International dose-assurance in radiation technology. IAEA Bulletin 24 (1982) No. 3, 21-27.
5. MILLER, A., CHADWICK, K.H. and J.W. NAM. Dose assurance in radiation processing plants. pres. 4th International Meeting on Radiation Processing. Dubrovnik, Oct. 1982 (in press).

II Short Communications, Theses, Internal Reports, Patents.

1. LEENHOUTS, H.P., CEBULSKA-WASILWESKA, A. and K.H. Chadwick. The influence of storage of *Tradescantia* cuttings on the radiation sensitivity. Netherlands Radiobiological Society, 5 February 1982, Amsterdam. Abstract in: Int. J. Radiat. Biol. 42 (1982) 77.
2. CEBULSKA-WASILWESKA, A. Investigations carried out at ITAL during an IAEA-fellowship in the period January 1981 - September 1982. Association Euratom-ITAL, Technical and Preliminary Research Report No. 94; October 1982.
3. LEENHOUTS, H.P. The development of a theoretical approach to permit the comparison of the carcinogenic acitvity of ionizing radiation and chemicals. Koningin Wilhelmina Fonds, Netherlands Cancer Foundation, Rijswijk, October 1982.

4. CEBULSKA-WASILWESKA, A., LEENHOUTS, H.P. and K.H. CHADWICK. Synergisme van EMS met straling bij mutaties in *Tradescantia*. Nederlandse Vereniging voor Stralingshygiëne, 12 February 1982, Bilthoven. Abstract in: NVS-nieuws 7 (1982) 12.

5. LEENHOUTS, H.P. and K.H. CHADWICK. Radiation Quality and the nature of the biological lesion. G.S.I. Workshop on Models of the Energy Deposition of Ionizing Radiation and the Biological Response. Darmstadt, 7-8 June 1982.

Progress Report
1982

Contractor:
Centre d'Etude de l'Energie
Nucléaire, CEN/SCK
Avenue Plasky 144
B-1040 Bruxelles

Contract no.: BIO-E-451-81-B

Head(s) of research team(s):
Dr. A. Léonard
Département de Radio-
biologie, CEN/SCK
Boeretang 200
B-2400 Mol

General subject of the contract:
Experimental studies on the teratogenic effects of small doses of radiation delivered during the preimplantation period.

List of projects:

1. Studies on sensitivity of embryos to low doses of radiation during the first day of pregnancy.

Title of project nr 1 : STUDIES ON SENSITIVITY OF EMBRYOS TO LOW DOSES OF RADIATION DURING THE FIRST DAY OF PREGNANCY

Head of project and scientific staff : A. LEONARD, P. JACQUET

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1. Compared study of the egg radiosensitivity at different times during the first cellular cycle

In a preliminary autoradiographic study, we found that in the super-ovulated zygote of the Balb/c strain, DNA synthesis begins at approximately 19 hours after HCG injection (i.e. 7 hours after ovulation), reaches its maximum at 23-24 hours and is terminated at about 26 hours after HCG injection. On the basis of these results, irradiation of pregnant females was performed at the time of maximal DNA synthesis (24 hours post HCG) or after DNA synthesis (27.5 hours post HCG), and the embryonic radiosensitivities found at these two times were compared with those found last year for the fertilization stage (15 hours post HCG) and the early pronuclear stage (19 hours post HCG). For each time, radiosensitivity was evaluated by killing pregnant females one day after X-irradiation, and by following the development of their embryos in culture, so that mortality at the different stages up to the blastocyst implantation could be recorded. Irradiation was most effective when administered at 19 hours post HCG. Such a treatment increased the mortality prior the first cleavage and, thereafter, from the 8-cell (100 rads) or morula stage (25, 50 rads). Blastocyst hatching and implantation were also impaired. Irradiation at other times was much less harmful for the embryos, which died mainly from the blastocyst stage. Finally, radiosensitivities of the mouse zygote at the different times studied can be estimated as follows : fertilization : +++ ; pronuclear stage before DNA synthesis : +++++ ; maximum DNA synthesis : + ; DNA synthesis terminated : ++ .

2. Cytogenetic effects of radiation

Balb/c female mice were induced to superovulate and mated with males of the same strain. 19 hours after HCG injection, i.e. at the time of maximum egg radiosensitivity, they were irradiated with 50 rads to the whole body. Immediately after irradiation, they were killed and eggs at the 1-cell stage were removed from the oviducts and put into medium containing colchicin. First metaphase chromosomes were prepared on the following day according to the technique of Tarkowski. Nearly 30% of the cytogenetically analysable embryos contained one or more aberration, mainly chromosomal fragments. These aberrations were not present in the control metaphases. These studies show that loss of genetic material following X-irradiation at the zygote stage represents probably the main cause of embryonic mortality occuring during the following preimplantation stages, or during implantation.

Publications

P. Jacquet, G. De Clercq and G. Kervyn
Studies on the sensitivity of the mouse egg to low doses of X-irradiation, in : Proc. Int. Symp. "Developmental Effects of Prenatal Irradiation", München nov. 1980, Kriegel et al. (eds.), Fischer, Stuttgart - New York, pp.51-57 (1982).

P. Jacquet, G. Kervyn and G. De Clercq
In vitro studies on the mouse zygote radiosensitivity,
in : Proc. Symp. EULEP "Effects of Prenatal Irradiation with Special Emphasis to Late Effects ", Bordeaux July 1982, in press.

P. Jacquet, G. Kervyn and G. De Clercq
In vitro studies on the mouse egg radiosensitivity from fertilization up to the first cleavage.
- Mutation Res., accepted for publication.

Communications

P. Jacquet
Morphological development after irradaition of the mouse embryo during the preimplantation period.
EULEP Symposium, Reisensburg near Ulm, February 25, 1982.

Progress Report
1982

Contractor:

Medical Biological Laboratory TNO
P.O. Box 45
NL-2280 AA Rijswijk

Contract no.: BIO-E-403-81-NL

Head(s) of research team(s):

Dr. P.H.M. Lohman
Medical Biological Laboratory
TNO
P.O. Box 45
NL-2280 AA Rijswijk

General subject of the contract:

The genetic and biochemical basis of radiation sensitivity in human and other cells in culture.

List of projects:

1. Genetic analysis of DNA repair.
2. Biochemical analysis of DNA repair.
3. Consequences of DNA damage and repair.

PREAMBLE

Human cell variants believed to be deficient in DNA repair, continue to constitute a major focus of the work of the contracting laboratories. Further genetic analysis has revealed the existence of three complementation groups for Cockayne syndrome and a further complementation group (H) in xeroderma pigmentosum (XP). Hypermutability of Cockayne syndrome cells following UV has been confirmed in a third cell strain. In the A, D, G and H complementation groups of XP it has been possible to correct the repair defect after microinjection of human cellular extracts. A gene involved in the A group complementation process has been localized on chromosome 1. As a pointer to the future, it has also been possible to obtain transfer and expression of a gene originating in an inactive X-chromosome.

A method has been developed for determining the ability of human cells to effect repair of potentially lethal radiation damage. Among cells shown to be deficient in such repair have been those from a patient with Gorlin's syndrome and those from an apparently normal lymphoma patient who showed a severe erythemal response to radiotherapy.

At the biochemical level, work has progressed in two areas concerned with the more sensitive detection of DNA lesions. Firstly, the UV-endonuclease method for detecting sensitive sites has been developed by incorporating elution techniques and fluorescent dyes, thus eliminating the need for radioisotopic labelling and permitting *in vivo* experimentation. A second approach has involved the development of a procedure for making monoclonal antibodies with a high affinity for specific DNA lesions. The use of monoclonal antibodies has enabled the detection of pyrimidine dimers in human epithelial cells exposed to gamma radiation.

Significant progress has been made in the study of the mechanisms of action of DNA repair inhibitors such as aphidicolin, bringing out the importance of the metabolic state of the cell (whether stationary or exponentially growing). The role of inhibitors of poly ADP-ribose polymerase in repair of damaged DNA, as well as their influence on frequencies of chromosome aberrations, sister chromatid exchanges and point mutations has been studied in detail. Other biochemical studies have concerned the involvement of DNA ligases I and II in DNA repair and a human cell mutant has been characterized with properties consistent with a deficiency in DNA ligase I activity.

The work has been carried out in a joint programme by:

D. Bootsma *et al*, Erasmus University, Rotterdam (BIO 404 NL).
B. A. Bridges *et al*, MRC, Brighton (BIO 414 UK).
P. H. M. Lohman, TNO, Rijswijk (BIO 403 NL).
J. W. I. M. Simons, University of Leiden (BIO 407 NL).

Title of project No.1 : Genetic analysis of DNA repair
Heads of project and scientific staf: Dr. P.H. Pouwels and Dr. G. Veldhuisen

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In this project which is aiming at the identification of genes involved in the repair of UV damage in eukaryotic cells (in particular human cells), recombinant DNA techniques are used to clone repair genes in a microorganism and characterize the gene products, with the ultimate goal the cloning of human repair genes. This study was started by cloning 2 prokaryotic repair genes, viz the v gene of bacteriophage T4 and the phr gene of E.coli. Gene v of bacteriophage T4 codes for T4 UV-endonuclease, an enzyme involved in the excision of pyrimidine dimers. The phr gene codes for an enzyme responsible for the photoreactivation (phr) of these lesions.

During the second year of the contract we have continued our attempts to clone the v gene by insertion of the "Sal E fragment," obtained by digestion of T4 DNA with the restriction enzyme Sal E, or parts thereof (presumably carrying the v gene of T4), into plasmid DNA. For the presence of recombinant DNA plasmids in transformed bacteria was screened by hybridization with ^{32}P-labelled T4 Sal E DNA. Further analysis of the recombinant plasmids showed that the T4 DNA insertions were relatively small (2.5 - 4.0 kb) and originate from the left end of the Sal E fragment. It could be demonstrated that the T4 tk gene coding for the enzyme thymidine kinase and previously localized on the lefthand side of the same Sal E fragment, is present in some of the recombinant DNA plasmids. The presence in transformed bacteria of the v gene, which is supposed to be located on Sal E and to the right of tk, could not yet be demonstrated.

The Sal E fragment also was inserted into λ L47 DNA, after cleavage with restriction enzyme Xho or partial digestion with restriction enzymes Eco RI or HindIII. After selection for UV resistant phages on hcr$^-$ host bacteria, phages were obtained containing the uvrB gene from E. coli. This result is consistent with the recent observation by Young et al. (J. of Virol. 41, 345, 1982) demonstrating the presence of transducing particles in preparations of T4 alc mutants. We will pursue our attempts to clone

the T4 v gene by insertion of defined fragments of Sal E DNA from T4 phage into λ 1149, 1150 or 1151,in which smaller fragments can be incorporated than in λ L47, and screening by hybridization with ^{32}P-labelled Sal E DNA.

The subcloning of the phr gene, initiated during the first year of the contract, was continued. This resulted in a reduction of the size of this fragment from 40 to 2.0 kb, which makes it accessible for direct sequence analysis. Presently it is attempted to determine the boundaries of the phr gene on the fragment by shortening the ends of the fragment and following the effect thereof on the expression of the phr gene.

When cloning of the phr gene and/or the v gene has been achieved, their transfer to cells from xeroderma pigmentosum patients will be attempted in order to study the effect on the repair of UV damage in these cells. To facilitate expression of these genes in human cells an introductory study is being made of the requirements for optimal expression of a prokaryotic gene in a mammalian cell. Towards this goal a cell-transformation assay was set up for mouse L cells. In this mouse cell system expression of an easily assayable prokaryotic gene (merA, confering mercury resistance to the host organism) will be studied. The expression of the merA gene will be brought under control of the promotor of the metallothioneine gene, MT. This well characterized eukaryotic promotor is relatively strong and has the additional advantage to be inducible.

Title of project No.2 : Biochemical analysis of DNA repair

Heads of project and scientific staf: Dr. P.H.M. Lohman and Dr. G.P. van der Schans

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This project aims at the identification of various lesions in mammalian cells and at the elucidation of their repair. Special attention is given to mutant cells of mammalian origin that are defective in certain repair pathways of identified DNA lesions. The agents used in these studies for the induction of lesions are ionizing radiation and irradiation with ultraviolet light of different wavelengths.

Ionizing radiation damage

As stated in the previous progress report, the mutant human cells used in this study are obtained from patients suffering from ataxia telangiectasia (A-T), a hereditary disease; these patients show an elevated radiosensitivity extending from the clinical to the cellular level.

We studied the repair of radiation damage in the presence or absence of specific repair inhibitors. For repair of DNA lesions the cell has different enzyme systems; one of them -excision repair- uses specific DNA-polymerases. Three different DNA polymerases are known in mammalian cells (α, β and γ). Presumably, polymerase α is mainly involved in the normal DNA replication, but also has an important function in the gap-filling step of the long patch type of excision repair. Most likely polymerase β plays a role in excision repair, whereas polymerase γ is involved in the synthesis of mitochondrial DNA. By influencing the long patch repair by a specific inhibitor, information may be obtained about the relevance of this particular repair system for the repair of specific DNA lesions.

In this study the polymerase inhibitor Ara-C (1-β-arabinofuranosyl-cytosine) inhibiting polymerase α, has been used. Ara-C appeared to influence the repair of DNA damage in cultured human cells both after γ-irradiation and after exposure to UV-light, resulting in an accumulation of incision breaks (due to the incorporation of Ara-C the rejoining step in the excision repair process is inhibited). In general it is assumed that damage caused by γ-rays is repaired via short patch repair, that is differently from that induced by UV. The results with Ara-C, therefore, suggests that γ-radiation also induces UV-type damage in human cells, presumably thymine dimers. In that case it should be possible to demonstrate the presence of thymine dimers. Indeed we observed that after irradiation of human cells with γ-rays, a fraction of lesions in the isolated DNA of these cells can be removed by using a photoreactivating enzyme, which is specific for thymine dimers. This also could be confirmed in vivo in chicken-embryo cells, which possess their own photoreactivating enzyme. Also results obtained with Ara-C in cells derived from patients suffering from xeroderma pigmentosum, a hereditary disease resulting in hypersensitivity to UV, confirmed the hypothesis that γ-radiation induces UV-type of DNA damage.

In collaboration with Dr. Y. Shiloh (Hebrew University, Jerusalem, Israel) the induction and repair of single-strand and double-strand breaks were studied in normal human cells and in A-T cells that had been

subjected to a short treatment with the radiomimetic and antitumor agent, neocarzinostatin (Ncs). As for survival, A-T cells are more sensitive to Ncs than normal human cells. Initially, the induction of both single-strand and double-strand breaks occurred very rapidly, but after 5 min the rate slowed down and no further increase occurred after 10 min. This levelling off is probably due to the balance obtained between continuing induction of strand breaks and strand breakage repair, and between the formation and resealing of secondary breaks accompanying excision repair of other types of damage. The kinetics of single-strand and double-strand break repair, studied after a brief treatment, were biphasic and comparable to results found after γ-irradiation.There was no difference between the control cells and A-T cells with regard to both the kinetics of single-strand and double-strand break rejoining and the fraction of breaks remaining open. This is in accordance with previous results and makes it less probable that there is a simple quantitative reduction in the rejoining ability in A-T cells, as the cause for the hypersensitivity to Ncs.

Ultraviolet light (UV) induced damage

Irradiation of cultured human cells with UV leads to DNA damage, which may result in cell death or mutations. In most studies UV-C (wavelength < 290 nm) has been used as the damaging agent, which induces pyrimidine dimers as the major photoproducts. With long wavelength UV (UV-A; > 320 nm), however, predominantly non-dimer lesions are currently held responsible for cytotoxic and mutagenic effects. For the effect of the UV-region of sunlight on human skin, studies solely involving irradiation with UV-C do not appear too relevant, as wavelengths shorter than 290 nm normally do not reach the earth's surface.

Our investigations aim at a comparison of the effects of UV-A and UV-C in relation to dimer induction and at a possible interaction between the two forms of irradiation. In cultured human fibroblasts, UV-C was found to induce pyrimidine dimers (detected as UV-endonuclease sensitive sites), whereas equicytotoxic doses UV-A did not. Slightly cytotoxic doses of UV-A induced single strand DNA breaks (detected with "alkaline elution") which were repaired rapidly, but no evidence was obtained about the occurrence of an excision repair process as can be measured by the autoradiographic assay of unscheduled DNA synthesis (UDS). Moreover, unlike in cells irradiated with UV-C, no accumulation of incision breaks during post-irradiation treatment with hydroxyurea and Ara-C, was detected. UDS induced by UV-C, however, was reduced on immediate subsequent irradiation with UV-A. Apparently, in human cells UV-A induced DNA lesions interfere with repair of UV-C induced DNA lesions or UV-A leads to photoreactivation of these DNA damages.

Preliminary experiments are carried out to study the photoreactivating capacity of human epithelial cells. For this purpose a sensitive technique for the detection of UV-endonuclease susceptible sites, using DNA elution methods, is in development. Further, monoclonal antibodies raised against the different types of pyrimidine-dimers will be produced in order to be able to discriminate between these different types of dimers with respect to their photoreactivability in different cell types.

Publications

Schans, G.P. van der, H.B. Centen and P.H.M. Lohman, Studies on the repair defects of Ataxia telangiectasia cells, Proc. Nato Advanced Study Institute EMBO Lecture Course: Chromosome damage and repair, Bergen 1980, Ed. E. Seeberg and K. Kleppe, Plenum Publishing Corp., N.Y., 1981, page 355-359.

Schans, G.P. van der, H.B. Centen and P.H.M. Lohman, DNA lesions induced by ionizing radiation, Progress in Mutation Research, Vol 4. Eds. A.T. Natarajan et al., 1982, Elsevier Biomedical Press pp. 285-299.

Schans, G.P. van der, H.B. Centen and P.H.M. Lohman, The induction and repair of double strand DNA breaks in normal human and Ataxia telangiectasia cells exposed to ^{60}Co-γ-radiation, 4-nitroquinoline-1-oxide or bleomycin., In: Ataxia telangiectasia - a cellular and molecular link between cancer, neuropathology and immune deficiency, Eds. B.A. Bridges and D. Harnden, John Wiley and Sons, 1982, pp. 291-303.

Title of project No.3 : The consequences of DNA damage and repair

Heads of project and scientific staf: Dr. P.H.M. Lohman and Dr. G.P. van der Schans

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In this project the attention is focussed on the individual sensitivity of human cells towards ionizing radiation. It is our aim to adapt more simple, fast and sensitive biochemical techniques to obtain data that are relevant for the radiosensitivity of cells. This will involve the development of techniques by which various types of radiation damage in the DNA of mammalian cells can be reliably detected.

DNA damage in mammalian cells introduced by ionizing radiation can be varied in different ways. Possibilities, as described in the previous report, are to irradiate cells in the presence of protective agents or to irradiate cells with fast neutrons instead of γ-rays. In both cases large shifts result in the spectrum of the different types of radiation damage in the DNA of the cells, which are reflected also in the ability to kill cells.

Another possibility to change the spectrum of lesions is to apply specific inhibitors of repair after γ-irradiation (see also project 2). When Ara-C, an inhibitor of polymerase α was applied, no increase was found in incision breaks after γ-irradiation of X-P cells (known to be deficient in carrying out the incision step during repair of UV-type damage). In A-T cells, however, some increase was found but less than in normal cells, suggesting that some types of damage may not be recognized in A-T cells by certain repair processes or are more slowly repaired than in normal strains.

A new method is in development in which the alkaline elution method for the detection of single-strand breaks is combined with the fluorometric detection of the amount of DNA by means of the fluorescent dye Hoechst 33528. With this method we shall be able to detect DNA damage after exposure in vivo. Particularly, the influence of protective agents on the induction of radiation damage in vivo will be studied in this way.

Publication

Schans, G.P. van der, M.C. Paterson and W.G. Cross, Induction and repair of DNA breaks after exposure of cultured human fibroblasts to fast neutrons or ^{60}Co-γ-rays. Submitted to Int. J. Rad. Biol.

Progress Report
1982

Contractor:
Institut Curie
Section Biologie
Rue d'Ulm 26
F-75231 Paris Cédex 05

Contract no.: BIO-E-397-81-F

Head(s) of research team(s):
Dr. E. Moustacchi
Section Biologie
Institut Curie
Centre Univ. - Bât. 110
F-91405 Orsay

General subject of the contract:
Nuclear and mitochondrial genetic lesions induced by radiations in a lower eukaryote : mutagenic and recombinogenic processes in relation to replication and repair.

List of projects:

1. Repair, nuclear and mitochondrial mutagenesis : relation with DNA replication.
2. Repair, recombination and mitotic cycle after X-ray irradiation in yeast.

Title of project nr 1 REPAIR NUCLEAR AND MITOCHONDRIAL MUTAGENESIS : RELATION WITH DNA REPLICATION.

Head of project and scientific staff : Drs. E. MOUSTACCHI, M. HEUDE, C. CASSIER, R. CHANET, N. MAGANA-SCHWENCKE and T. SAEKI.

Radiations induced functions in yeast.

The possible modulations of proteolytic activities in relation to induction of DNA lesions and their repair were unexplored in the unicellular eucaryotic organism Saccharomyces cerevisiae. On the other hand, a specific proteolytic activity associated with the "SOS" phenomenon has been described in E.coli whereas in embryonic mammalian cells the induction of a protease, the plasminogen activator, following the production of DNA lesions has been reported. In the first case the activity is associated to a DNA-binding protein, the RECA protein, whereas in the second case the activity is not directly linked to DNA processing.

Among the five most well known proteinases of S.cerevisiae, we found that proteinase B activity increases up to three times in wild type RAD^+ yeast cells after a dose of 250 nm UV leaving 40 % survivors. This post-UV increase in proteinase B activity is dose-dependent and is inhibited by cycloheximide. This last observation indicates that an inducible process is involved.

Carboxypeptidase Y, aminopeptidase I and II and proteinase A were either only moderately increased or were unchanged following irradiation in both wild type and in repair-defective mutants. A mutant defective in excision-repair, rad1-3, demonstrated the same response as the isogenic wild type with respect to proteinase B activity.

In contrast following the same UV-treatment the level of proteinase B remains almost constant in a mutant blocked in a general error-prone repair system, rad6-1, and in a mutant defective in a more specific mutagenic repair pathway pso2-1.

It is obvious that the induction of proteinase B activity after UV-treatment cannot be equated to the induction of the RECA protein in

E.coli. However the correlation found between the block in mutagenic repair and the lack of UV-induction of protease B activity leads to question on the possible role of certain protease activities in mutagenic repair in eucaryotic cells. In this connection, it is of interest to notice that specific protease inhibitors such as antipain and leupeptin have been shown to suppress radiation-induced malignant transformation (Kennedy and Little, 1978) or chemically induced tumorigenesis in mouse skin (Hozumi et al., 1972). Both antipain and leupeptin behave as non-alkylating inhibitors of the serine-type of proteinases such as the yeast proteinase B.

As a result of misreplication or misrepair of photodamage, it is known that there is a premature termination of mRNA and this leads to uncomplete polypeptide fragments. It can be tentatively proposed that an increase of certain proteinases is necessary for the degradation of such abnormal polypeptides. Why such a response is impeded in strains unable to perform mutational repair of radiolesions is still an open question currently investigated with the aid of proteinase B defective mutants (kindly provided by Drs. E. Jones and K. Wolf).

Use of repair-defective yeast mutants to characterise the nature of lesions induced by antitumoral drugs.

If a mutant blocked in the repair of a specific lesion is more sensitive than the wild type, it can be reasonably inferred that the agent tested is likely to produce in vivo principally the concerned unrepairable lesion. Such rapid tests of growth inhibition can then guide the biochemical detection of the lesions and allow to study the effects of simultaneous exposure to ionizing radiations and the drug as well as the role of oxydo-reductive processes. This method applied to isogenic strains defective in repair of DNA strand breaks (rad52) or of DNA interstrand cross-links (pso2) or in excision of DNA adducts (rad3) has been successfully applied to neocarzinostatine, BD40 a new derivative of ellipticines and to a family of new derivatives of psoralens (mono and bifunctional pyridopsoralens).

Title of project nr 2 : ANALYSIS OF THE MECHANISMS LEADING TO RECOMBINOGENIC EVENTS.

Head of project and scientific staff : Dr. Francis FABRE ;
Mrs. A. BOULET-CLAVAUD.

Resolution of the conversion structure

The demonstration in mitotic yeast that most of the conversion events occur during the G1 phase raises a puzzling question : how are the crossing over (C.O.) that lead to the segregation of heterozygotes alleles, related to conversion ? The cleavage of the isomerized form of the structure leads to exchange of outside markers, but if this occurs in G1, they will remain heterozygotes. Because there is a coincidence between CO and conversion, M. Esposito (PNAS, 75, 4436, 1978) proposed that the structure is not cleaved but resolved by replication. Thus the formal result is the same as CO in G2. Because we knew, from our experiments with conditional cell cycle mutants, that the structure can be established early in G1, this model seemed to us unlikely : the crossed structures should have to persist a too long period of the cycle. In collaboration with H. Roman (Seattle), we decided to ask how the G1 induced conversion structure is resolved. The experiments consists in the genetic analysis of convertant colonies sectored for an outside marker. Thus, all the chromosomes present in the cell during conversion, are recovered and analysed. The rationale is that, if resolution is mediated by replication, only two of the 4 chromatides can be involved in reciprocal exchanges. On the contrary, if resolution is mediated in G1 by endonucleolytic cleavage and if a coincidental G2 event occurs afterwards, 3 chromatides could be involved. We found a number of cases that can be explained only by the cleavage of the structure in G1, followed by a G2 CO. Thus, we conclude that the G1 induced structure are cleaved before DNA replication. This may however be different for spontaneous events, if they occur a short time before replication. The coincidence between G1 conversion and crossing over may be explained either by the persistence of the induced ability of the cells to recombine (see next section) or

else by a molecular trace of the G1 event, retained after replication.

Inducible recombination ability : mitotic stability of the induced state

In mitotically dividing yeasts, radiation induced DNA lesions are a signal for the induction of a recombinational mechanism (Fabre & Roman, PNAS, 74, 1667, 1977). We have asked if, as it is for the "SOS" in Escherichia coli (Defois et al., M.G.G., 148, 125, 1976), repression occurs within the first cycle following treatment. Experimental conditions were set up that allow the study of the kinetics of the formation of convertants as a function of the generations that follow the treatments (γ rays or UV). The results show that, if a fraction of the derepressed cells recover their original state within the first 5 generations, the rest of the population remains derepressed during at least 10 generations, and perform gene conversion at a high rate. This implies the involvement of a switch control mechanism. The biological consequences of this memory of DNA damaging treatments may be of importance, since it results in the establishment of cell lines with increased genetic instabilities.

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

. Announced as "in press" in 1981 report.

- The fate of 8-Methoxypsoralen photoinduced crosslinks in nuclear and mitochondrial yeast DNA : Comparison of wild type and repair-deficient strains. N. MAGANA-SCHWENCKE, J.A.P. HENRIQUES, R. CHANET and E. MOUSTACCHI. Proc. Natl. Acad. Sci. USA, 79, 1722-1726 (1982).

- Mutagenesis and repair in yeast mitochondrial DNA. E. MOUSTACCHI and M. HEUDE. In : "Molecular and Cellular Mechanisms of Mutagenesis", Edited by J.F. Lemontt and W.M. Generoso, Plenum Publish. Corp. USA, Chapter 19, pp. 273-301 (1982).

- Aspects of radiation-induced mutagenesis and malignant transformation. E. MOUSTACCHI. In : "Trends in Photobiology", Edited by C. Helene, M. Charlier, T. Montenay-Garestier and G. Laustriat, Plenum Publish. Corp. USA, pp. 217-228 (1982).

. 1982 publications.

- Proteolytic activities in yeast after UV irradiation. I. Variation in proteinase levels in repair proficient RAD^+ strains. J. SCHWENCKE and E. MOUSTACCHI. Molec. Gen. Genet., 185, 290-295 (1982).

- Proteolytic activities in yeast after UV irradiation. II. Variation in proteinase levels in mutants blocked in DNA-repair pathways. J. SCHWENCKE and E. MOUSTACCHI. Molec. Gen. Genet., 185, 296-301 (1982).

- Cytotoxic and mutagenic effects of neocarzinostatin in wild type and repair-deficient yeasts. E. MOUSTACCHI and V. FAVAUDON. Mutation Res., 104, 87-94 (1982).

- Les lésions génétiques et leur réparation. E. MOUSTACCHI. Le Courrier du CNRS, n° 48, 17-21 (1982).

II. Short Communications, Theses, Internal Reports, Patents...

- "Contrôle génétique de la mutabilité induite et de la conversion induite chez la levure : Influence des gènes pso qui gouvernent la sensibilité à la photoaddition des furocoumarines". C. CASSIER. Doctorat de 3ème Cycle soutenu le 5 Mars 1982 (Université de Paris-Sud), Jury : Ph. VIGIER, J.L. ROSSIGNOL, C. HELENE et E. MOUSTACCHI.

- Rapport de la Commission Scientifique sur la gestion des combustibles irradiés pour le Conseil de la Sureté Nucléaire, Ministère de la Recherche et de l'Industrie par J. ANCELLIN, J. BENARD, R. CASTAING, J. DUPORT, C. FREJACQUES, R. GUILLAUMONT, J. LEFEVRE, E. MOUSTACCHI, J.R. SCHAPIRA, J. TEILLAC, J.C. ZERBIB et P. ZETTWOOG. 82 pages et 25 Annexes (1982).

- "Pyrido (3,4-c) psoralènes, obtention, applications en cosmétologie et à titre de médicaments et compositions cosmétiques et pharmaceutiques les contenant". E. BISAGNI, J. MORON, D. AVERBECK, L. DUBERTRET, D. PAPADOPOULO, J. BLAIS, P. VIGNY, N. MAGANA-SCHWENCKE, E. MOUSTACCHI, S. NOCENTINI and F. ZAJDELA. Brevet français 82 03 157 (25/02/1982).

Progress Report
1982

Contractor:
Rijksuniversiteit Leiden
Stationsweg 46
NL-2300 RA Leiden

Contract no.: BIO-E-421-81-NL

Head(s) of research team(s):
Prof. A. T. Natarajan
Dep. Radiation Genetics
and Chemical Mutagenesis
Wassenaarseweg 72
NL-2333 AL Leiden

General subject of the contract:
Development of in vivo mouse models for human X-ray sensitive diseases.

List of projects:

1. Breeding of mouse mutants.*
2. Screening of mouse mutants for chromosomal radiosensitivity.
3. Genetic tests on radiosensitive mouse mutants.

*Project 1 : see A.G. Searle MRC Harwell BIO-E-429 UK

Title of project nr. 2: Screening of mouse mutants for chromosomal radiosensitivity.

Head of project and scientific staff: Prof.Dr. A.T. Natarajan and Dr. P.P.W. van Buul.

Using the bone-marrow micronucleus test at two dose levels (50 and 100 rad X-rays) and two sampling times after irradiation (18h and 27h) as screening method, the following mouse mutants were studied for their chromosomal radiosensitivity: gyro (Gy), jimpy (jp), lurcher (Lc), fidget (fi), grizzle-belly (Sl^{gbh}), postaxial hemimelia (px), contrasted (Sl^{con}), scabby (Scb) and p-black-eyed sterile (p^{bs}). Of these mutants the sex-linked genes jp and Gy were tested in both heterozygous and hemizygous conditions, Lc in heterozygous and all the others as homozygotes.

Although the full scheme of 2 males and 2 females for each point has not yet been reached in all cases, the earlier observations for the mutants brindlet (Mo^{br}), viable brindlet (Mo^{vbr}), ochre (Och), sprawling (Swl) and varitint-waddler (Va) (see 1981 report) of a small but significant higher sensitivity of males compared to females could be confirmed with all mutations examined sofar.
This sex difference was more pronounced in the sex-linked mutants gyro and jimpy.

The mutants lurcher, fidget, postaxial hemimelia and scabby showed clearly higher radiosensitivities than normal mice whereas p-black-eyed sterile mice were less sensitive. All the other mutants did not deviate from the normal pattern of radiosensitivity. In general the differences between radiosensitive and normal mice were small compared to those reported for the hereditary human radiosensitive diseases, such as ataxia telangiectasia and Blackfan Diamond.

Progress Report
1982

Contractor:
Università di Roma
Policlinico Umberto I
I-00100 Roma

Contract no.: BIO-E-475-81-I

Head(s) of research team(s):
Prof. B. Nicoletti
Facoltà di Medicina
Policlinico Umberto I
I-00100 Roma

General subject of the contract:
Studies of the molecular basis of post-replication repair.

List of projects:

1. Characterization of the gene of pR plasmid involved in "error prone" repair in E. Coli.

Title of project n.BIO-E-475-81-I: Characterization of the gene of pR plasmid involved in "error prone" repair in E.coli.

Head of project:F.Gigliani

Scientific staff:R.Elli,L.Marcucci,P.A.Battaglia,C.Oppi

The TP120 plasmid is known to determine enhanced UV survival in E.coli wild type,UVrB and PolA mutants but not in RecA mutant. In a previous work it has been obtained a plasmid,named pR,by restriction of TP120. This new plasmid contains the origin of replication as well as the genes responsible for ampicillin and UV resistance and it codes for a 22.000 mw protein involved in the "error prone repair" process in E.coli wilde type.

During the last year the localization of the gene in the plasmid has been determined and the pR size was properly reduced to the purpose of analyzing the gene DNA sequence.

The results obtained are the following:

1. UV survival curves of 65 pR::Tn5 clones

E.coli cell strain C600 carrying the pR plasmid were infected with phage b221c185Tn5(Kan) at multiplicity of 5phages/cell.The Tn5 transposon, once inserted into a gene, inactivates its function through mutation by insertion. We analyzed for UV survival 65 Ap^+ Kan^+ transductants selected on agar plates containing Ap (20 ug/ml) and Kan (30 ug/ml). The UV survival curves of C600 cells containing pR::Tn5 plasmid have shown that 18 Pr::Tn5 clones have lost their ability to protect the cells against UV killing. The other 47 clones have shown the same or a higher UV survival as bacterial cells containing the intact plasmid (Fig.1).

2. DNA restriction analysis of pR::Tn5 clones

In order to localize the insertion sequences of the transposon into pR DNA, the DNAs of pR::Tn5 clones were extracted according to Humprey et al. and digested with appropriate restriction enzymes; the digestion products were separated by electrophoresis on 1% agarose orizontal slab gel in Tris-borate buffer pH8.2.

The results obtained from the DNAs of 7/18 clones,in which the Tn5 insrtion had inactivated the UV resistance, indicate that the UV function is localized into the BamHI-SmaI fragment of the pR genome (see Fig.2).

Preliminary results of the DNA restriction analysis of 3/47 clones that have mantained the UV resistance suggest that the Tn5 transposon is not inserted into the Bam-Sma fragment.

3. Isolation of DNA restriction fragments containing the UV$^+$ function

In order to study the mechanism of the expression of the UV gene we are constructing a new smaller plasmid which mantains only the replicon, the Ap resistance and UV function.

Our results suggest that all these three functions are localized in the HindIII-SmaI fragment; therfore we have carried out the following experiment:

- double digestion of pR DNA with HindIII and SmaI endonucleases.
- separation of HindIII-SmaI fragment on 1% low melting point agarose and recovery of this fragment as described in "Molecular cloning" (Maniatis T. and Fritsch E.F. and Sambrook J. 1982).
- purification of the eluted DNA by chromatografy of DEAE-Sephacel.

The following steps are the annealing of this fragment and the transformation of E.coli C600 in order to test if this new plasmid has mantained the three functions.

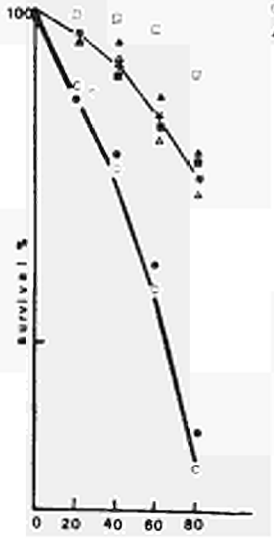

Fig.1 UV survival of C600 strain transformed with either pR or pR::Tn5 DNA.
○ C600; ✱ C600+pR; ● C600+2pR::Tn5; ▲ C600+53pR::Tn5; △ C600+54pR::Tn5; ■ C600+6pR::Tn5; □ C600+55pR::Tn5

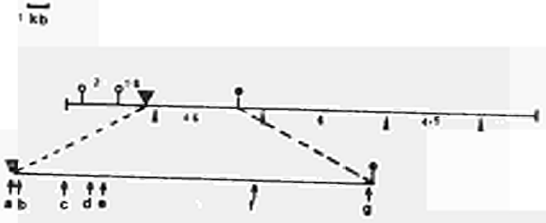

Fig.2 Restriction map of the pR plasmid cleavage sites for the restriction endonuclease Bam H1 ▽, BglII ▼, SmaI ▼, EcoRI ▽ are shown for HindIII linearized pR form.
Arrows indicate the insertion sites of Tn5 transposon in Bam H1-SmaI fragment:

a	2pR::Tn5
b	11pR::Tn5
c	1pR::Tn5
d	7pR::Tn5
e	76pR::Tn5
f	75pR::Tn5
g	69pR::Tn5

List of publications in 1982

I. Short Communications, Theses, Internal Reports, Patents...

1) Congresso del Gruppo di Biologia cellulare della SIBRES (Norcia aprile 1982)

2) First European Congress on Cell Biology (Parigi 18-23 luglio 1982)

3) XIX Convegno Nazionale della SIBBM (S.Gimignano 23-25 settembre 1982)

- Internal Reports

1) Progress report (Istituto Biologia generale Febbraio 1982)

2) Progress report (Istituto Biologia generale Giugno 1982)

3) Seminar (Istituto Superiore Sanità Ottobre 1982)

Progress Report

1982

Contractor:
Università di Roma
Città Universitaria
I-00185 Roma

Contract no.: BIO-E-400-81-I

Head(s) of research team(s):
Prof. G. Olivieri
Ist. di Genetica
Università di Roma
Città Universitaria
I-00185 Roma

General subject of the contract:
Studies on induced chromosome aberrations for a better evaluation of genetic risk to man.

List of projects:

1. Mechanisms of chromosome breakage and rejoining in Drosophila and their relationships with known repair processes.

Title of project nr 1. Mechanisms of chromosome breakage and rejoining in Drosophila and their relationships with known repair processes

Head of project and scientific staff: M. Gatti, S. Pimpinelli, S. Bonaccosri, C. Bove and G. Olivieri

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Isolation and characterization of sex-linked temperature sensitive lethals affecting mitotic cell division in Drosophila melanogaster.

Fifteen sex-linked temperature sensitive lethal and semi-lethal mutations in Drosophila melanogaster have been isolated that affect the fidelity of mitotic chromosome baheviour. These mitotic mutants were recovered from among a collection of 168 temperature sensitive (ts) lethal and semilethal mutants which were in turn found among 4,925 ethyl methanesulfonate -treated X-chromosomes. These X linked ts lethals were screened for those defective in functions necessary for regular mitotic chromosome transmission by constructing males hemizygous for each ts lethal and heterozygous for the autosomal recessive somatic cell marker mwh, rearing them under semi-restrictive conditions and examining wings of those individuals surviving to adulthood for an increased frequency of mwh clones. Those mutations producing elevated levels of chromosome instability during growth of the wing imaginal disc were also examined using somatic cell markers for their effects of chromosome behaviour in the abdominal histoblasts. Fifteen mutations affecting both wing disc and histoblast cells were detected. We infer that these mutations identify loci generally required for the fidelity of mitotic chromosome transmission. Mapping and complementation tests showed that these mutations identified 15 loci. One mutant was an allele of a locus (mus-101) previously identified by mutagen sensitive mutants (Boyd et al.,Genetics 84: 485, 1976) and a second mutant was an allele of the previously identified lethal locus zw-10 (Judd et al.,Genetics 71: 139, 1972).

The results of the cytological and genetical studies show that the effects of individual mutants on the fidelity of chromosome transmission were due to

elevated frequencies of either chromosome breakage, nondisjunction or a failure of chromosome condensation. Thus these mutations define a broad spectrum of functions required for the normal execution of the mitotic chromosome cycle.

Isolation and characterization of autosomal mutants affecting essential mitotic functions in Drosophila melanogaster

On the basis of our recent studies on lethal alleles at the mus-105 and mus-109 loci (Baker et al., PNAS 79: 1205, 1982) we proposed that alleles of essential mitotic loci should be found among lethals that die at the end of the larval period. Such late lethals have been isolated by three groups (Shearn et al., PNAS 68: 2594, 1971; Stewart et al., Dev.Biol. 27: 71, 1972; Kiss et al., Theoret.Appl. Genet. 48: 217, 1976) who have kindly made their mutant collections, totaling about 100 late lethals, available to us. Of the 41 lethals we have examined to date, 21 cause brofound disruptions in mitotic chromosome behaviour. This initial group of mutants screened has identified loci that are important for chromosome condensation, spindle formation, separation of daughter nuclei, and the maintenance of chromosomal integrity. Thus the phenotypes of late lethality together with poorly developed imaginal discs are almost diagnostic of a lesion in an essential mitotic function. We are proceding with the cytological screening of the approximately 60 remaining late lethals strains and should finish in about one year.

List of publications in 1982

I.

Gatti, M. (1982). Sister chromatid exchanges in Drosophila. In sister chromatid exchanges (S.Wolff ed.). Wiley and Sons, New York and London, 267-296.

Baker, B.S., D.A. Smith and M. Gatti (1982). Region-specific effects on chromosome integrity of mutants at essential loci in Drosophila melanogaster. Proc. Natl.Acad.Sci. U.S. 79: 1205-1209.

II.

Ciafré, S., E. Costa and M. Gatti (1982). Autosomal mutants affecting somatic chromosome stability in Drosophila melanogaster. Atti Ass. Genet. Ital. 27: 107-108.

Progress Report
1982

Contractor:
Università di Roma
Città Universitaria
I-00185 Roma

Contract no.: BIO-E-450-81-I

Head(s) of research team(s):
Prof. G. Olivieri
Ist. di Genetica
Università di Roma
Città Universitaria
I-00185 Roma

General subject of the contract:
Relationships between chromosome structure and induced chromosome rearrangements.

List of projects:

1. Sensitivity to chromosome aberrations and SCE's of different heterochromatic regions and of euchromatic - heterochromatic junctions.
2. Studies on the genetic control of the stability of different chromosome regions.

Title of project nr 1 - Sensitivity to chromosome aberrations and SCE's of different heterochromatic regions and of euchromatic - heterochromatic junctions.

Head of project and scientific staff: G. Olivieri, F. Pelliccia, A. Micheli, G. Belloni

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A number of experiments have been done with human lymphocytes in order to st dy repair mechanisms in this system, particularly as regards phase G_2 of the cel cycle.

a) Study of the distribution in the human genome of premutational damage repaire in G_2

In recent years it has been shown that in chromatid lesions induced by various mutagen treatments repair phenomena of a marked extent can occur in late G_2. This is at present the subject of extensive research. Two situations particularly point up the phenomenon:

a. G_2 cells which undergo mitotic delay have a lot fewer chromatid aberrations (C than cells which after mutagen treatment go on to mitosis without delay.

b. in cells that have undergone previous mutagen treatment exposure to hydroxyure and caffeine in the last hours of G_2 increases the frequency of CA 8-10 times.

We fell that an examination of the break points of the CA of cells with high and low CA frequency both in situation a. and in situation b. would help understand repair phenomena in G_2. The study was done in human lymphocytes either irradiated (situation a: comparison of cells with and without mitotic delay) or treated with thiotepa (situation b: comparison of cells with or without post-tre tment with hydroxyurea and caffeine).

The results obtained show a different distribution of breaks in the genome o cells with high and low frequencies of CA, which would indicate a non random distribution of repair phenomena.

b) Mitotic delay and repair in human lymphocytes

In human lymphocytes we have found it interesting to compare the frequency of induced chromatid aberrations (CA) in cell which both do and do not undergo mitotic delay.

Experiments were done on human lymphocytes by irradiating the cultures with various doses of X-rays (40R to 480R) and fixing at the various irradiation times (0-10h). Bearing in mind the fact confirmed in our experiments that in an irradiated G_2 population the number of cells that do not undergo mitotic delay- that is, that had come through the transition point (TP) -decreases with increased dose, using the data on the mitotic index, we were able to compare induced CA in cells presumably irradiated in the same stage of the cycle but which did or did not undergo mitotic delay. It was seen that after mitotic delay the frequency of induced CA decreases drastically. Whether as a result of or simultaneously with mitotic delay is yet to be seen.

c) Effects of $_3$HTdR or TdR at low concentrations on chromatid aberrations induced in human lymphocytes exposed to X-rays.

It is known that a radionuclide incorporated into a cell produces various kinds of genetic damage. Particularly well known are the effects of tritium incorporated into DNA, mainly by way of tritiated thymidine. We have found it of interest to study the damage caused by a second mutagen in a cell subjected to chronic irradiation after the incorporation of tritium.

Experiments were done on human lymphocytes in which the induction of chromatid aberrations with X-rays was studied in the presence of $_3$HTdR or TdR at equimolar concentration (1.5×10^{-8}M). At the concentrations used TdR alone or labelled with tritium can alter the effect of X-rays, decreasing the frequency of chromatid breaks. It is not fully clear whether at least part of the effect observed were due to the incorporation of tritium in itself.

Title of project nr 2 - Studies on the genetic control of the stability of different chromosome regions.

Head of project and scientific staff: M. Gatti, S. Pimpinelli, S. Bonaccorsi, C. Bove

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A gene controlling condensation of heterochromatin in Drosophila melanogaster

We have recently isolated a temperature sensitive lethal of D.melanogaster which maps in the same position of the mutagen sensitive locus mus-101 previously identified by two homozygous viable mutants confering hypersensitivity to killing by methyl-methanesulfonate (Boyd et al.,Genetics 84: 485, 1976). Complementation tests involving all known phenes - MMS sensitivity, female sterility and mitotic chromosome instability detected both genetically and cytologically - showed our ts lethal to be an allele at the mus-101 locus. Thus it has been named mus-101^{ts1}.

To characterize the effect of mus-101^{ts1} cytologically third instar larvae which had been reared at 18°C were shifted to the restrictive temperature of 29°C for various periods of time and metaphase chromosome preparations made by squashing ganglia in acetic orcein. mus-101^{ts1} cells kept at 18°C are cytologically normal and are indistinguishable from wild type control cells. However after exposure at restrictive temperature there is a dramatic effect on heterochromatin condensation, while euchromatin is unaffected. The frequency of cells with a complete failure of heterochromatin condensation is about 30% after 12-24 hours at 29°C.

Autoradiographic experiments showed that uncondesed heterochromatic regions incorporate tritiated thymidine during the S phase and that the failure of condensation can be induced in G_2 cells.

The simplest hypothesis which can account for these observations on mus-101^{ts} is that this locus codes for either a structural or an enzymatic protein which is specifically required for heterochromatin condensation. Our results on

mus-101^{tsl} strongly suggest that mutagen sensitivity and repair defects shown by mus-101 alleles are secondary effects due to the altered chromatin structure present in these mutants. It is conceivable that mutations at the mus-101 locus also affect chromatin organization in interphase nuclei and either render it more susceptible to mutagen damage or less available to repair.

List of publications in 1982

I.

- Gatti M., S. Bonaccorsi, S. Pimpinelli and M. Coluzzi (1982). Polymorphim of sex chromosome heterochromatin in the Anopheles gambiae complex. In "Recent Developments in the Genetics of Insect Disease Vectors", (W.W. Steiner ed.) Stipes Publishing Company, Champaing,pp 32-48.

II.

- Belloni G., S. Ciafré, S. Pimpinelli and M. Gatti (1982). Mutagen specificity of Mytomycin-C in somatic cells of Drosophila melanogaster. Atti Ass. Genet.Ital. 27: 407-408.
- Bonaccorsi S. (1982). Cytologicl dissection of Drosophila hydei heterochromatin. Atti Ass.Genet.Ital. 27: 427-428.
- Dimitri P. (1982). Cytogenetic analysis of the centromeric heterochromatin of chromosome 2 of Drosophila melanogaster. Atti Ass.Genet.Ital.27: 429-430.
- Pimpinelli S. and M. Gatti. Cytogenetic organization of the fertility factors of the Y chromosome of Drosophila melanogaster. Atti Ass.Genet.Ital. 27: 425-426.

Progress Report
1982

Contractor:
University College of Swansea
Singleton Park
GB-Swansea SA2 8PP

Contract no.: BIO-E-411-81-UK

Head(s) of research team(s):
Dr. J. M. Parry
Department of Genetics
University College of Swansea
GB-Swansea SA2 8PP

General subject of the contract:
Radiation damage, repair and genetic change in eukaryotic cells.

List of projects:

1. Radiation damage and repair in yeast cultures undergoing mitotic and meiotic cell division.
2. Comparative study of genetic change in eukaryotic cells irradiated during mitotic and meiotic cell division.

Project No. 1: Radiation damage and repair in yeast cultures undergoing mitotic and meiotic cell division.

Head of Project and scientific staff. R. Waters, J.M. Parry, S. Kelly, C. Merrill and J. Fox.

a) DNA repair studies in yeast using the cdc9 strain which possesses a temperature sensitive DNA ligase (R. Waters and J. Fox)

The production of single strand breaks at the restrictive temperature in UV irradiated cdc 9 cells and their absence at the permissive temperature was reported in 1981. We have now shown that the frequency of single strand breaks observed at the restrictive temperature is approximately equal to the number of M. luteus UV endonuclease sensitive sites observed in such UV irradiated cells. This indicates that the strain cdc 9 has attempted to repair the majority of dimers induced by the UV dose range of 0 - 20 $J.m^{-2}$. Thus in spite of the non-ligation of lesions undergoing repair the cells still undertake the repair of subsequent damage, and excision repair in this yeast strain must be considered as being uncoupled. This is in contrast to what has been observed in human cells following UV irradiation where the prevention of ligation can be achieved by DNA polymerase inhibitors, and results in the aborted repair of only a fraction of the dimers induced.

The cdc 9 assay is now being developed for extension of its use to the study of lesion formation and their repair in synchronized mitotic and meiotic yeast cells.

b) The cloning of the genes mediating DNA repair in Saccharomyces cerevisiae. (S. Kelly and J. M. Parry).

During 1982 we have been undertaking a project to clone the yeast genes responsible for the mediation of DNA repair after radiation exposure. In this work use has been made of the plasmid YEP-13 which contains inserts for the 2μ plasmid and leucine-2 gene of yeast together with resistance markers for amphicillin and tetracycline. The YEP-13 plasmid we are using carries a series of 5-10Kb inserts derived from the DNA of wild type (repair proficient) yeast.

Using the YEP-13 plasmid we have undertaken a study to identify specific plasmids carrying wild type yeast genes which complement the extensive range of yeast repair mutants available in our collection and a number of repair deficient bacterial strains. In the case of the yeast strains it has been necessary to produce a new range of cultures

carrying specific repair mutations and non-revertable mutants of leucine-2 which may be used as a selective marker for the incorporation of the YEP-13 plasmid into a yeast cell.

Using repair deficient strains of bacteria we have identified a number of plasmid isolates which carry yeast genes capable of complementing the bacterial mutations rec A and uvr A. In both cases repair deficient bacterial cultures carrying specific YEP-13 plasmids show wild type responses to X-rays and UV light respectively. These plasmids will now be used to determine the homology between specific repair genes of yeast and bacteria.

c) Inhibitors of DNA repair (R. Waters)

Studies using DNA repair inhibitors have been continued with an investigation of their effects in UV irradiated human fibroblasts from Cockayne Syndrome patients. This was carried out in conjunction with the M.R.C. Cell Mutation Unit at Sussex. Two strains from CS sibs appeared to carry out the incision step more rapidly than normal cells, but other CS strains in the same complementation group were normal. Hence, although the heterozygous parents of the CS sibs showing this difference did not exhibit repair differences it is more likely that the differences seen are the consequence of genetic factors other than the defective CS genotype.

Studies by other workers involving inhibitors of poly ADP-ribose polymerase have shown that they can slow down the rejoining of single strand breaks induced by X irradiation and increase the cell death observed in mammalian cells. It has been suggested that poly ADP-ribose polymerase activity influences the action of ligase II, and the blocking of the former enzyme reduces ligation.

Experiments in our laboratory indicate that this is not the only way by which poly ADP-ribose polymerase activity influences DNA repair. Following the exposure of normal human fibroblasts to the chemical carcinogens 4NQO or 3me 4NQO inhibitors of poly ADP-ribose polymerase increase the rate of cell growth. Hence an effect opposite to that seen after X irradiation occurs, and the results cannot be explained by a reduction in the activity of ligase II.

Project No. 2: Comparative study of genetic change in eukaryotic cells irradiated during mitotic and meiotic cell division.

Head of Project and scientific staff. J. M. Parry, E. M. Parry, N. Danford, S. Kelly, C. Merrill, A. Lafi, F. Ali.

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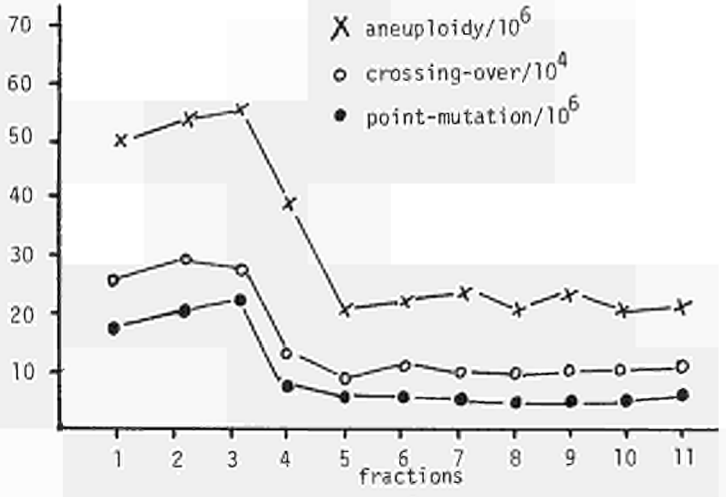
a) Comparison of the induction of chromosome aneuploidy, crossing over and point mutation by X rays in mitotically dividing yeast cells. (F. Ali and J. M. Parry).

We have utilized a series of yeast strains in which induced chromosome aneuploidy, cross-over and point mutation may be assayed in the same cell to study the induction of these events in X irradiation mitotic cultures of yeast. Cells were separated using a zonal roter into samples containing homogenous fractions representing specific stages during the cell cycle. The results of this study are shown in Figure 1 which shows the relative frequencies of these events after exposure to an X-ray dose of 60Krads.

Fig. 1



The results obtained indicate that the maximum frequencies of all three events were induced to a maximal extent during G_1 with a fall in the levels of induction during S phase.

The relative frequencies of the 3 genetic effects have also been compared after exposure of X-ray treated cells to the protease

inhibitor antipain. At the concentrations of antipain used in our experiments there was little or no effect of the presence of antipain upon cell viability. In contrast antipain exposure resulted in decreases in the levels of the 3 genetic events with does modification factors ranging from 1.4 to 3.6. The relative reductions in the levels of the three events were in order of crossing-over < aneuploidy < point mutation.

b) In vivo mouse meiotic aneuploidy assay (N. Danford)

We have been continuing our studies upon the induction of meiotic chromosome aneuploidy in mice carrying the X-linked coat colour marker, Tabby. Males were irradiated with 50 and 100 rads of X-rays. The treated males were crossed either with XX females heterozygous for the Tabby marker, or XO females. The preliminary results of these experiments are given in Table 1. The progeny which were suspected to have resulted from sex chromosome aneuploidy in the treated males were further examined cytogenetically and were shown to have the expected karyotypes.

Table 1. The number of progeny indicating the occurrence of sex chromosome aneuploidy in male mice treated with 50 or 100 rads X-rays.

	Dose(rads)	No. matings	No. progeny	No. showing sex chromosome aneuploidy
a)	0	75	621	0
	50	63	467	1
	100	65	454	1
b)	0	151	1029	0

a) Experimental data b) Breeding stocks

c) Sensitivity to X-rays and UV during meiotic cell division in yeast

Reduced sensitivity to the lethal effects of X-rays and UV was found during meiotic G1 phase compared to mitotic G1 phase and a similarity between the response of GO phase cells and G1 phase meiotic cells to UV induced lethality. Cells during meiotic S phase and prophase I exhibited relative resistance to X-ray and UV light induced lethality as in the mitotic cell cycle suggesting the operation of a similar sister-chromatid exchange repair process. The increased resistance of meiotic G1 phase cells may reflect differing amounts of damage associated with different chromatin forms or alternatively a greater level of interhomologue exchange repair in G1 prior to meiosis in comparison to mitotic G1 phase in diploid cells.

List of Publications in 1982

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N. Danford and J.M. Parry. The effects of 4CMB, 4HMB and BC in the micronucleus test. Mutation Res., 100, 1982, 353-56.

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Progress Report
1982

Contractor:
United Kingdom Atomic Energy
Authority, UKAEA
Charles II Street 11
GB-London SW1Y 4QP

Contract no.: BIO-E-460-81-UK

Head(s) of research team(s):
Dr. D.H. PEIRSON
Env.& Medical Sciences Div.
AERE
Harwell, Didcot
GB-Oxon OX11 ORA

General subject of the contract:
Studies in microdosimetry, cellular radiobiology and track structure.

List of projects:

1. Theoretical microdosimetry.

Title of Project nr 1 Theoretical microdosimetry

Head of project and scientific staff: P D Holt

The object of this project is to construct a model to interpret data on the dose-response relation of mammalian cells for radiations of different quality and at different dose-rates, in order to be able to predict the the response at very low doses and at very low dose-rates. A study has been made of the Repair-Misrepair model of cell survival (C. A. Tobias et al, Lawrence Berkeley Laboratory Report LBL 11220, 1980) and an attempt has been made to use the model to fit single-dose, split-dose and mixed-radiation survival data previously obtained in this laboratory for V79 Chinese hamster cells exposed to gamma-rays and fast neutrons.

Two types of repair process are postulated in the model, in which the instantaneous rates of repair are proportional to the density of unrepaired lesions and the square of this density respectively. For gamma-ray irradiation (Case I) the first type of repair is correct repair and the second type is misrepair, leading to cell death. For neutron irradiation (Case II) a fraction of the first type is also misrepair. On this basis it was difficult to interpret mixed-radiation data since the total density of lesions would be made up partly of neutron-induced lesions and partly of gamma-ray-induced lesions, and it was not clear what would be the correct value to assume for the fraction of linear repair that was misrepair.

An alternative model of cell killing has therefore been developed. It is based on the assumption that cell death is due to the interaction of pairs of sub-lesions which have a limited interaction range. The two sub-lesions of a pair may be produced by the same ionizing particle or by two different ionizing particles. The survival formula for both gamma-ray and neutron irradiation is identical to that of Case II of the RMR model but the formalism lends itself to the interpretation of mixed-radiation survival data. Fits have been obtained to our experimental data and a paper has been prepared for publication.

Munson et al (Int. J. Radiat. Biol. 13, 205, 1967) exposed several mutants of the bacterium E. coli to radiations of different LET's and interpreted their results on the assumption that cells could be killed by damage to either one or both strands of the DNA, but that in radio-

resistant strains single-strand damage could be repaired. The two-ion version of Lea's target theory, which was previously developed in this laboratory (P. D. Holt, Proc. Fourth Symp. Microdosimetry, 1973) has been adapted to this model. It predicts that at LET_∞'s above about 100 keV μm^{-1} two radiations with the same LET_∞ will not necessarily have the same RBE, and also that at these high LET's there will be a decrease in inactivation cross section with increasing LET_∞. Both these predictions are confirmed by published experimental data.

Publications

P.D. Holt The application of target theory to the inactivation of bacteria at high LET. Eighth Symp. on Microdosimetry, Jülich, 1982.

P.D. Holt, J.C. Asquith and C.C. Paice. A new model for cell killing by radiation and its application to the effect of dose fractionation. To be submitted to Int. J. Radiat. Biol.

Progress Report
1982

Contractor:
Gesellschaft für Strahlen- und
Umweltforschung mbH, GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

Contract no.: BIO-E-394-81-D

Head(s) of research team(s):
Prof. Dr. W. Pohlit
Abt. für Biophysikalische
Strahlenforschung, GSF
Paul-Ehrlich-Strasse 20
D-6000 Frankfurt 70

General subject of the contract:
Investigation of radiation induced damage to DNA in eukaryotic cell systems and its possible relationship to survival.

List of projects:

1. Quantitative determination of unrepaired and misrepaired DNA double - strand breaks and their possible relevance to radiation killing of yeast cells.
2. Measurement of induction and repair of double - strand breaks in mammalian cells.

Title of project no.1: Quantitative determination of unrepaired and misrepaired DNA double-strand breaks and their possible relevance to radiation killing of yeast cells.

Head of project and scinetific staff: Dr.D.Frankenberg, Dr.M. Frankenberg-Schwager

The studies of kinetics of DNA double-strand break (dsb) repair in irradiated yeast cells have been extended to a temperature-sensitive yeast mutant (rad 54-3/rad 54-3). This mutant is proficient to repair dsb at the permissive temperature of 23°C yielding a shouldered survival curve and is dsb repair-deficient at the restricitve temperature of 36°C (1) yielding an exponential survival curve (fig.1). Using this mutant the kinetic of dsb repair at 23°C can be investigated at the survival level. Fig.1 shows that the repair of dsb induced by a dose of 60 Gy after various periods of time in growth medium at 23°C (immediate plating conditions); repair of dsb was stopped by shifting the cells to 36°C. Similar experiments are currently performed under delayed plating conditions.

We have postponed to 1983 the studies on the effect of caffeine on dsb repair. Instead, we have performed the following studies scheduled for the 1983 programme: The RBE-value of cell survival is dependent on both the LET and the radiation dose. It seems that two factors determine the actual value of RBE: one factor is of physical nature and is due to the pattern of energy deposition for a given radiation and to its dose; the other factor is of biological nature since it is known that repair-proficient cells show higher RBE-values than cells which are deficient in the repair of damage induced by ionizing radiations (2). The role of repair contributing to the actual value of RBE of alpha particles was studied with the help of the above mentioned temperature-sensitive mutant. This mutant exhibits liquid holding (LH) recovery of colony forming ability (fig.2) in dependence of the LH-period at 23°C. Thus, with this mutant it is possible to obtain survival curves involving no repair of dsb (immediate plating (IP), 36°C), partial repair of dsb (IP, 23°C) and gradually increased levels of dsb repair by delayed plating (DP) after LH-periods of 24, 48 and 72h. The RBE-values of densely ionizing alpha particles (mean LET $\bar{L}_{100}$ = 65 keV/µm) in relation to sparsely ionizing 30 MeV electrons ($\bar{L}_{100}$ = 0.1 keV/µm) have been determined in dependence of the level of dsb repair (table I). The results are:

1. The RBE-value is low (1.6) and independent on dose when no repair of dsb is involved.
2. The RBE-value becomes dependent on dose when repair of dsb occurs. This dose-dependency is the more pronounced the higher the level of dsb repair.
3. The RBE-value for a given surviving fraction becomes gradually bigger (up to 7) with a gradual increase in the level of dsb repair.

The mathematical analysis of survival curves after LH-recovery for 72h on the basis of measured initial dsb and their repair by recombination shows that dsb which are irreparable by a recombinational process cannot represent the only damage leading

(1)M.Budd et al,Mutat.Res.103,1982,19. (2)V.G.Petin et al,Mutat.Res.82,1981,285

to cell killing (3).Instead, misrepaired dsb may contribute essentially to this effect. In fact, it is demonstrated experimentally that after 72h LH-recovery both more small and more long DNA molecules are observed than in unirradiated cells (3). This may be due to fragments and dicentrics arising from the misrepair of dsb. Therefore, the evaluation of the mean number of unrepaired dsb in irradiated yeast cells after dsb repair under LH-conditions for 72h and after dsb repair on the nutrient agar plate is ambiguous.

Table I: RBE-Dependency on repair of dsb for alpha particles

Surviving fraction S	IP, 36°C	IP, 23°C	DP,24h 23°C	DP,48h 23°C	DP,72h 23°C
0.9	1.6	3.0	3.7	5	7
0.7	1.6	3.0	3.7	4	5
0.5	1.6	3.0	3.7	3.4	4
0.37	1.6	3.0	3.7	3.2	3.6
0.1	1.6	3.0	3.7	2.8	3.0

0 — increase of dsb repair in homozygous rad 54-3 yeast — maximum

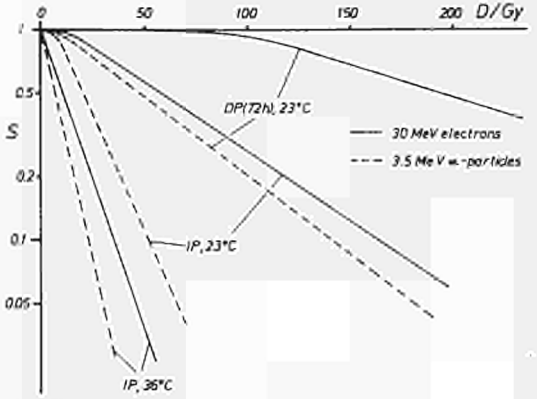

Fig.1

Survival curves of the diploid yeast 54-3 which is temperature-sensitive with respect to its ability to repair DNA dsb. At the restrictive temperature of 36°C exponential survival curves are obtained for 30 MeV electrons and for 3.5 MeV alpha particles. At the permissive temperature of 23°C shouldered survival curves are obtained for both types of radiation. (IP=immediate plating, DP=delayed plating).

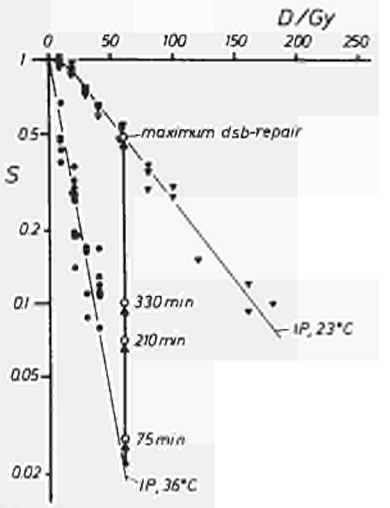

Fig.2

Increase of survival due to DNA dsb repair in the yeast mutant 54-3 as a function of incubation time in growth medium at 23°C. Repair was stopped by shifting cells on the nutrient agar to 36°C. Irradiation: 30 MeV electrons.

(3) D.Frankenberg, Proc.8thSymp. Microdos., 1982, in press

Project 2.

Measurement of induction and repair of double strand breaks in mammlian cells.

Head of project: Dr.P.E.Bryant.
Scientific staff: Dr.M.Nüsse, Dr.H.Schorn.

The repair of double strand breaks (dsb) in X-irradiated Ehrlich ascites tumour cells has been followed over a period of several hours using the unwinding method calibrated against the neutral sucrose gradient centrifugation method. The influence of DNA synthesis inhibitors on this repair; 9-ß-D-arabinofuranosyadenine (ß-ara A) and aphidicolin (APD) were shown to cause inhibition of dsb repair in a concentration dependent manner (1,2). These studies complimented survival studies which had previously shown that both ß-ara A and APD can cause inhibition of repair of PLD in this cell line. At low concentrations (<2µg/ml) of APD, repair of PLD in exponentially growing cells was promoted and the repair of dsb was not inhibited (2).

In experiments designed to simulate procedures used in PLD repair experiments, the numbers of dsb remaining unrepaired were measured in X-irradiated stationary cells after a 7h incubation in the same medium, followed by a 24h incubation in fresh growth medium (24h would be required for the cells to reach their first mitosis, had they been lightly irradiated). The results showed that the numbers of residual dsb were related to dose in a non-linear fashion. At higher doses more dsb remaining per dose were observed (figure 1). This relationship was similar to that found using the neutral sucrose gradient centrifugation method (3). Treatment of stationary cells with ß-ara A for 7h after X-ray exposure, followed by 24h in fresh growth medium, resulted in a larger number of residual dsb (ref.1, and figure 1). It has been shown that treatment of cells with ß-ara A after irradiation causes loss of the shoulder of the survival curve and an inhibition of PLD repair. The dsb repair experiments suggest that part of this effect may result from the inhibition of dsb repair during ß-ara A treatment, leaving a larger number of residual dsb which may lead to chromosome deletions (acentric fragments) at mitosis. Preliminary experiments also suggest that during the ß-ara A induced inhibition of dsb repair in X-irradiated cells more misrepair events take place between dsb, leading to exchange type chromosome aberrations. Experiments to investigate this further are in progress.

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3.Blöcher,D. and Pohlit,W.,1982,Int.J.Radiat.Biol.42,329.

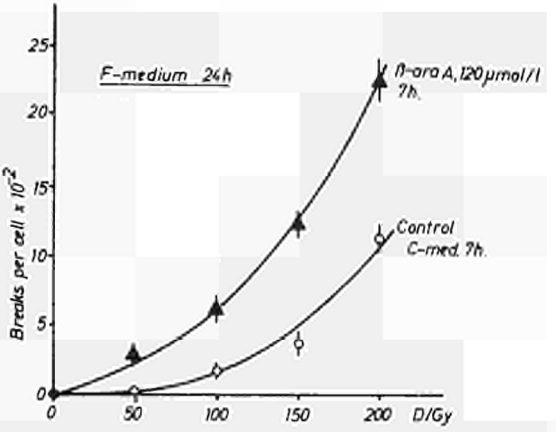

Figure 1. Residual dsb in cells after X-irradiation.

Investigation of the influence of membrane-specific drugs on various cellular end-points. (Dr.H.Schorn).

The influence of the neuropharmcological drugs chloropromazine (CPZ) and procaine have been shown to have a sensitizing effect on the X-ray killing of cells under hypoxic conditions, but not under aerobic conditions. Procaine produced in addition some radioprotective effect under oxic conditions. This resulted,in the presence of CPZ or procaine, in a diminution of the oxygen effect at the survival level.

In order to investigate the possible role of SH-compounds in this effect, the influence of CPZ on both protein-bound SH (PSH) and non-protein-bound SH (NHSP) compounds was studied. It was found that in exponentially growing Ehrlich ascites tumour cells CPZ resulted in a marked depletion of both NPSH and PSH. It is therefore possible that the reduced protective effect of hypoxia on X-ray killing in the presence of these membrane-specific drugs is related to the depletion of SH-compounds. Cells genetically deficient in the synthesis of the SH-compound glutathione have been shown to have an oxygen ratio of near 1.0 (1).

Structural changes were also observed after CPZ treatment. Using stereoscopic electron microscopy, cells were shown to have shorter microvilli after CPZ treatment. Blebs also occured as areas without membrane extensions.

Using the DNA unwinding method, the repair of both single strand breaks (ssb) and double strand breaks (dsb) in the DNA of EAT cells after X-irradiation under aerobic and anoxic conditions and in the presence or absence of CPZ. Preliminary experiments show that at drug concentrations producing a sensitization at the survival level, no change could be found in the rates of repair of either ssb or dsb as compared with the control untreated cells.

1. Morse,M.L. and Dahl,R.H.,1978, Nature 271,660.

List of publications in 1982

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Ahnström,G., and Bryant,P.E.,DNA double strand breaks generated by the repair of X-ray damage in Chinese hamster cells. Int.J.Radiat.Biol.,41, 671-676,(1982).

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Iliakis,G., Nüsse,M.,and Bryant,P.E. Effects of aphidicolin on cell proliferation, repair of potentially lethal damage and repair of DNA strand breaks in Ehrlich ascites tumour cells exposed to X-rays. Int.J.Radiat.Biol.,42,417-434(1982)

Bryant,P.E. and Blöcher,D. Repair of double strand breaks in X-irradiated Ehrlich ascites tumour cells and the effects of the DNA inhibitor ß-ara A. Proceedings of Symposium: Molecular and cellular action of ionizing radiation, Munich, Neuherberg, W.Germany, March(1982).

Bryant,P.E. The effects of the DNA polymerase inhibitor ß-ara A on double-strand break repair and the frequency of chromosome bridges and fragments observed at first anaphase after X-irradiation of Erlich acsites tumour cells. Proceedings of the 17th Annual Meeting of the European Society for Radiation Biology, Bordeaux,France,(1982).

Schorn,H., Bertsche,U.,Nüsse,M. and Purohit,S.C., Radiosensitization of anoxic EAT cells by membrane-specific drugs. Proceedings of the 17th Annual Meeting of the European Society for Radiation Biology, Bordeaux, France(1982).

Progress Report
1982

Contractor:

Gesellschaft für Strahlen-
und Umweltforschung mbH.
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

Contract no.: BIO-E-513-82-D

Head(s) of research team(s):

Prof. Dr. W. Pohlit
Abt. für Biophysikalische
Strahlenforschung, GSF
Paul-Ehrlich-Strasse 20
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General subject of the contract:

Chromosomal damage and cell repair after ionizing irradiation.

List of projects:

1. Chromosomal damage and cell repair after ionizing irradiation.

Title of project no. BIO-E-513-82-D

Chromosomal damage and cell repair after ionizing irradiation

Head of project and scientific staff:

Prof. Dr. W.Pohlit and Dr. U.Bertsche, Gesellschaft für Strahlen-und Umweltforschung mbH, Frankfurt(M), Germany.

The aim of this work is to study the influence of repair of potentially lethal damage (RPLD) on chromosomal damage after treatment with sparsely and densely ionizing radiations. Priority is directed to investigations at low doses ($D < 1$ Gy) and low dose rates ($D < 0.05$ Gy/h), since in these ranges more relevant data for radiation protection purposes can be gained than at higher doses. RPLD is expected to play a considerable role in the biological response after low doses if high dose rates are administered. Therefore the contribution of RPLD should be measurable by dose rate experiments and by inhibition of repair using drugs specific for RPLD. This then would allow to separate reparable damage from that part of damage which cannot be modified by conditions favoring RPLD ('irrepairable damage') and to estimate effects on cell inactivation or chromosome breakage at very low doses where up to now precise determinations are difficult.
Whereas the test for loss of colony forming ability (abbrev. CFA-loss) is the usual method for cell inactivation at high doses ($D > 1$ Gy), a test for chromosomal breakage would fit much better to the required sensitivity at low doses. However, classical procedures where different types of chromosome aberrations are counted are time consuming and not well suitable in investigations which involve more than one parameter. Therefore, chromosomal damage was scored using the micronucleus (MN-) test known in applications for screening of mutagenic substances where considerable loss of genetic material (micronucleus) occurred. This loss causes cell inactivation in most cases.

The basic characteristics of the MN-test were studied in Ehrlich ascites tumour cells (EATC) in exponential or plateau phase of growth. The cells were treated either with X-rays or with Americium-241-alpha particles (mean energy : 4.5 MeV) at various doses in the low or in the high dose region and incubated afterwards in nutrient medium to allow duplication

and formation of micronuclei.

After fixation and staining with a specific fluorescent DNA dye, Hoechst 33258, cell preparations were scored for MN in a fluorescence microscope. At least 3o MN but mostly 100 MN were counted in each sample. The fraction of micronucleated cells (with 1 and more MN) was determined to the total cell number. The technique allowed scoring of MN with volumes of less than 1 % of the nuclear volume. The following results were obtained:

1) Kinetics of MN formation:

Exponential growing EATC were irradiated at high dose rates with X-rays and allowed to proceed for different time intervals afterwards. With increasing time the MN-fraction increased up to a plateau observed immediately after the 1st division (duplication of total cell number). These results hold in general also after alpha-irradiation.

2) Dose response
Irradiation with 150 kV X-rays at high dose rates resulted in a linear response between 0 and 0.8 Gy (lowest dose: 0.1 Gy) and in a quadratic dose dependence at higher doses. After alpha-particle irradiation with comparable high dose rates linearity was found between 0 and about 0.1 Gy (lowest dose 0.01 Gy).
From the comparison of X-ray and alpha-particle irradiation effects in the linear region a RBE = 5.4 (+ 1.3, -0.9) was determined.

3) Reproducibility and sensitivity
The MN-response was tested for reproducibility after alpha-particle irradiation in three different runs. Results agreed within 10 %, if the control values (spontaneous MN-induction in unirradiated samples) were subtracted.
Sensitivity of the MN-test was found to depend strongly on culture conditions because of spontaneous MN-fraction. Cultures in exponential growth showed 1 % in controls, and plateau-phase cultures (3 day of growth) 2 %. Therefore only in exponential growing cultures the low dose limits of about 0.05 Gy in X- and 0.01 Gy in alpha-particle irradiations could be achieved.

4) Relationship to loss of colony forming ability
After X-irradiation at high dose rate with D = 3 Gy and inhibition of RPLD a MN-fraction of 65 % was found. CFA-loss was determined under similar circumstances to 95 %. Thus, at least 2/3 rd of all CFA-loss events could be attributed to MN-formation. The remaining 1/3 rd may not come only from further MN's which arise separately in the 2nd and 3rd division after irradiation but also from other events like the formation of binucleated cells, the fraction of which increased from 2 % (control) to 8 % (3 Gy).

5) Repair of potentially lethal damage
RPLD was observed in irradiated cells at doses D = 1 Gy and D = 3 Gy after X-irradiation at high dose rates by a significant decrease of the MN--fraction within 2 hours. ß-arabinofuranosyladenine, a specific inhibitor of DNA-polymerases known to inhibit RPLD on the CFA-level, increased MN--fraction significantly. The kinetics of repair and fixation of PLD were qualitatively similar in the MN-response and the CFA-loss test.

Progress Report
1982

Contractor:
Université Libre de Bruxelles
ULB
Av. F. D. Roosevelt 50
B-1050 Bruxelles

Contract no.: BIO-E-420-81-B

Head(s) of research team(s):
Prof. M. Radman
Dept. de Biologie Moléculaire
ULB
Rue des Chevaux 67
B-1640 Rhode-St.-Genèse

General subject of the contract:
Genetic effects induced by radiations and chemical carcinogens : mechanisms common to DNA repair, replication, recombination and mutagenesis.

List of projects:

1. Radiation induced mutagenesis and chromosomal rearrangements : molecular mechanisms of their induction and prevention and their role in carcinogenesis.

Title of project nr 1

Radiation induced mutagenesis and chromosomal rearrangements : molecular mechanisms of their induction and prevention and their role in carcinogenesis.

Head of project and scientific staff : M. Radman,

P. Caillet-Fauquet, P. Lecomte*, Z. Trgovcevic (1 month visitor),

C. Dohet*(Research Assistant).

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1) The molecular basis of the accuracy of DNA replication and induced mutagenesis.

A proofreading mechanism responsible for base selection by DNA polymerase was identified : it acts at an intermediate step in DNA synthesis [DNA.dNMP.PPi by the abortion of the non complementary nucleotide in the form of regenerate dNTP (PPi exchange). The inhibition of this mechanism (by PPi-ase or manganese) allows efficient in vitro mutagenesis on intact and damaged template, thus mimicking the effect of SOS induction during in vivo mutagenesis. Validation of this mutagenic mechanism in vivo is under investigation.

2) Characterization of mutants affecting DNA replication accuracy.

The effect of conditional thermosensitive mutations affecting DNA replication and mapping in (or close to) the E.coli gene coding for DNA polymerase III (polC gene) on spontaneous and UV induced mutagenesis was studied in relation to recA and lexA mutations. These mutator mutations affected differently the three studied mutational targets ($his^- \rightarrow his^+$; rifS → rifR ; valS → valR) indicating mutational specificity effects. The results clearly indicate a strong interference between the spontaneous mutator effect of the mutation in polC gene and induced mutagenesis and offer a promising experimental system to study the enzymology of mutagenesis.

3) Interference between DNA repair, DNA mismatch correction and SOS induction (in collaboration with G. Maenhaut-Michel - see also report of Euratom Contract BIO-C-359-81 B).

The inducible SOS mutator effect, measured in undamaged bacteriophage λ genomes replicating in UV irradiated E.coli host, was found to be higher in mismatch correction deficient mutants mutH, mutL and mutS and lower in the adenine methylation deficient (dam^-) mutant, than in the wild type E.coli. We conclude that a large proportion of the SOS induced "untargeted" mutations (which occur as mixed mutational clones) are removed by the mismatch correction system. The loss of potential mutants (both spontaneous and SOS induced) observed in dam^- mutants is proposed to result from a selective killing (mismatch stimulated suicide) of mismatch bearing chromosomes as the consequence of undirected repair of spontaneous or SOS induced replication errors. Consequently, the SOS mutator effect on undamaged DNA appears to result from decreased fidelity of DNA synthesis.

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* Present address : Institut Jacques MONOD, Université Paris VII (France).

4) The effect of UV lesions on the transmission of genetic markers.

Phage λ heteroduplex DNA carrying two mismatched genetic markers and UV lesions in only one, both or none of the two strands were used to infect E.coli hosts. Genetic markers carried by the irradiated strand were preferentially lost. The effect of DNA lesions on sister molecule exchange and mismatch correction is now being studied.

5) Chromosomal effects of a tumor promotor. (in collaboration with P. Jeggo, London, and A. Mogg, Brighton).

A Chinese Hamster V79 cell line heterozygous for a recessive mutation in the APRT gene (i.e. APRT +/-) was used to study the chromosomal effects of a non-mutagenic tumor promoter TPA. No evidence for mitotic recombination or gene deletion generating the change APRT +/- → APRT -/- was obtained. Instead different degrees of the shut-off of the $APRT^+$ gene were found in different TPA treated clones. The hypothesis of TPA-induced DNA methylation as the mechanism of gene inactivation is now being tested in collaboration with Dr. D. Drahovsky, University of Frankfurt, Germany.

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- Maenhaut-Michel, G. and Caillet-Fauquet, P. (1982)
2-aminopurine, a base analogue of adenine, induces in E.coli an error-free repair acting upon UV lesions in double stranded DNA. Mutation Res. 97, 242.

- Maenhaut-Michel, G. and Caillet-Fauquet, P. (1982)
2-aminopurine induced DNA repair in E.coli. Molec. Gen. Genet. 188, 143-148.

Progress Report
1982

Contractor:
Centre National de la
Recherche Scientifique
CNRS
Quai Anatole France 15
F-75007 Paris

Contract no.: BIO-E-427-81-F

Head(s) of research team(s):
Dr. A. Sarasin
Inst. de Rech. Scient.
sur le Cancer, CNRS
B.P. n°8
F-94800 Villejuif

General subject of the contract:
Genetic effects induced by radiation and chemical carcinogens : mechanisms common to DNA repair, replication, recombination and mutagenesis.

List of projects:

1. Analysis of SOS functions induced in mammalian cells by radiations and chemical carcinogens.

Analysis of some SOS functions induced in mammalian cells by radiations or chemical carcinogens.

Head of project : Dr. Alain Sarasin

Scientific staff : Alain Sarasin ; Alain Gentil ; Mauro Mezzina ; François Bourre ; Jacques Armier ; Leela Grosjean.

During the past year, we have continued to investigate the effect of ultraviolet-induced lesions (essentially pyrimidine dimers) on the mutagenesis and the genetic recombination of Simian Virus 40 DNA (SV40) used as a molecular model for studying analogous processes for mammalian cell DNA.

Mutagenenis of UV-irradiated SV40

Mutagenesis of UV-irradiated SV40 is measured as a reversion frequency from a thermosensitive phenotype (tsA58 : an early mutant or tsB201 : a late mutant) towards a wild-type phenotype. Mutation frequency is directly proportional to the UV-fluence and is strongly increased when host cells have been pretreated by UV-light or chemical carcinogen. This result has been taken as an example of possible SOS functions induced in mammalian cells.

We carried out a molecular analysis of UV-induced revertants of tsA58 virus which shows :

(1) No detectable variation in the size of the revertant genomes (deletion, addition, recombination) has been observed ;

(2) The original tsA58 mutation is still present in the revertant genomes (a point substitution from G:C to A:T) ;

(3) At least one base-pair substitution per revertant genome has been mapped and sequenced in the T antigen gene around the tsA58 mutation site ;

(4) These base-pair substitutions have been shown to be responsible for the reversion phenotype using a marker-rescue technique ;

(5) These base-pair substitutions are all localized opposite a potential thymine dimer site (9 revertants have been sequenced).

These results indicate clearly, and for the first time in animal cells, that pyridimine dimers are directly responsible for a targeted-

mutagenesis in a reversion type of mutation assay. Pretreatment of host cells by a cell DNA-synthesis inhibitor strongly increases this mutagenic potentiality of pyrimidine dimers.

Recombination process between UV-irradiated SV40

By using again SV40 mutants as a model system, we have also studied genetic recombination in mammalian cells in relation to UV-induced lesions. The recombination assay consists of infecting the same monkey kidney cells with two thermosensitive SV40 mutants which belong to the same complementation group, such as tsA58 and tsA7 or tsA30. After one lytic cycle at the permissive temperature of 33° C the survival of virus progeny is determined both at 33° C and 41° C. The recombination frequency is calculated by the survival ratio 41° C over 33° C after subtraction of the reversion frequency of each ts mutant towards the wild type phenotype.

Results have shown that the recombination frequency is very low (around 10^{-5}) when both viruses and host cells are intact. However, UV-irradiation of one of two viruses or of the two viruses increases the recombination frequency proportionally to the UV-fluence. For example, a 1200 J/m^2 UV-dose to the virus gives rise to a recombination frequency of 10^{-3}. This result, which has also been shown for other viruses such as *Herpes simplex* virus, indicates that inhibition of the replication fork by pyrimidine dimers produces abnormal molecules which are good substrates for a recombination process. In our experiments, UV-irradiation (10 J/m^2) or mitomycin C-treatment of host cells 24 hours before infection did not increase the recombination frequency in the viral progeny. This last result seems to indicate that genetic recombination of two ts SV40 mutants cannot be induced by pretreating the host cells with DNA-damaging agent or that infection with UV-damaged viruses is sufficient by itself to induce such a recombinogenic process by an indirect way.

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Progress Report
1982

Contractor:

Medical Research Council
MRC
Park Crescent 20
GB-London W1N 4AL

Contract no.: BIO-E-429-81-UK

Head(s) of research team(s):

Dr. A. G. Searle
Radiobiology Unit
MRC
Harwell, Didcot
GB-Oxon OX11 ORD

General subject of the contract:

Development of in vivo mouse models for human X-ray sensitive diseases.

List of projects:

1. Breeding of mouse mutants.
2. Screening of mouse mutants for chromosomal radiosensitivity.*
3. Genetic tests on radiosensitive mouse mutants.

* Project 2: see A.T. Natarajan, Univ. Leiden BIO-421-NL

Title of project nr 1: Breeding of mouse mutants

Head of project and scientific staff: Dr. A.G. Searle

The following mutants with behaviour and/or other defects have now been bred and transported to Leiden in sufficient quantities to allow their initial screening for radiosensitivity differences to be completed:- fi, jp, Lc, Mo^{br}, Mo^{vbr}, Och, p^{bs}, px, Rtd, Scb. Breeding of Gy, Sw1 and Va heterozygotes is also nearly complete; extra heterozygotes of Gy and Va are being bred so that the more severely affected Gy hemizygous males and Va homozygotes can be bred at Leiden and also tested. Attention is now concentrated on the breeding of mice carrying mutant alleles at the W and Sl loci, associated with white-spotting, macrocytic anaemia and inviability, as well as the Rw and Ph loci adjacent to W and with some similar properties, because of reports of increased somatic radiosensitivity. In addition, the mutant wasted (wst), with distinct similarities to ataxia telangiectasia, has been imported from the United States and will be bred in similar fashion.

Progress Report
1982

Contractor:
Rijksuniversiteit Leiden
Stationsweg 46
NL-2300 RA Leiden

Contract no.: BIO-E-407-81-NL

Head(s) of research team(s):
Dr. J.W.I.M. Simons
Dep. Radiation Genetics
and Chemical Mutagenesis
Wassenaarseweg 72
NL-2333 AL Leiden

General subject of the contract:
The genetic and biochemical basis of radiation sensitivity in human and other cells in culture.

List of projects:

1. Genetic analysis of DNA repair.
2. Biochemical analysis of DNA repair.
3. Consequences of DNA damage and repair.

PREAMBLE

Human cell variants believed to be deficient in DNA repair, continue to constitute a major focus of the work of the contracting laboratories. Further genetic analysis has revealed the existence of three complementation groups for Cockayne syndrome and a further complementation group (H) in xeroderma pigmentosum (XP). Hypermutability of Cockayne syndrome cells following UV has been confirmed in a third cell strain. In the A, D, G and H complementation groups of XP it has been possible to correct the repair defect after microinjection of human cellular extracts. A gene involved in the A group complementation process has been localized on chromosome 1. As a pointer to the future, it has also been possible to obtain transfer and expression of a gene originating in an inactive X-chromosome.

A method has been developed for determining the ability of human cells to effect repair of potentially lethal radiation damage. Among cells shown to be deficient in such repair have been those from a patient with Gorlin's syndrome and those from an apparently normal lymphoma patient who showed a severe erythemal response to radiotherapy.

At the biochemical level, work has progressed in two areas concerned with the more sensitive detection of DNA lesions. Firstly, the UV-endonuclease method for detecting sensitive sites has been developed by incorporating elution techniques and fluorescent dyes, thus eliminating the need for radioisotopic labelling and permitting *in vivo* experimentation. A second approach has involved the development of a procedure for making monoclonal antibodies with a high affinity for specific DNA lesions. The use of monoclonal antibodies has enabled the detection of pyrimidine dimers in human epithelial cells exposed to gamma radiation.

Significant progress has been made in the study of the mechanisms of action of DNA repair inhibitors such as aphidicolin, bringing out the importance of the metabolic state of the cell (whether stationary or exponentially growing). The role of inhibitors of poly ADP-ribose polymerase in repair of damaged DNA, as well as their influence on frequencies of chromosome aberrations, sister chromatid exchanges and point mutations has been studied in detail. Other biochemical studies have concerned the involvement of DNA ligases I and II in DNA repair and a human cell mutant has been characterized with properties consistent with a deficiency in DNA ligase I activity.

The work has been carried out in a joint programme by:

D. Bootsma *et al*, Erasmus University, Rotterdam (BIO 404 NL).
B. A. Bridges *et al*, MRC, Brighton (BIO 414 UK).
P. H. M. Lohman, TNO, Rijswijk (BIO 403 NL).
J. W. I. M. Simons, University of Leiden (BIO 407 NL).

Title of project nr. 1.
Genetic analysis of DNA-repair

Head of project and scientific staff :
Dr. J.W.I.M. Simons, Dr. A.T. Natarajan and
Dr. R. Cupido.

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1. Experiments directed at the concomitant induction of mutation and cell transformation.

   A BHK 21/13 cell line was obtained from Dr. Revell (Institute of Cancer Research, England) which had been cultured for only 100 generations since the original isolation of clone 13. This line is still characterized by a normal contact inhibition. The conditions for the selection of transformed clones have been sorted out, which resulted in a standardization with respect to cell density, pH and serum concentration which allow a 100% recovery of transformed cells in reconstruction experiment. After treatment with 4 NQO cell transformation was induced. After a chronic treatment with 4 NQO cell transformation was induced in the absence of cytotoxicity. Induction of cell transformation was also observed after treatment with ENU. The expression time curve for the induction of cell transformation appeared to be characterized by an optimum. The same cell line lends itself for the selection of both ouabain-resistant and 6-thioguanine resistant mutants.

2. Experiments directed at the isolation of radiation sensitive mutants. CHO cells were treated with ENU in order to induce repair-deficient mutants. After five days expression time the cells were irradiated with a small dose of UV and incubated in a medium containing BUdR and Hoechst stain 33258. Subsequently they were irradiated with long wave ultraviolet light in order to kill the cells which had incorporated BUdR by repair replication. After this treatment the surviving fraction of the population is 10^{-3} and theoretically the fraction of repair sensitive mutants in the population is enriched 100-200 fold. After two rounds of enrichment clones have been isolated and tested for UV-sensitivity. Although some clones appeared to be somewhat more sensitive to UV than wild type cells (about 2-fold), sofar no real radiation sensitive mutants have been obtained.

3. One more patient of Blackfan Diamond was analyzed during this year and

found to be radiosensitive similar to an earlier case reported by us.

Title of project nr. 2.
Biochemical characterization of radiosensitive mutants

Head of project and scientific staff :
Dr.Ir. A.A. van Zeeland

The effects of aphidicolin, an inhibitor of polymerase alpha, on UV-induced repair replication.

Aphidicolin (APC) is an inhibitor of polymerase alpha, the enzyme involved in semiconservative DNA replication in mammalian cells. It has no effect on polymerase beta or gamma, when tested in vitro. This differential inhibitory effect of APC has been used by several investigators to study the question which of the polymerases is involved in DNA-repair replication after excision of DNA damage. These investigations however have led to conflicting results. Ciarrochi et al. (1979) reported the inhibition by APC of DNA repair whereas Pedrali-Noy and Spadari (1980) did not find such an inhibitory effect using Hela cells. Using permeabilized Hela cells in which semiconservative DNA synthesis was blocked by the addition of hydroxyurea Hanaoka et al. (1979) found an inhibition of repair replication by APC. We have carried out experiments in which we measured the effects of APC on UV-induced repair replication using the CsCl density labelling technique. These experiments were carried out with human diploid fibroblasts (growing as well as stationary cells) and with Hela cells. The results show that in growing fibroblasts APC (5 ug/ml) virtually completely blocks semiconservative DNA synthesis, as judged from the presence of radioactivity in neutral CsCl gradients at the position of DNA in which thymidine in one of the strands has been substituted by bromodeoxyuridine (BUdR). However in these growing fibroblasts no inhibition was found of DNA repair replication. In contrast with the situation in growing fibroblasts, we observed a strong inhibtion of DNA repair replication in human fibroblasts held in stationary phase. We hypothesized that this differential inhibitory effect of APC is caused by differences in the size of deoxyribonucleotide triphosphate pooled between growing and resting cells. An indication for this were the results obtained with Hela cells. APC caused only an inhibitory effect on UV-induced DNA repair replication in the presence of hydroxyurea (10 mM). It is known that hydroxyurea blocks the enzyme ribonucleotide reductase, as

a consequence of which the size of dNTP's in the cell are reduced.

Effects of 3-aminobenzamide on DNA repair in human cells.

It has been shown by several investigators that 3-aminobenzamide (3AB), an inhibitor of poly(ADP-ribose)polymerase is able to increase the amount of excision repair measured as unscheduled DNA synthesis in human lymphocytes treated with UV-light or alkylating agents as well as in diploid human fibroblasts treated with dimethylsulphate (DMS). In addition it has been observed that 3AB inhibits the repair of single strand breaks or alkali labile sites induced by dimethylsulphate in mouse L1210 cells and in human fibroblasts. A possible explanation which has been put forward is that the inhibition of poly(ADP-ribose) polymerase prevents the rejoining step in excision repair. Under these conditions nick translation at repair sites might take place resulting in a larger size of the repair patches. If the number of repair sites is not changed the amount of incorporation of repair label will be increased. We have carried out experiments in which we investigated the effect of 3AB on the size of repair patches induced by UV-irradiation or dimethylsulphate in human diploid fibroblasts. Repair replication was determined using the CsCl density gradient technique. Human fibroblasts (VH-16) were cultured for 2-3 days in medium containing 0,3 uCi/ml ^{32}P (as orthophosphate) and subsequently grown to confluency in medium without radioactivity (MEM plus 15% foetal calf serum). One hour prior to the mutagenic treatment, BrdUrd (10^{-5} M) and FUdR (10^{-6} M) were added to the medium. At the time of treatment the medium was removed and the monolayers were rinsed with phosphate buffered saline. The cells were then either irradiated with shortwave UV-light or incubated for 20 min in medium without serum containing 0-600 uM DMS, BrdUrd (10^{-5} M), FUdR (10^{-6} M). 3AB and/or hydroxyurea were included where indicated. Following the treatment the cells were incubated in medium containing 5 uCi/ml ^{3}H-BrdUrd (25 Ci/mmole), 10^{-5} M unlabelled BrdUrd and 10^{-6} M FUdR. 3AB and/or hydroxyurea were present where indicated. After a 4-hour repair period the cells were lysed and neutral plus alkaline CsCl density gradients were used to determine the amount of repair replication. In those cases where the size of the repair patches were analyzed, two neutral CsCl gradients were used, prior to sonification of the DNA and analysis on an alkaline CsCl gradient. In each

experiment the specific activity of the DNA expressed in cpm ^{32}P/ug DNA was determined and the amount of repair replication was expressed as cpm ^{3}H/ug DNA.

The results obtained after UV-irradiation show that the presence of 3 mM 3AB in the medium during the repair period had no effect on the amount of repair replication observed in these cells. The size of the UV-induced repair patches was determined on alkaline CsCl gradients. Sonification of the DNA causes a shift in the ^{3}H-BrdUrd labelled DNA to a position in the gradient corresponding with a higher density than the ^{32}P-labelled parental DNA. This is because the ^{3}H-BrdUrd-labelled repair patch becomes a significant part of the total length of the DNA fragment. The results show that the presence of 3AB during the repair period does not effect the shift of the repair label in the alkaline CsCl gradients and therefore 3AB does not influence the size of the UV-induced repair patch. The results obtained with DMS in the same cell strain are similar to those obtained with UV-light. Also here there is no effect of 3AB on the amount DNA repair replication. In some instances 3AB has been added to the cells 30 min before treatment with DMS. In those experimens also hydroxyurea (10 mM) was present from that time on until the end of the repair period. In none of the experiments there was a potentiating effect of 3AB on DNA repair replication. The alkaline CsCl gradients show that 3AB has no effect on the size of DMS induced DNA repair patches.

Title of project nr. 3.
Consequences of DNA damage and repair

Head of project and scientific staff :
Dr. A.T. Natarajan, Dr.Ir. A.A. van Zeeland, Dr. J.W.I.M. Simons, Drs. I. Enninga.

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1. Inhibitors of poly(ADP-ribose)polymerase such as 3-aminobenzamide (3AB) and benzamide (B) increase the frequencies of spontaneously occurring sister chromatid exchanges (SCEs) by 5 to 10 fold. This increase occurs only in the second cell cycle when BU containing DNA is used as template for DNA synthesis. 3AB increases the frequencies of chromosomal aberrations induced by X-rays, increases the frequencies of SCEs induced by methylmethanesulfonate and ethylmethane sulfonate, but not those induced by UV and mitomycin C in CHO cells. Though 3AB treatment increases the frequency of SCEs, it does not increase the rate of point mutations occurring spontaneously or induced by EMS or ENU, indicating that SCEs and point mutations are not directly related processes.
2. Fibroblasts from patients of ataxia telangiectasia, Fanconi's anemia, xeroderma pigmentosum and Huntington's chorea respond similar to normal human fibroblasts with regard to induction of SCEs by 3-aminobenzamide. Though 3AB does not induce chromosomal aberrations in CHO cells or normal human fibroblasts under conditions that induce a several fold increase in the frequencies of SCEs, in cells derived from Fanconi's anemia and ataxia telangiectasia, the frequencies of spontaneous chromosomal aberrations are increased by 3AB.
3. Aphidicolin, an inhibitor of DNA polymerase -increased the frequencies of chromosomal aberrations induced by X-rays in G_0 lymphocytes, but not in G_1 stage of exponentially growing CHO cells, which indicate that the metabolic status of the cells during treatment with inhibitors is an important factor in the extent of potentiation obtained. Parallel biochemical experiments have confirmed this conclusion (see report Van Zeeland).

   When CHO cells are treated with UV or alkylating agents in G_1 and challenged in G_2. Just prior to fixation with inhibitors of DNA synthesis (aphidicolin, araC) or repair (caffeine), a significant potentiation of the frequencies of chromosomal aberrations was obtained.

These results indicate that the lesions induced in G_1 persist (in original or modified form) till G_2, the repair of which can be inhibited at that stage.

4. Human diploid skin fibroblasts were cultured at 30° C in order to investigate whether this leads to liquid holding conditions and whether the cells can be synchronized in this way. It was indicated that cells which are in S-phase at the time of switching to low temperature complete their DNA-synthesis before becoming arrested in G_1. The arrested cells were stimulated to proliferate synchronously by restauration of the optimal growth temperature (37° C). At 30° C excision repair is operative although somewhat reduced in comparison with repair at 37° C.

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Progress Report
1982

Contractor:
National Radiological
Protection Board, NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

Contract no.: BIO-E-413-81-UK

Head(s) of research team(s):
Dr. H. Smith
Biology Department
NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

General subject of the contract:
The production of chromosome aberrations in human lymphocytes by ionizing radiations.

List of projects:

1. Dependence of radiation induced chromosome aberrations on radiation quality.
2. Studies of break repair kinetics in the production of chromosome aberrations in human lymphocytes.

Title of project nr 1

Dependence of radiation induced chromosome aberrations on radiation quality

Head of project and scientific staff :

A. A. Edwards

Problems concerned with the dosimetry of accelerated charged particles have arisen during the year. The estimates of the proton and the helium-3 fluences using an ionisation chamber disagreed with the estimates made using thermoluminescent devices. Non-uniformity of the beam was a contributory cause and setting up procedures have been instituted to obviate this. A residual non-uniformity still exists, the centre of the beam having a 20% higher intensity than the edges. All attempts to reduce this variation have so far failed. It is thought that the remaining discrepancy between the physical dosimetric methods will disappear when the energy spectrum of the beams are measured properly and the effects of the low energy tail are taken into account.

Two series of exposures have taken place during the year both with 22 MeV helium-3 ions corresponding to an LET of about 24 keV/μm. The first series failed due to poor cell yields in the cultures. The second attempt was successful at most dose points and microscope analysis has begun. Microscope slides from the previous year's irradiation with protons of energy 8.6 MeV (LET = 5.2 keV/μm) have been scored and the yields, contrary to expectation, are slightly lower than those observed for X-rays at 250 kVp. Repeat irradiations at some higher doses have been made to check this finding but as yet they have not been scored.

Title of project nr 2

Studies of break repair kinetics in the production of chromosome aberrations in human lymphocytes

Head of project and scientific staff :

D. C. Lloyd

A range of doses up to 5 Gy of Co-60 γ radiation has been delivered to human blood maintained at 37°C over dose delivery times of 5 mins, 1, 3, 6 and 12 hours. Some difficulties were experienced in viability of cells exposed for the longest time but these were overcome by modifications, especially to the volume of head space in the specimen tubes.

Lymphocytes from the blood have been analysed for unstable chromosome aberrations; dicentrics, centric rings and acentrics; and analyses are completed for the 5 mins, 1, 3 and 6 hour exposures. For the 12 hour exposure the microscope analysis is almost completed.

A preliminary examination of the results prior to a detailed computer analysis of dose-response relationships shows that with increasing exposure time the aberration yields fall. By comparison with the acute exposure data the decline in yields is most marked at the higher doses. This accords with the hypothesis that for a linear quadratic dose effect relationship dose rate affects the quadratic term of the equation. When the results are fully analysed it is hoped that the work will enable a time related function, which depends on a chromosome lesion repair time, thought to be $\sim$2 hours, to be applied to the quadratic terms of the yield equations.

Progress Report
1982

Contractor:

Rijksuniversiteit Leiden
Stationsweg 46
NL-2300 RA Leiden

Contract no.: BIO-E-406-81-NL

Head(s) of research team(s):

Prof. F. H. Sobels
Dep. Radiation Genetics
and Chemical Mutagenesis
Wassenaarseweg 72
NL-2333 AL Leiden

General subject of the contract:
The genetic effects of radiation in eukaryotes.

List of projects:

1. Radiation genetics with Drosophila melanogaster.
2. Radiation cytogenetics with mammalian cells in vivo and in vitro.
3. Improvement of mutational assay systems and discrimination between point mutations and deletions in mammalian cells.

Title of project nr. 1.
Radiation genetics with Drosophila melanogaster.

Head of project and scientific staff :
Dr. J. Eeken, Drs. W. Ferro
Dr. B. Leigh, Dr. A. Schalet
Prof.Dr. K. Sankaranarayanan
Prof.Dr. F.H. Sobels

1.1. GENETIC RESPONSE OF A RADIATION SENSITIVE EBONY MUTANT -

a) As an extension of our earlier work on maternal effects, in the first series of experiments irradiated males with appropriate autosomal markers were crossed to Canton-S or ebony females. The results show that the frequencies of autosomal (2-3) translocations are higher when ebony females are used. These data are consistent with our earlier findings on maternal effects with respect to the X-ray induction of sex-linked recessive lethals and dominant lethals.

b) In another series of experiments, males carrying ring-X chromosomes were irradiated in N_2 or O_2 (500 R to 4000 R) and mated to Canton-S or ebony females. The frequencies of ring-X chromome losses (= XO males) were scored. The currently available results suggest that with Canton-S females (13 experiments), the frequencies of XO males are higher after irradiation in O_2 than after irradiation in N_2. In the ebony crosses (10 experiments), the situation is not clearly established. However, at lower exposures (500 and 1000 R), the situation seems to be similar to that involving Canton-S females.

c) Biochemical data on repair of endonuclease sensitive sites after UV irradiation of primary cell cultures suggest a slight but consistent difference between ebony and Canton-S (i.e., the cells from the ebony strain are less proficient in photorepair after UV irradiation). Experiments on the repair of single-strand breaks after X-irradiation show, that the rate of repair may be slightly slower in ebony than in Canton-S. However, the X-ray doses necessary to demonstrate the difference are well above those used in genetic tests.

d) We investigated whether the Canton-S and ebony strains also differ in their genetic response to chemical mutagens: MMS, ENU, DEB, DEN and 2,4,6-Cl_3-PDMT, and differences were only found with the last two chemicals. With DEN, the ebony strain gave lower frequencies of sex-linked recessive lethals in post-meiotic male germ cell stages than the Canton-S strain.

With 2,4,6-Cl_3-PDMT, the ebony strain was more sensitive than the Canton-S strain, particularly with respect to the response of the spermatids. These data have been interpreted on the assumption of differences in the enzymatic activation of DEN versus that of 2,4,6-Cl_3-PDMT.

1.2. RADIATION-INDUCED CHROMOSOMAL CHANGES IN MEIOTIC AND POST-MEIOTIC MALE GERM CELLS - The effects of neutrons on various stages of spermatogenesis have been reported in the literature, but there was no example of a continuous series of samples from spermatogonia to spermatozoa. This information was required for extension of the studies with neutrons. One-day old wild-type (M56i, Amherst) males were irradiated with 7.5 Gy of 0.5 MeV neutrons (REP-TNO, Rijswijk) or 15.0 Gy of 100 kV X-rays. Treated germ cells were then sampled by mating the males for a series of five 2-day broods, with 5-6 fresh virgin M-6 females per male per brood. The frequencies of recessive lethals were obtained by scoring the F_2 cultures. An RBE of 2.5 was found in the first three broods. An RBE of 1.0 was found in the last two broods, but it was difficult to get sufficient numbers of chromosomes to test because of the sterility of the irradiated males in these broods. The highest frequencies of recessive lethals were found in brood 3.

1.3. THE ROLE OF DNA REPAIR PROCESSES IN THE INDUCTION OF MUTATIONS AND CHROMOSOMAL ABERRATIONS BY X-IRRADIATION -

A. Experiments were designed to examine whether genetic damge induced in spermatozoa and late spermatids after irradiation in O_2 is qualitatively different from that induced in an N_2 atmosphere. Ia. Experiments with irradiated spermatozoa and the mei-9^a mutant (excision repair deficient) have been completed (Ferro, Mutation Res., 1983). In these studies, adult Muller-5 males were X-irradiated in N_2 (2000 R), air (500-2000 R) or O_2 (1000 R) and mated to mei-9^a or mei$^+$ females. Irradiation in air or in N_2 led to significantly higher yields of recessive lethals when the irradiated males were mated to mei-9^a females. After irradiation in O_2 however, the yields were similar with both kinds of females. No significant differences in the frequencies or reciprocal translocations were observed between the mei-9^a and mei$^+$ groups after irradiation of the males in N_2, air or O_2. These results support the notion that the kinds of genetic damage induced in mature spermatozoa in air or N_2 are qualitatively similar at least with respect to the component(s) that lead to the production of sex-linked recessive lethals, but clearly different when

induced in an O_2 atmosphere.

Ib) Similar studies with the mei-41^{D5} mutant (post-replication repair-deficient) and the mei^{+} strains provide no evidence for differential maternal effects for sex-linked recessive lethals irrespective of the gaseous atmosphere (N_2, air or O_2) used during irradiation of the males. However, there was a statistically significant increase in the frequencies of autosomal translocations after irradiation in air, with the mei-41^{D5} females relative to the mei^{+} females; no such effect could be demonstrated after irradiation in N_2 or O_2.

II. Late spermatids were irradiated in M-5 pupae. The pupae were irradiated in N_2 (1250 R) followed by a post-treatment of N_2 or O_2 and irradiated in O_2 (400 R) followed by a post-treatment of O_2. Immediately after eclosion irradiated individuals were crossed to females of the following genotype: mei^{+}, mei-9^{a}, mei-41^{D5} or mus 101^{D1}.

The results showed that the frequency of recessive lethal mutations was increased after irradiation in nitrogen when treated males were crossed to mei-9^{a} females as compared to mei^{+} females. No increase was detected after irradiation in oxygen in a similar comparison. When irradiated individuals were crossed to mei-41^{D5} females, there was no effect on the frequencies of recovered recessive lethals, regardless whether irradiation was performed in a nitrogen or oxygen environment. When irradiated individuals were crossed to mus-101^{D1} females, the frequency of recessive lethals was reduced, after irradiation in nitrogen as well as after irradiation in oxygen. A slight but consistent reduction in the frequency of recessive lethals with O_2 post-treatment after irradiation in nitrogen was found irrespective of the genotype of the females used.

The frequency of translocations was increased at the borderline of significance in nitrogen and oxygen when mei-41^{D5} females were used, as compared to mei^{+} females; whereas this frequency was decreased significantly when mus-101^{D1} females were used and was most pronounced after irradiation in oxygen.

B Experiments to determine the radiosensitivity of immature oocytes stages (stage 7) of mei-9 mutant have now been completed. In these experiments, the second chromosomes of the mei-9 and mei^{+} females were made lethal-free and irradiated with X-ray exposures of 500, 1000 and 2000 R. The frequencies of second chromosome lethals were determined. The main results are as follows: 1) the frequency in the controls is higher in

mei-9 than in mei$^+$; 2) after irradiation with 500 or 1000 R, the frequencies with mei-9 females are higher than in mei$^+$ females (about 2-fold); and 3) after 2000 R, no gametes could be tested with mei-9 females. These results are consistent with what has been recorded with respect to maternal effects with these two strains and show that mei-9 females are deficient in the repair of X-ray-induced genetic damage leading to recessive lethals.

1.4. ANALYSIS OF MUTATIONS INDUCED BY A FREQUENTLY OCCURRING MUTATOR GENE - We investigated a frequently occurring mutator gene, MR, that enhances spontaneous and X-ray induced mutation frequency in a specific way. The mutation induction does not affect all genes equally; certain genes (singed, sn and raspberry, ras) are mutated much more frequently than others. Therefore, the mutation to sn or ras can be used as a parameter of specific MR-activity. Since it is now known that MR is involved in a transposition process, and MR-induced mutations are due to the insertion of a DNA-fragment, these mutations should be unstable, and revert to wild type at a high frequency. Indeed we found that our MR-h12-induced sn mutations (and ras mutations) do revert to wild type, but only if an active MR is also present. The frequency of sn to sn$^+$ reversion is about 1.7% (Eeken, 1982). This frequency is an order of magnitude higher than the MR-specific induction of sn. Therefore, the reversion of an unstable sn mutation is a much more convenient parameter of MR-activity than the induction of sn. Using this reversion-system we attempted to determine the effect of 1) DNA-repair deficiencies, and 2) the chemical mutagens ENU and MMS on the MR-mediated transposition process. The results show once more that MR-activity can be modified by DNA-repair deficient pathways (see our 1980 report), and that ENU but not MMS influences the reversion frequency.

We found that the presence of MR in males can lead to a sensitization for X-rays (see our report 1980). To determine whether this sensitization is due to an interference of MR with the maternal DNA-repair system, irradiated males were crossed to MR-carrying females. No influence of MR on the recovery of X-ray-induced sex-linked recessive lethals could be detected in this way (Eeken, 1982).

1.5. COMPARATIVE STUDIES ON THE GENETIC EFFECTS OF NEUTRONS AND X-RAYS ON THE GERM CELLS OF MALES - A preliminary analysis of neutron- and X-ray-induced mutations at the yellow, white, forked and vermillion loci produced by irradiation of spermatozoa has been published (Leigh et al., 1982). The reported indication for a qualitative difference between the

genetic effects of neutrons and X-rays has been extended by a more detailed genetic analysis of white locus mutations obtained in the work cited above and subsequent irradiations. Of 23 X-ray-induced white mutants studied, only 7 (30%) were multi-locus deficiencies, whereas 17 of the 25 (68%) neutron-induced white mutants analyzed were multi-locus deficiencies. Furthermore, all mutants were subjected to the spotted-white test that discriminates between functionally active and inactive mutants, and 4 additional neutron-induced mutants were identified as white locus deficiencies. In total then, the majority (70%) of the X-ray mutants retained some white locus function, but only a minority (16%) of the neutron mutants were functionally active.

In contrast to the clear differences observed for white mutants, the analysis of forked locus mutants thus far shows that no more than 1/3 of both the 24 X-ray-induced mutants and 30 neutron-induced mutants can be multi-locus deficiencies.

Title of project nr. 2.
Radiation cytogenetics with mammalian cells in vivo and in vitro.

Head of project and scientific staff :
Dr. P.P.W. van Buul
Prof.Dr. A.T. Natarajan
Dr. A.D. Tates
Dr.Ir. A.A. van Zeeland

2.1. FACTORS INFLUENCING THE FREQUENCIES OF CHROMOSOMAL ABERRATIONS, POINT MUTATIONS, AND SISTER CHROMATID EXCHANGES INDUCED BY IONIZING RADIATION IN MAMMALIAN CELLS. We have studied the influence of 4 classes of inhibitors: a) inhibitors of precursors of DNA synthesis, (e.g. hydroxyurea), b) inhibitors of DNA alpha polymerase (e.g. cytosine arabinoside, aphidicolin), c) inhibitors of DNA repair (e.g. caffeine) and d) inhibitors of poly (ADP-ribose)polymerase (e.g. 3 aminobenzamide and benzamide) on the frequencies of chromosomal aberrations induced by X-rays in Chinese hamster ovary cells (CHO) and human peripheral blood lymphocytes and fibroblasts. Hydroxyurea, aphidicolin and araC increase the frequencies of X-ray induced chromosome/chromatid breaks but not exchanges. Caffeine and benzamide, on the other hand, increase the frequencies of all classes of chromosomal aberrations, including exchanges, induced by X-rays, which indicates that the rejoining of the breaks is not inhibited by this class of agents. We have also demonstrated that the metabolic status of the cell during treatment with the inhibitors is an important factor determining the extent of potentiation achieved. For example, aphidicolin is very effective in potentiating induced chromosomal damage in stationary cells (human lymphocytes in G_0) as compared to exponentially growing CHO cells. Similarly, 3 aminobenzamide is very effective in potentiating X-ray induced chromosomal aberrations in unstimulated G_0 human lymphocytes, but ineffective in G_2 stage of the cell cycle. Parallel biochemical experiments using UV as chromosome damaging agent and aphidicolin as DNA repair inhibitor have supported the above conclusion about the existence of a difference in response between stationary and growing cells to this inhibitor (van Zeeland et al., 1982).

In a survey of 25 individuals' response to 100 rad X-rays given in G_2 stage of the cell cycle in blood lymphocytes, it was found that the frequencies of aberrations varied between individuals by a factor of two.

The influence of a post-treatment with 1 mM caffeine on the frequencies of chromosomal aberrations induced by X-rays in these individuals also demonstrated the existence of a high inter-individual variability with regard to caffeine potentiation (Natarajan et al., 1982).

A large experiment involving human lymphocytes irradiated with low doses of neutrons (0-25 rad) was scored this year under an IAEA co-ordinated research project. These results will be evaluated in 1983. This experiment is a follow up of an earlier low dose experiment with X-rays (see 1981 report). The other planned experiments with neutrons could not be carried out because a reliable neutron source was not available to us.

Adapting the method suggested by Albertini (1982), we have been able to reduce the background of $HGPRT^-$ variant frequency from 1 in 10^4 to 1 in 10^5 in human lymphocytes. This involves the freezing and thawing of isolated lymphocytes, which presumably kills the already stimulated lymphocytes. We have validated the usefulness of this system by measuring the frequencies of $HGPRT^-$ variants in lymphocytes of patients treated with cytostatic drugs and comparing them to the frequencies in controls.

2.2. COMPARISON OF CYTOGENETIC DAMAGE IN SOMATIC AND GERM CELLS. The induction of reciprocal translocations was studied in rhesus monkey (Macaca mulatta) stem cell spermatogonia at low doses (25 rad X-rays) and low dose rates (0.2 rad/min). At 25 rad we analyzed a total of 1890 cells from 3 monkeys and 6 testes. The observed frequency of 0.21% translocations exactly fitted the linear dose-response relationship previously obtained at doses of 50 and 100 rad (see table 1) and suggests that for very low acute exposures linear extrapolation seems to be justified. A change in the dose rate from 30 rad/min X-rays to 0.2 rad/min reduced the frequency of translocations in the rhesus monkey by a factor of two (table 2). This situation is essentially the same as that observed for the mouse (Searle et al., Mutation Res. 15, 89, 1972). A new group of rhesus monkeys has been irradiated with 25 rad acute X-rays or 100 rad chronic γ-rays (0.02 rad/min). The recovery from radiation damage is followed by regular biopsying. When sufficient recovery occurs, preparations from meiotic stages will be made and analyzed for the presence of translocation configurations.

Spermatocyte analysis of irradiated marmosets (Callithrix jacchus) indicated, at doses of 100 rad X-rays and lower, the same radiosensitivity for translocation induction in stem cell spermatogonia as that of the

rhesus monkey (see table 1). Thus, we could not confirm the earlier observation of a higher radiosensitivity of marmoset spermatogonia by Brewen and Preston (Primates in Medicine 10, 199, 1978).

Dose-response studies (300, 400, 500, 600, 700, 800 and 900 rad X-rays), for the induction of translocations in stem cell spermatogonia of T70H translocation heterozygous mice compared to normal mice, are in progress.

Combined treatments of Adriamycin or Cyclophosphamide and 900 rad X-rays, with 24 h between exposure to the chemical mutagen and irradiation, showed that cyclophosphamide produces a much stronger sensitization of stem cell spermatogonia, with respect to the induction of translocations by 900 rad X-rays, than Adriamycin. Since cyclophosphamide acts mainly on differentiating and differentiated spermatogonia and Adriamycin on all cell types, including stem cell spermatogonia, these results suggest that the sensitization of stem cell spermatogonia is probably produced by damaged differentiated or differentiating spermatogonia rather than damaged stem cell spermatogonia. Further experiments using other exposure levels are necessary to substantiate these first observations.

Earlier studies (see 1980 report) demonstrated that dwarf mice (dw) were less sensitive to the induction of micronuclei in bone-marrow cells than normal mice and that treatment with porcine growth hormone (pGH) plus L-thyroxine (T_4) could restore normal radiosensitivity in dwarfs. New experiments using lower X-ray exposures (100 rad instead of 300 rad) and pGH from another origin, fully confirmed the previous results and indicated that pGH alone was less efficient in restoring normal radiosensitivity in dwarf mice than T_4 alone or combined pGH and T_4 treatments. Study of the induction of translocations in stem cell spermatogonia of dwarfs by 250 rad of X-rays revealed a lower sensitivity of dwarf mice (2.7% vs. 4.6% in normals). In addition, it turned out that treatment of dwarf mice with single or combined treatments of T_4, pGH or prolactin (PRL) did not change the radiosensitivity of stem cell spermatogonia with respect to the induction of chromosomal translocations. The effect of hormone treatments on the induction of chromosomal aberrations by X-rays in dwarf mice bone marrow cells using metaphase analysis is under investigation at present.

Table 1. Induction of translocations in stem cell spermatogonia of two primate species following different X-ray exposures.

Dose in rad	Species	No.of individuals	No.of testes	No.of cells	% Translocations
0	rhesus monkey	22	23	3600	0.08
	marmoset	4	4	230	0
25	rhesus monkey	3	6	1890	0.21
50	rhesus monkey	9	13	3480	0.46
	marmoset	1	2	260	0.39
100	rhesus monkey	7	11	4650	0.86
	marmoset	1	2	345	0.87
200	rhesus monkey	7	9	3350	0.99
	marmoset	2	4	880	0.68

Table 2. Effect of dose-rate on the induction of translocations in rhesus monkey spermatogonia following 100 rad X-rays.

Dose-rate in rad/min	No. of monkeys	No. of testes	No. of cells	% Translocations
30	7	11	4650	0.86
0.21	4	7	3870	0.34

Title of project nr. 3.
Development of mutational assay system and discrimination between point mutations and deletions in mammalian cells.

Head of project and scientific staff :
Dr. J.W.I.M. Simons
Drs. A.G.A.C. Knaap
Drs. W.F. Witterland

3.1. DEVELOPMENT OF MUTATIONAL ASSAY SYSTEMS -

a. Development of a mutational assay system for the selection of APRT (adenine-phosphoribosyl-transferase)-deficient mutants in human diploid skin fibroblasts: cells from a human diploid cell strain heterozygous for APRT-deficiency were treated with 1.5 mM ENU for 1 hour at 37° C and the mutagenized cells were seeded at 0, 4, 7 and 10 days after treatment in order to determine the time which is needed for the expression of induced mutations at the APRT locus. Optimal mutant frequencies were obtained in cells which had been growing for 7 and 10 days after mutagenic treatment. These results indicate that an expression time of 7 days is sufficient for the expression of induced mutants. A first experiment has been performed to determine the X-ray sensitivity of the APRT locus. The results indicate that the radiation sensitivity of this locus is comparable to the radiation sensitivity of the HPRT-locus.

b. Development of an assay system for reactivation of the human inactive X-chromosome (in cooperation with Department of Human Genetics, Leiden). Three HPRT-deficient human x Chinese hamster hybrid cell lines, in which the human inactive X-chromosome was retained, have been investigated. Two of them occasionally and irreproducibly gave rise to HPRT containing cells. This HPRT contained both human and hamster determinants, which indicates that reactivation had occurred. A third hybrid, in which the HPRT-deficient Chinese hamster parent is known not to be revertible (cell line E36), gave rise to reactivated cells after treatment with 5-azacytidine. The reactivants had about 1/8 of the amount of enzyme of human cells and cellogel electrophoresis demonstrated that the electrophoretic mobility was like human HPRT.

c. Experiments on the possible use of tetrazolium stain for the selection of G6PD (glucose 6-phosphate dehydrogenase)-deficient cells: the aim of this research is to make more markers available for mutation studies and to develop a method which can detect large deletions which involve both

the G6PD and HPRT loci. The deposition of insoluble formazan crystals within the cell, as a consequence of the reduction of tetrazolium salts, appeared highly toxic for the cells (V-79 Chinese hamster cells). The killing activity of NBT (nitro blue tetrazolium), induced by the use of G6P (glucose 6-phosphate) as a substrate, was compared in G6PD-proficient and G6PD-deficient cells. (The G6PD-deficient cells were obtained from Dr. Wildt, Freiburg.) Although the G6PD-cells proved to be more sensitive to the action of the staining mixture considerable cell killing also occurred in the absence of the substrate in both $G6PD^+$ and $G6PD^-$ cells indicating the occurrence of enzymes other than G6PD which are able to reduce NBT. The action of these enzymes was largely prevented by incubating the cells in phosphate buffered saline for two hours prior to the staining reaction in order to deplete the substrates present in the cell. As this procedure appeared to interfere with the killing effect of G6P, glucose was used as a substrate instead. Under these conditions a strongly enhanced killing was obtained for the G6PD proficient cells as compared to the G6PD-deficient cells.

3.2. DISCRIMINATION BETWEEN POINT MUTATIONS AND DELETIONS - Experiments on the revertibility of HPRT-deficient mouse lymphoma cells: five spontaneous mutants have been investigated. Three of them did not revert, which means that their reversion frequencies, if present, would have been smaller than 8×10^{-9}, 2.7×10^{-9} and 1.4×10^{-9}. Two exhibited spontaneous revertant frequencies of 7×10^{-9} and 33×10^{-9}. Both of these mutants could be induced to revert with 0.5 mM ENU, which led to a 3 fold higher revertant frequency. These spontaneous mutants contrasted strongly with a mutant induced by the mismatching agent 6-hydroxy-amino-purine. This mutant had a spontaneous revertant frequency of 5×10^{-9} which could be raised after the treatment with ENU to $15{,}000 \times 10^{-9}$. Seven of the latter revertants have been isolated. All of them contained the same amount of HPRT-activity which was also the same as the HPRT-activity in the parental cell line. In addition, the electrophoretic mobilities of these enzymes appeared to be normal.

Publications I.

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Progress Report
1982

Contractor:

Contract no.: BIO-E-396-81-D

Universität Köln

D-5000 Köln 41

Head(s) of research team(s):

Prof. Dr. P. Starlinger
Institut für Genetik
Weyertal 121
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General subject of the contract:

Isolation of the Zea Mays transposable controlling element.

List of projects:

1. The role of transposing DNA elements in spontaneous and radiation-induced mutagenesis in Zea Mays.

Isolation of the Zea mays transposable controlling element.

Prof.P.Starlinger, Institut für Genetik der Universität zu Köln, Weyertal 121, 5000 Köln 41, FRG

The origin of transposable elements in maize in strains not formerly known to contain such elements is correlated to the occurance of chromosome breaks. These breaks can - among other causes - be produced by ionizing radiation. The creation of a permanent system of mutability by ionizing radiation is of interest for the evaluation of radiation risks.

One possibility for the creation of transposable element systems by ionizing radiation is the activation of formerly silent elements of this kind. A prerequisite for the identification of such elements is their cloning.

Methods for cloning are usually dependent on the availability of a gene product (RNA or protein). No such products were known at the beginning of this study for transposable elements. We therefore chose to isolate such elements as DNA associated with a mutant locus but not with the wild type locus of a gene that could be isolated by conventional methods. For this purpose, we chose endosperm sucrose synthase of maize.

In the last two years, we have isolated DNA clones containing the wild type locus of the gene and of mutant loci from two mutants. These latter share homology with the wild type clone in a contiguous segment. Adjacent to it, non-homologous DNA is found extending to the end of the inserted DNA.

Experiments of this type cannot prove that the DNA foreign to the wild type locus is indeed transposable element DNA.

Experiments to obtain this proof by appropriate genetic crosses are on their way. In the meantime, we have spent some time characterizing clones and have obtained the following results:

1. DNA segments near the breakpoint between wild type and foreign DNA in the two mutants are similar but not identical to each other (hybridization occurs, but restriction patterns differ).
2. In one of the mutants, the DNA near the breakpoint has an interesting substructure, consisting of two interdigitating pairs of inverted repeats. One of these repeats has been partially sequenced. It can be shown to have its endpoint exactly at the breakpoint with wild type DNA. Part of this repeat is composed of a smaller subunit consisting of the hexanucleotide CCGTTT and near relatives of it.
3. DNA from this region of the foreign DNA has been used as a probe to look for the presence of similar sequences in genomic DNA. It could be shown that these probes hybridize to up to 40 bands. Analysis of the restricition pattern on these bands shows that the different copies of this DNA in genomic DNA are similar but not identical to each other.

Should the DNA indeed come from a transposable element, we could draw the following conclusions:

1. Many copies of this DNA are present in the genome. This lends credibility to the hypothesis of the activation of silent transposable elements.
2. The differences between different copies of this DNA make it possible that some of them are active while others are not.
3. Transposable element families consisting of similar but not identical elements have been characterized in Drosophila. One of these, the FB-element, has certain similarities to the DNA studied by us.

I.

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Courage-Tebbe U, Döring HP, Geiser M, Starlinger P, Tillmann E, Weck E, Werr W: Two partial copies of heterogeneous transposable element Ds cloned from the shrunken locus in Zea mays L. In: Manipulation and Expression of Genes in Eukaryotes (Nagley P, Linnane AW, Peacock WJ, Pateman JA, eds.) Academic Press Sydney, pp. 221-228

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II.

Döring HP, Geiser M, Weck E, Courage-Tebbe U, Tillmann E, Starlinger P: Ds at the shrunken locus. Maize News Letters 56: 40

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Progress Report
1982

Contractor:
Università di Roma
Città Universitaria
I-00185 Roma

Contract no.: BIO-E-399-81-I

Head(s) of research team(s):
Prof. R. Strom
Ist. di Chimica Biologica
Università di Roma
Città Universitaria
I-00185 Roma

General subject of the contract:
Repair of DNA in human and animal cells.

List of projects:

1. DNA methylation and repair.

Title of project nr BIO-E-399-81-I:"DNA methylation and repair"

Head of project and scientific staff : Prof. Roberto STROM;
Dr.E.Whitehead; Dr.A.Bozzi; Dr.D.Carotti; Dr.G.Cerio;
Dr.A.Lucano; Dr.F.Palitti; Dr.S.Scarpa.

DESCRIPTION OF RESULTS.

Our project is concerned with the interactions of the natural methylation of eukaryote DNA in 5-methyl cytosine with repair of radiation damage.

We have partially purified (200x) a DNA methylase from human placenta. We have shown that it methylates cytosine of DNA in the 5 position. We are investigating its specificity towards different DNA's and have prepared and used for this purpose aza cytidine-substituted human DNA which contains hemimethylated sites, the repetitive Alu sequence from the rapidly renaturing fraction of human DNA (which is also hypothesized to contain these hemimethylated sites), the repetitive alpha-sequence from human DNA, and are now starting to prepare various synthetic polydeoxynucleotides. These investigation serve both to define the specificity of the methylase, necessary for understanding its function, and to obtain a better assay for the enzyme than those currently in use. We have evidence from this work that the enzyme prefers hemimethylated sites, suggesting a function as "maintenance methylase".

Using HeLa cells, in collaboration with the laboratory of Prof.P.Volpe of the Center for respiratory viruses of the Italian National Research Council in Rome, we have been investigating whether the cytosine bases of excision repair patches produced after u.v. irradiation of the cells become methylated. The experiments involve a standard technique of separating new semiconservatively synthesized strands from repair synthesis in old strands, by density labelling. To rigorously identify methylated cytosine in the repair patches it is necessary to incorporate labelled cytosine and then measure the radioactivity in the 5-methyl-C of the old strands after their separation and hydrolysis. We have not been able to achieve sufficient incorporation of radioactive base for this purpose by using labelled uridine, cytidine or deoxycytidine as precursors. Using methyl-labelled methionine, incorporation of radioactivity from this precursor into old DNA can hardly be rigorously identified with methylation of repair patches. Analysis of mono- and di-nucleotides

from suitable DNA hydrolysated offers now several ways round these problems,as better-incorporated precursors can be used.

Using L5 myoblasts in cell culture, we have studied the effect of 3-deazaadenosine, an inhibitor of DNA methylation processes, on cell differentiation. A more pronounced stimulation of cell fusion and differentiation into multinucleated myofibers was observed.

In collaboration with dr. G.Mariutti from the Istituto Superiore di Sanità in Rome and with Prof.B.Mondovì from the Institute of Applied Biochemistry of the University of Rome, we have been investigating how, in V-79 Chinese hamster fibroblasts in cell culture, DNA repair synthesis following exposure to u.v. radiation is inhibited by supranormal temperatures. This inhibition appears to specifically affect the repair synthesis of DNA as compared to replicative one.

In collaboration with the laboratory of Prof.M.Rossi,of the Institute of Organic and Biological Chemistry of the University of Naples, we have been investigating the allosteric kinetics of dCMP aminohydrolase. Allosteric control of this enzyme has been shown by other workers to influence the mutation rate in human cells, by controlling deoxyribonucleoside triphosphate ratios so as to minimize the error rate of DNA polymerase. We have studied competition,by monophosphodeoxyribonucleotides, for substrate sites in this enzyme. Since kinetics are co-operative, classical competitive inhibition analysis cannot be applied, but we have successfully applied a linkage approach to account quantitatively for our results.

LIST OF PUBLICATIONS IN 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

1) S.Scarpa, A.Bozzi, R.Strom, G.L.Cantoni:"Effect of 3-deaza-adenosine on L5 myoblast differentiation in culture". Ital.J. Biochem., to be published (1983)

2) A.Bozzi, G.Mariutti, B.Mondovì, R.Strom:"Effect of hyperthermia on DNA repair synthesis in Chinese hamster fibroblasts V-79 exposed to ultraviolet light". Ital.J.Biochem., to be published (1983)

3) S.Mastrantonio, R.Nucci, C.Vaccaro, M.Rossi, E.P.Whitehead: "Analysis of competition for substrate sites in an allosteric enzyme with co-operative kinetics: effect of dAMP and dUMP on donkey spleen deoxycytidylate aminohydrolase". Submitted for publication (1983)

II. Short Communications, Theses, Internal Reports, Patents...

1) G.Mariutti, M.Vischetti, B.Mondovì, A.Bozzi:"Effetto combinato dell'ipertermia e delle radiazioni u.v. su fibroblasti di hamster cinese V79-753". Comunicaz.Conv.Naz.Assoc.Ital.Protez. contro le radiaz. (AIRP); Genova,23-25 sept.1982.

Progress Report
1982

Contractor:
Landbouwhogeschool
Salverdaplein 10
NL-6701 DB Wageningen

Contract no.: BIO-E-477-81-NL

Head(s) of research team(s):
Prof. Dr. J. Sybenga
Vakgroep Erfelijkheidsleer LH
Gen. Foulkesweg 53
NL-6703 BM Wageningen

General subject of the contract:
Genetic background damage accompanying fast neutron and X-ray induced chromosomal aberrations.

List of projects:

1. Genetic background damage accompanying fast neutron and X-ray induced chromosomal aberrations.

Title of project nr BIO-E-477-81-NL 1.
Genetic background damage accompanying fast neutron and X-ray induced chromosomal aberrations.

Head of project and scientific staff:
J. Sybenga

Genetic background damage is defined, in this context, as damage induced at loci other than the specific locus at which the primary observation is made. The specific damage is a reciprocal translocation, recognized at mitosis as a gross change in chromosome morphology and at meiosis by forming characteristic quadrivalents. The background damage can be induced simultaneously anywhere in the background but is studied in the chromosomes of which the homologues are involved in the translocation. In a segegrating M_2, there will be a shortage of translocation homozygotes when damage in the rearranged chromosomes exceeds that in the homologous not-rearranged chromosomes. When the reversed is the case, more damage has been induced in the normal homologues. If neutrons are more efficient than X-rays in inducing rearrangements compared to "general" damage, they will less frequently produce rearrangements which segregate with a shortage of homozygous normals. The damage induced in the rearranged chromosomes can be analysed specifically in segregation after selfing a backcross with the untreated parental line.

Soaked seeds of an inbred spring type of rye (normally a cross-fertlizer) were irradiated with 1, 2 and 3 Gy of fast neutrons and 10, 20 and 30 Gy of X-rays at ITAL, Wageningen. Of each M_1 plant a single M_2 seed was used in order to avoid complications resulting from chimerism. The M_2 plants were checked cytologically on recognizable chromosome rearrangements in the roottips and at meiosis. In spite of the large number of M_2 seeds (neutrons 297, 349 and 128 respectively, X-rays 267, 399 and 198 respectively) and the relatively high doses (considerable phenotypic damage, including sterility) the number of recognizable translocations which can be classified at mitosis was low. Several more meiotically recognizable translocations were detected but these can not be used in rye as it is practically impossible to score se-

gregating progenies on the basis of meiotic analysis. Scoring of semi-sterility is not possible as (in rye) translocation heterozygosity induces only limited sterility and the level of fertility is low already in the inbred lines. The X-ray series which was particularly low in translocations was enlarged by a 60 and 90 Gy additional irradiation, but the neutron series could not be extended because of the close down of the reactor at ITAL. The additional experiment gave rise to two more translocations only. It was decided, therefore, to use the material prepared at ITAL in a parallel experiment on barley, which is a self fertilizer. The yield of translocations was higher than in rye, and use can be made of all translocations as semisterility is sufficient to recognize heterozygotes in segregating populations. The homozygotes must be separated into translocation and normal types by crossing to normal and scoring the resulting hybrids on heterozygosity. A few preliminary results are now available on some M_3 segregations in rye.

neutrons			X-rays		
norm.	het.	hom.	norm.	het.	hom.
4	18	17	12	23	11
15	27	14	33	56	25
9	15	12	11	20	8
42	14	0			

Further translocations and backcrosses will follow soon on rye. No results are available yet for barley.

The three X-ray translocations completed so far segregate exactly according to expectation. Two of the four neutron translocations shown here segregate different from expectation, one with a significant deficit of normals, the other with a significant deficit of translocation homozygotes. These very preliminary results suggest that neutrons do not produce less background damage per translocation than X-rays, in rye. This agrees with the conclusions of Ramulu and Sybenga (Arabidopsis Information Science 16; 27-34; 1979) on Arabidopsis and Leigh, van Steenbrugge and Robinson (Mutation Res. 84; 101-105; 1981) on Drosophila.

No publications in 1982.

Progress Report
1982

Contractor:

Rijksuniversiteit Leiden
Stationsweg 46
NL-2300 RA Leiden

Contract no.: BIO-E-408-81-NL

Head(s) of research team(s):

Prof. P. van de Putte
Lab. Molecular Genetics
and Biochemical Lab.
Wassenaarseweg 64
NL-2333 AL Leiden

General subject of the contract:

The genetic control and enzymology of DNA repair of radiation damage in prokaryotes and eukaryotes.

List of projects:

1. Mechanism of the excision - repair process in Escherichia coli and its regulation.
2. Mechanism of mutagenesis : role of radiation in the activation of transposable elements.
3. Mechanisms of DNA repair in human cells.

HEAD(S) OF RESEARCH TEAM(S):

Prof. P. van de Putte
Laboratory of Molecular Genetics
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Wassenaarseweg 64
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GENERAL SUBJECT OF THE CONTRACT:
The genetic control and enzymology of DNA repair of radiation damage in prokaryotes and eukaryotes.

List of projects:

1. Mechanism of the excision-repair process in Escherichia coli and its regulation.
2. Mechanism of mutagenesis: role of radiation in the activation of transposable elements.
3. Mechanisms of DNA repair in human cells.

Summary project 1.

The excision-repair process is a major pathway for the removal of damage from DNA and therefore plays an important role in the maintenance of the integrity of the genetic information. The first enzymatic step of this process, incision, is accomplished by the uvrA, uvrB and uvrC gene products. The cloning and amplification of these genes has been described in the last years reports. In 1982, the research effort of the contractor has been devoted mainly to the study of the regulated expression of the different uvr genes. All three genes were found to be regulated in a lexA-recA dependent fashion. The fine-tuning of this regulated expression was studied in detail for each of these genes.

Summary project 2.

The mutator phage Mu causes mutations by random insertions into the host DNA by illegitimate recombinations and provides a model system for insertional and for non-repairable DNA alterations caused by the transposition process. The main early promoter, which determines the level of Mu transposition, was mapped further and a second promoter for the transcription of the Mu repressor was detected.
The site specific recombination genes gin (of bacteriophage Mu) and pin (of E.coli) have been sequenced. Specific domains are highly conserved and appear important for the recombination process.
Pin mediates an inversion of 1600 bp of E.coli DNA flanking the pin gene. It is located on a UV inducible DNA element.

The Mu gene mom is regulated by a positive regulator encoded by Mu itself and by methylation of its promoter by E.coli Dam protein.
The DNA lesions which are responsible for most of the mutation induction by cis-$Pt(NH_3)_2Cl_2$, an antitumor agent, were determined. GAG and GCG sequences appear to be hotspots where the Pt compound induces intrastrand crosslinks. UvrB and RecA are involved in the repair processes leading to the fixation of the mutations. The secundairy structure of the DNA seems to play an important role in the formation of Pt-DNA lesions. It was found that pKM101, a plasmid which enhances mutagenesis and is often used on 'test' strains, drastically alters the mutation spectrum induced by cis-Pt. The mutagenesis with pKM101 becomes more untargeted.
Transposable elements and transposition processes are believed to play a role also in higher organisms: in differentiation, regulation, mutagenesis and carcinogenesis. We have started to find transposable elements in mammalian cells, which may play a role in the above mentioned processes.

Summary project 3.

Repair processes in human cells will be studied along several lines.

a. First, the possible complementation of the repair defects in different repair deficient human cell lines (Xeroderma pigmentosum) by the Escherichia coli uvrA, uvrB and uvrC gene products will be analysed. In 1982 the cloned uvrA and uvrB genes have been correctly tailored in order to allow their expression in a eucaryotic system. In the case of uvrA a SV40-uvrA shuttle plasmid has been constructed. Plasmids overproducing the UvrA and UvrB proteins have been constructed and the uvrA gene product has been purified and a start has been made with complementation studies in Xp cells.
b. Secondly, in the middle of 1982 a new project has been started with the purpose to clone human genes which are induced after UV irradiation of human epidermal keratinocytes. In 1982 a human keratinocyte culture system has been set up (in collaboration with Dr. M. Ponec, Leiden), polyA messenger RNA from these cells was isolated and newly synthesized proteins after UV irradiation were visualized by 2D PAGE.
c. In a third project, a beginning has been made with the study of the expression of the uvrA, uvrB and uvrC genes in Saccharomyces cerevisiae, followed by a complementation analysis of different excision repair defective yeast strains (rad^- mutants).

Title of project nr. 1: Mechanism of the excision-repair process in Escherichia coli and its regulation.

Head of the project : Prof.Dr. P. van de Putte

Scientific staff : Dr. C.A. van Sluis, Drs. J.A. Brandsma, Drs. E.A. van den Berg

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uvrA, ssb (Brandsma)

1. In order to study the in vivo regulation of the uvrA gene a set of plasmids, carrying deletions of different sizes in the uvrA regulatory region (pUVR-A-series, see project 3, progress report 1981) were used. The fusion of the different truncated promoters, present on these plasmids, to the galK structural gene enabled the quantification of uvrA transcription.
   UvrA gene expression proved to be dependent on the genes lexA and recA. In contrast with other procaryotic promoters, uvrA transcription already occurred when only the '-10' sequence of the uvrA promoter was fused to galK. The '-35' sequence (commonly known as the RNA polymerase binding site) merely functions as an 'amplifier sequence' which increases uvrA expression, as soon as SOS induction occurs. Indeed, the lexA repressor binding site is overlapping with the '-35' region. This type of regulation allows a basal level of uvrA expression in the absence of SOS inducing signals. The same conclusions could be drawn from a qualitative analysis of uvrA transcription using the S1-mapping technique.

2. The ssb gene is located on the E.coli chromosome near the uvrA gene, but is transcribed in the opposite direction. In order to investigate the expression of this gene, a number of galK fusion plasmids were constructed carrying various parts of the ssb gene and the intergenic region including the uvrA operator (LexA binding site). Plasmids carrying exclusively the presumptive ssb promoter, show a basic level of expression. However, the presence of extra sequences in the direction of the uvrA gene (including the uvrA operator) renders the expression of the ssb gene inducible by UV and mitomycin C. Presently the sequences responsible for this phenomenon are being precisely mapped using the Bal31 method.

3. Because ssb is involved in the RecA promoted formation of intermediate structures in recombination, experiments were carried out to measure the effect of intracellular Ssb protein levels on recombination. A cooperation with Dr. A. Cohen (Jerusalem) allowed us to establish that Ssb^+ plasmids greatly increase interplasmidic recombination.

4. A cooperative project with Dr. J.T. Pembroke (University of Galway, Eire), who visited our laboratory on an EMBO fellowship, concerned the nature of the UV-sensibilization of E.coli by the incJ plasmid R391. The region responsible on R391 for the UV-sensibilization was cloned on a smaller plasmid. R391 had no effect on the induction of the uvrA gene, suggesting that it does not act via the RecA-LexA pathway.

uvrB (van den Berg)

1. The in vivo regulation of the uvrB promoters P1 and P2 was studied

using the S1 nuclease mapping technique. Although only the P2 promoter contains a binding site for the lexA repressor, transcription from both promoters is strongly induced by UV irradiation. Transcription from P1 is much more abundant compared to P2, both before and after irradiation. To establish whether transcription from P1 is regulated by repression at the SOS box in P2, we constructed plasmids carrying various deletions in this region. Two of these mutants were characterized by sequence analysis and their transcriptional activity in vivo was measured by S1 nuclease mapping. It was found that deletion of part of the SOS box in P2 results in constitutive transcription from P1 in uninduced cells, which does not significantly increase upon UV irradiation.

2. Several plasmids were constructed in which part of the uvrB regulatory elements are fused to the β-galactosidase (lacZ) gene. LacZ expression controlled by the P2-P1 region is inducible by UV in a RecA-LexA dependent fashion. Fusion of the promoter regions of the deletion mutants described in 1. with lacZ results in high basal levels of lacZ expression which are only slightly induced by UV. These results are in agreement with the in vivo transcription data. However, when a more extended region 300 bp upstream of P2 is included in the fusion plasmid, cell growth and plasmid stability are severely affected. Such a plasmid could only be maintained in some UvrB and UvrC mutants but even then lacZ expression was highly variable. We attempt to clarify the cause of this instability both by analysis of spontaneous stable plasmid mutants and by the comparison of the chromosomal uvrB and uvrC mutants that allow stable maintenance of the plasmid.

3. It has been reported that SOS induction of several din genes and SOS dependent mutagenesis is impaired in uvrB mutants when they are treated with agents that cause crosslinks in DNA (like cis-Pt and MC). Experiments are in progress to compare SOS induction with such agents in several uvrB mutants by using a plasmid in which the inducible uvrA promoter is fused to the galK gene (see Brandsma).

4. Using a plasmid which overproduces the uvrB gene product (see project 3) we started with the purification of the uvrB protein. By selective precipitation from cell extracts with Polimine P, followed by affinity chromatography on DEAE cellulose we obtained a preparation that was already 90% pure. Several alternative steps for the final purification are under study.

uvrC (van Sluis)

1. The elucidation of the uvrC gene's structure (in particular the location of the 70 K structural gene) has been pursued further by the establishment of the DNA sequence of the uvrC regulatory region situated on plasmid pCA95051 (see report 1981), and the presumptive amino-terminal end of the gene. Presently, plasmids are constructed, harbouring the uvrC structural gene fused to strong prokaryotic promoters, in order to overproduce and purify the uvrC gene product (see as well project 3 in this respect).

2. The study of uvrC regulation has been continued using fusion plasmids (galK and Km^R). Induction of uvrC occurs in a RecA-LexA dependent mode but delayed as compared to other din genes. Additionally, nalidixic acid does not induce uvrC expression. Preliminary results indicate that uvrC expression is strongly decreased in Uvr^- strains. Our results

indicate that uvrC is probably not directly regulated by the LexA protein. The absence of a SOS-box in the 400 basepair region preceeding the 70 K structural gene supports this assumption.

3. The start of uvrC transcription was established by S1-nuclease mapping of in vivo synthesized RNA. RNA originating from plasmid pCA95051, harbouring the 300 bp PvuII-BglII regulatory region, initiates at approximately 220 basepairs upstream from the start of the uvrC structural gene, just outside the 1.9 kb BglII fragment (report 1981). Using plasmids harbouring extra sequences 5' to the PvuII-BglII region allowed us to detect as well longer transcripts. Presently we try to explain the origin of these longer transcripts.

4. The molecular basis of the variability of the phenotype of different uvrC mutants is still obscure. Therefore one particular filamentous strain PAM3124 was investigated (in cooperation with Dr. Green, Brighton and Dr. Johnson, Palo Alto). Both filament production and UV-deficiency are reversed by the introduction of $uvrC^+$ plasmids. Since plasmids carrying the 70 K uvrC gene express also a gene coding for a 28 K protein (with unknown function) (see report 1981), and as in addition the genes are closely linked, we want to assess whether the 28 K protein plays a role in the expression of uvrC and/or filamentation. This last aspect will be carried out in collaboration with Dr. D'Ari, Paris).

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Title of project nr. 2: Mechanism of mutagenesis: role of radiation in the activation of transposable elements.

Head of project : Prof.Dr. P. van de Putte

Scientific staff : Dr. M. Giphart-Gassler, Drs. N. Goosen, Drs. M.M. Groenen, Drs. R.H.A. Plasterk, Drs. J. Brouwer and Dr. J. Tasseron-de Jong

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1. Integration and regulation of bacteriophage Mu.

The region in the Mu genome ± 800-1200 bp from the left end is the main regulatory region of the phage. Here the promoters for the Mu A transpose and that for the Mu repressor are located. At least three Mu gene products influence the level of transcription for these promoters, (1) the repressor (2) the Ner protein (3) the Cim protein. We have studied how these gene products regulate, where they bind and where transcription intiation takes place.

From DNA sequence analysis by Goosen in collaboration with Allet and by Priess a presumed promoter sequence for the c gene has been found. By cloning various parts of the repressor end of the Mu DNA it became obvious that this promoter alone is not functional but that a second promoter, approximately 200 bp upstream, is necessary for the expression of the repressor. By further subcloning and digestion with Bal31 this promoter will be localized precisely. A very strong repressor binding site was located in the leftmost HindIII fragment and by fusing the HindIII site to the Tc gene we were able to bring Tc expression under repressor control. The position of the p_R promoter could be mapped further with promoter-up mutations (pip-mutations), which all are the result of the same single base change. This promoter is now independent of the E.coli hip (himc) enzyme. Hip (himc) is a host enzyme essential for the expression of Mu transposase. At least one of the him genes (himA) has been shown to be one of the din genes. It will be studied if by UV irradiation or SOS induction expression of Mu transposition genes is enhanced.

Moreover we have started the construction of a Ner phage, because until now all evidence on the function of ner is obtained from plasmid clones and not from the phage itself.

2. Site specific recombination systems in Mu and in Escherichia coli.

a. G-inversion

Inversion of the G-region of Mu is a site specific recombination event which is mediated by the Mu Gin product. To study the action of gin both in vitro and in vivo, plasmids have been constructed to quantitate G inversion in which the lacZ gene serves as a marker for G inversion. It appears that gin can mediate deletion formation when the inverted repeats, flanking the G region, are in a direct orientation. The frequency of this process is, however, much lower than normal G-inversion. Using these plasmids Gin mediated inversion could be compared quantitatively with homologous recombination systems such as pin (5).
The quantity of Gin protein seems to be the rate-limiting factor in the recombination process. Although we have announced the overproduction of Gin last year the level of overproduction appeared insufficient to start purification of the gin gene product. Gin seems to have no effi-

cient translation-intitiation signals. Therefore gin with its 2e triplet, was fused to translation-initiation signals of lacZ including its first 4 triplets. The product of the fusion gene remains functional. By recombining these structures in vitro to the p_L promoter of λ we hope to produce enough of 'Gin' in vivo. If so, we will start purification of this protein to study the site specific recombination reaction in vitro.

b. P-inversion in E.coli.

Last year we reported that gin mutants were complemented in some E. coli K12 strains and that we have been able to isolate three clones from the Carbon and Clarke colony bank which contain this function. Upon sub-cloning it could be determined that this pin gene catalyses an inversion of a 1600 bp region (P-region) in the E.coli DNA. as with gin also pin is located in the region flanking the P-region.
We have presumed that Pin was involved in phase-variation of the pilli or the fimbriae of E.coli because of its location near the pil genes. Recently, however, we have mapped pin definitely near purB, between purB and fabD. The reason for the initial false localization is that pin^+ cells loose the pin^+ character upon UV-irradiation with high frequency and initial mapping experiments were done with a pin^+ strain that had been irradiated. It has been reported that 'a cryptic plasmid' is located near purB which is excised upon UV-irradiation (Hill, J. Bacteriol. (1980) 144, 312-321). We have been able to show that pin and the P-region are located in this plasmid or defective prophage named e14. The function of e14 and that of pin remain to be clarified.
We like to emphasize here that nearly all experiments on DNA repair have been performed with AB1157 strains.
The AB1157 strain contains e14 and pin which is excised after UV-treatment. Such excision event might interfere with DNA repair phenomena.

c. Pin and Gin DNA sequence.

The DNA sequence of both Gin and Pin and the flanking regions, including the inverted repeats have been determined (6). The sequences are ± 70% homologous at the aminoacid level and resemble that of Hin in Salmonella typhimurium (± 60% homology). Comparison with the resolvase tnpR of Tn3 shows regions of homology between Hin, Pin, Gin and TnpR which seem essential for the recombination reaction (TnpR is evolutionary related to Gin but mediates preferentially deletions instead of inversions).

d. Regulation of mom expression.

The expression of the Mu modification gene mom has been investigated mainly by changing its regulatory region and changing host backgrounds. It has been proven that initiation of mom transcription occurs only: (1) If a Mu-encoded positive regulator protein is present and (2) if the mom promoter is methylated by Dam. The positions in the mom regulatory region, where these processes take place, could be determined on the DNA sequence level (7).

3. The mutation specificity of Pt-compounds with antitumor activity and the role of repair processes.

Recently we showed that the anti-tumor agent cis-$Pt(NH_3)_2Cl_2$ induces specific basepair substitutions in the lacI gene of wt E.coli cells at sites with a GAG or GCG sequence. This specificity was explained by the formation of intrastrand crosslinks at two guanines separated by a third base. Furthermore we showed that a higher treatment temperature stimulates the formation of these crosslinks. The repair of these lesions, which leads to the fixation of basepair substitutions, was showed to be dependent upon both UvrB and RecA.
In RecA cells a different type of mutations is induced. Mapping experiments have shown that these mutations are very specific and only occur in the last 160 bp of the lacI gene. Currently we are sequencing these mutants.
Experiments, in which plasmid DNA was treated in vitro with cis-$Pt(NH_3)_2Cl_2$, showed that the tertiary structure of the DNA is very important for the effect of the treatment upon the biological activity. Supercoiled DNA is much more sensitive then relaxed DNA, and the Pt-compound only induces mutations in supercoiled DNA.
Furthermore we studied the effect of the plasmid pKM101 upon mutation induction and cell-killing by cis-$Pt(NH_3)_2Cl_2$. pKM101 is a derivative of the clinical isolate R46, and is thought to carry genes that enhance the error-prone repair capacity of the host cell. We found that cells carrying pKM101 are more sensitive for the Pt-compound than normal wt cells and that mutation induction by cis-$Pt(NH_3)_2Cl_2$ is strongly enhanced by the presence of pKM101. Comparison of the mutational spectra after treatment of cells with and without pKM101, showed that the plasmid has also a strong qualitative effect on mutation-induction by cis-$Pt(NH_3)_2Cl_2$. In cells with pKM101 25% of the induced ambre and ochre mutants were derived from substituions at AT basepairs, that are hardly induced (3%) in cells without pKM101. Apparently the presence of pKM101 in the cells, results in non-targeted mutagenesis. Furthermore these experiments show that, in the case of Pt-damage, repair under influence of pKM101 differs from normal SOS repair.

4. Transposable elements in mammalian cells and their role in mutagenesis and repair processes.

In 1982 we have started a new line of research to study the importance of transposable elements and transposition processes in mammalian mainly human) cells. Our initial goal will be to trap transposons in DNA of plasmids brought by DNA-mediated gene transfer into the eukaryotic cells, thus showing that transposons exist and that active transposition occurs. We have already set up an experimental system which, in the future, not only will enable us to study transposition but will allow the study of all sorts of mutations at the level of DNA sequence.

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Title of project nr. 3: Mechanism of DNA repair in human cells.

Head of Project : Prof.Dr. P. van de Putte

Scientific Staff : Dr. C. Backendorf, Dr. K. Planqué, Drs. T. Kartasova

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1. Expression of uvrA, uvrB and uvrC in human cells (Backendorf)

a) The deletion mapping of the uvrA gene using nuclease Bal31 has been reported (progress report 1981).
In 1982 these results have led to:
- The construction of plasmids expressing the UvrA protein under control of the p_L promoter of phage λ(pPL-A9). Using strains harbouring this plasmid, 20-30% of the total cellular protein synthesis can be expressed as UvrA protein.
- The construction of eucaryotic shuttle vectors harbouring the uvrA gene under control of the early SV40 promoter (pEV1-uvrA). A splice junction and a polyadenylation site have been introduced into pEV1-uvrA
- The construction of yeast promoter-uvrA plasmids (see Planqué).
- The construction of plasmids where the uvrA promoter is fused to the galK structural gene (see project 1).

b) Purification of the UvrA protein. Raising of anti-uvrA antibodies.
The chromatography of protein extracts from cells harbouring plasmid pPL-A9 on a single-stranded DNA-cellulose column results in a > 95% pure UvrA preparation. This protein preparation has been used to produce anti-uvrA in rabbits (in collaboration with Dr. M. Giphart-Leiden). The antibodies will be used in 1983 to monitor the expression of UvrA in human or yeast cells.

c) Complementation studies.
Purified UvrA protein has been microinjected into several repair deficient human Xeroderma pigmentosum cells. No complementation was found. The negative result may be due to the fact that UvrA was injected into the cytoplasm. Presently the protein is injected directly into the nucleus of the human cells (in collaboration with the group of Prof. D. Bootsma,Rotterdam) (see as well Euratom project: BIO-E-404-81-NL).

d) Overproduction of the uvrB gene product.
Hybrid plasmids have been constructed where the uvrB promoter is replaced by the regulated p_L promoter of phage λ. We found that UvrB overproduction can only be achieved if a sequence of 30 nucleotides preceeding the uvrB structural gene is deleted. The sequence has probably an effect on ribosome initiation. Cells harbouring plasmid pPL-BZ, where this sequence has been deleted produce 10-20% of the total protein synthesis as UvrB. The purification of UvrB has been started (see project 1).

e) Localization of the start of the uvrC structural gene.
The Bal31 deletion mapping technique (see report 1981-uvrA) has been used to localize the start of the uvrC gene. Presently different subclones are being sequenced.

2. Cloning UV inducible human genes. (Kartasova)

In 1982 a cell culture system for human epidermal Keratinocytes has been developed in collaboration with Dr. M. Ponec (Leiden). UV surviving curves have been made and compared to the UV surviving of fibroblasts originating from the same donor. PolyA$^+$ messenger RNA was isolated and translated in an in vitro reticulocyte system. Newly synthesized proteins, after UV irradiation, could be visualized by using a 2D PAGE (O'Farrell system). These pilote experiments will enable us to determine the most suitable time after UV irradiation to isolate polyA$^+$ messenger RNA for the synthesis of cDNA.

3. Expression of uvrA, B and C genes in Saccharomyces cerevisiae. (Planqué)

In the yeast Saccharomyces cerevisiae a large number of excision repair mutants (rad$^-$ mutants) have been described. In order to determine whether the E.coli repair genes can complement the yeast mutations the uvrA, B and C genes will be introduced into yeast mutants using yeast cloning vectors. The approach is essentially similar to the one described for the introduction of procaryotic genes into human cells. In 1982 a beginning has been made with the construction of leu2-rad acceptor strains in collaboration with Dr. E. Moustacchi (Paris). Hybrid plasmids containing the uvrA gene fused to the yeast cytochrome C promoter were constructed. Pilote experiments, to determine the transformation efficiency of the different leu2-rad strains were performed.

Progress Report
1982

Contractor:
State University of Leiden
Stationsweg 46
NL-2300 AA Leiden

Contract no.: BIO-E-405-81-NL

Head(s) of research team(s):
Prof. Dr. A.J. van der Eb
Sylvius Laboratories
Wassenaarseweg 72
NL-2333 AL Leiden

General subject of the contract:
Molecular biology of the repair of DNA damage in mammalian cells, and its relationship with mutagenesis and oncogenic transformation.

List of projects:

1. Relationship between repair of DNA damage, mutagenesis and oncogenic transformation.
2. Identification of repair genes using recombinant DNA technology.

Title of project nr.1: Relationship between repair of DNA damage, mutagenesis and oncogenic transformation. (Collaboration with Dr.J.Cornelis and Dr.J.Rommelaere, Brussels).

Head of the project and scientific staff:
Prof.Dr.A.J. van der Eb, Dr.P.J. Abrahams
Ir.J.L.M. van der Lubbe, H. Braggaar.

UV-induced induction of SV40 replication in virus-transformed cells.

Treatment of monkey cells with DNA damaging agents induces a number of processes resulting in enhanced survival of UV-damaged virus and enhanced mutagenesis of unirradiated virus. To investigate whether these SOS-like functions are accompanied by other processes involving activation of the cellular genome, induction of SV40 replication in SV40-transformed cells was studied. It was found that SV40 replication was induced in transformed hamster, rat and human cells after fusion with UV-irradiated untransformed cells, and that the kinetics of virus-induction were similar to those of enhanced reactivation and enhanced mutagenesis. Optimum induction was found when fusion between UV-irradiated cells and SV40-transformed cells was carried out 24-48 hrs after UV-irradiation (of the untransformed cells). (Collaboration with Rommelaere and Cornelis, Brussels; finished spring 1983).

UV-induced mutations.

In order to characterize the nature of the mutations induced by UV-irradiation, SV40 DNA molecules have been constructed in which the early region is UV-irradiated and the late region is unirradiated, and vice versa. These"hybrid" DNA molecules will be used in the near future to answer the question whether mutations only occur in the UV-irradiated half of the genome (targeted mutagenesis),or also in the unirradiated other half. If mutations also occur in the unirradiated DNA-half it would indicate that the mutator activity is, at least partly, untargeted. Since the host cells were not irradiated, it would follow that the untargeted mutator activity is induced by the damage present in the infecting DNA itself.

To characterize the nature of the mutations induced in UV-damaged SV40 DNA in unirradiated host cells, a series of independently isolated revertants of the temperature-sensitive mutant tsBC245 have been studied by nucleotide sequence analysis (tsBC245 is a late mutant carrying a mutation in the VP1 gene). Only in one revertant the original mutation was reverted to the wild type sequence (a true reversion). In all other cases reversion was caused by secondary mutations. The results did not allow conclusion regarding specificity of the UV-induced mutations (see Table 1).

Mutant (or revertant)	Mutation (compared to wild type)	
	codon change	amino acid change
tsBC245	CCT → TCT	pro_{176} → ser
revertant 15001, 15002, 15004	GCT → GTT CCT → TCT	ala_{127} → val pro_{176} → ser
15005	GTT → GCT	val_{264} → ala
19502	CAT → CGA CCT → TCT	his_{132} → arg pro_{176} → ser
19503	GTG → GTA ACT → CCT CCT → TCT	val_{167} → val thr_{194} → pro pro_{176} → ser
19506	ATA → GTA GGC → AGC CAG → CCG CCT → TCT	ile_{35} → val gly_{67} → ser glu_{77} → pro pro_{176} → ser

Error-prone repair in human cells.

We have recently extended our studies on induction of error-prone repair mechanisms also to human cells, including cells derived from UV-sensitive Xeroderma pigmentosum (XP) patients. Experiments with diploid human fibroblasts and XP cells showed that the phenomenon of enhanced reactivation of UV-irradiated SV40 also occurs in human cells. No significant differences were found between normal cells and XP cells. For studies on enhanced mutagenesis SV40 is not a suitable virus as its efficiency of replication in human cells is too low. Therefore, we have chosen herpes simplex virus 1 (HSV1) as a probe to study mutagenic

activity in UV-irradiated human cells. An assay based on induction of forward mutations in the viral tk gene was used. The results indicate that UV-irradiation induces a transient mutator activity in human cells. The effect is maximal 2-3 days after UV-irradiation. Sofar, no significant differences have been found in the magnitude of the effect between XP cells and normal cells. This work is expected to be finished in the summer of 1983.

Publications

J.J. Cornelis, B.Klein, J.H.Luper, P.J. Abrahams, R.A.M. Hooft van Huysduynen and A.J. van der Eb.
The use of viruses to study DNA repair and induced mutagenesis in mammalian cells. In: Progress in Mutation Research vol.4 (DNA repair Chromosome alterations and chromatin structure (A.T. Natarajan et al. Eds.) Elsevier Biomedical Press (1982).

Title of project nr.2: Identification of repair genes using recombinant DNA technology. (Collaboration with Prof.D.Bootsma Rotterdam)

Head of project and scientific staf:
Prof.Dr.A.J.van der Eb, Dr.A.Pastink, Mw.B.Klein.

The identification of mammalian repair genes is based on the genotypic correction of the defect of repair-deficient cells following after introduction of the corresponding wild type gene. We have transfected XP-cells, defective in UV-repair, with DNA from repair-proficient cells, and have made attempts to select UV-resistant cells from the transformed cultures. The selection protocol consisted of UV-irradiating the cells both from the top and the bottom on 3 consecutive days. Control experiments with mock-transfected cultures showed that no XP cells survive the UV-selection procedure. Transfection of SV40-transformed XP-A cells (complementation group A) with DNA from repair-proficient hamster cells resulted in several UV-resistant colonies. None of these colonies, however, showed regained capacity of unsceduled DNA synthesis after UV-irradiation. The possibility that the negative results are due to loss of the hamster genetic informations is being studied. Further experiments have been started with normal human DNA. In order to carry out similar experiments with other XP cells,cultures of complementation groups C, D and H have been transformed with SV40 and SV40 DNA, and stable lines are being isolated.

Progress Report
1982

Contractor:
State University of Leiden
Stationsweg 46
NL-2300 AA Leiden

Contract no.: BIO-E-476-81-NL

Head(s) of research team(s):
Prof. Dr. A.J. van der Eb
Sylvius Laboratories
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General subject of the contract:
Molecular biology of the repair of DNA damage in mammalian cells, and its relationship with mutagenesis and oncogenic transformation.

List of projects:

1. Studies on host-cell reactivation of SV40, vaccinia virus and herpes simplex virus type 1 in human cells deficient in repair of DNA damage.

Title of project nr.1: Studies on host-cell reactivation of SV40, vaccinia virus and herpes simplex virus type 1 in human cells deficient in repair of UV-damage.

Head of project and scientific staff:
Prof.Dr.A.J. van der Eb
Dr.P.J. Abrahams

Previous work has shown that the defects in repair activity of Xeroderma pigmentosum (XP) cells can be quantified by measuring the extent of host-cell reactivation of UV-irradiated SV40 virus as a probe. This technique has been extended to other genetic diseases characterized by repair defects: Cockayne's syndrome (CS), Bloom's syndrome (BS) and Fanconi's anemia (FA). Host-cell reactivation of UV-irradiated SV40 and HSV-1 was studied in two complementation groups of Cockayne's syndrome. With both viruses, a lower survival was found in the CS cells than in normal cells. Only little difference was obtained with BS cells, using UV-irradiated virus. Studies with FA cells and SV40 DNA treated with platinum compounds (cross-linking agents) were started. FA cells appear to be more sensitive to certain platinum compounds than to psoralen + long wave UV, which was previously used with FA cells. If sufficiently large differences are found between the survival of treated SV40 in defective cells compared to that in normal cells, the possibility will be considered to use the host cell reactivation method for attempts to identify the defective gene.

Progress Report
1982

Contractor:

Medical Research Council
MRC
Park Crescent 20
GB-London W1N 4AL

Contract no.: BIO-E-412-81-UK

Head(s) of research team(s):

Dr. J. Vennart*
Radiobiology Unit
MRC
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GB-Oxon OX11 ORD

General subject of the contract:

The radiobiology of cultured mammalian cells.

List of projects:

1. Dose-effect relationships for cellular effects with different radiation qualities.
2. Development and characterization of mutation and transformation assays in cultured mammalian cells.
3. Studies on radiosensitive mammalian cells.

* From 1 November 1982: Professor G.E. Adams

Title of Project nr 1: Dose-effect relationships for cellular effects with different radiation qualities

Head of project and scientific staff: Dr. D.T. Goodhead (head), Dr. J. Thacker, Dr. R. Cox, Dr. D.E. Charlton

We have shown previously that ultrasoft X-rays can be used as a fine probe of mechanisms of radiation action in mammalian cells. Because of the very short secondary electron tracks which they produce throughout the irradiated cells, their relative biological effectiveness (RBE) places tight constraints on possible mechanisms of action and on the energy and spatial requirements for biological damage. Previous results indicate that the predominant mechanisms of radiation action of ultrasoft X-rays are common to conventional low-LET radiations such as hard X-rays and γ-rays. During 1982 we have completed our experimental study of the effectiveness of 0.28 keV carbon K X-rays in inducing chromosome aberrations in plateau phase (G_1) V79 hamster cells. The observed RBE is approximately 2.6 (relative to 250 kV X-rays) which is slightly less than that found previously for inactivation, and for induction of mutations, in these cells and in human fibroblasts. Nevertheless it appears that these very short secondary electron tracks (~14 ionisations, total range $\lesssim$7 nm) are highly effective in producing exchange aberrations (with a substantial linear term in the dose response). This implies either that there is a large probability of two chromosomes being sufficiently close together for a single track to damage both of them or that an exchange takes place between a single damaged chromosome and an undamaged chromosome. Preparations are in hand to extend these investigations to other phases of the cell cycle in BHK cells for which techniques are now available to distinguish pre-S, post-S and subdivisions of S phase cells in a population of asynchronous cells.

Detailed codes of electron track structure have been used to compare the spatial properties of energy deposition by ultrasoft X-rays (0.28 keV carbon K, 1.5 keV aluminium K and 4.5 keV titanium K) and 250 kV hard X-rays in small spherical water volumes of 1-10 nm diameter (collaborative work with Dr. D. Brenner of Los Alamos National Laboratory). These codes perform full Monte Carlo simulations of the relevant interaction processes (ionisations and excitations) of electrons. This comparison has revealed a close correlation between the previously observed RBEs of these radiations and the probability of their depositing 100 eV of energy (or greater) in a 3 nm diameter volume. These relatively large energy concentrations account for only about 5% of the total energy deposited by hard X-rays and about 20% of that of carbon X-rays. This has led us to hypothesize that the biological effects of low-LET radiations are due predominantly to relatively rare configurations of energy deposition closely related to the above threshold. Preliminary extensions of these track structure calculations to α-particles (also in collaboration with Dr. Brenner) indicate that this single putative critical property of radiation is not sufficient to explain the RBEs of high-LET radiations. This finding is in accord with earlier biological observations which imply that the effectiveness of high-LET radiations is predominantly due to

their producing initial critical damage which is significantly different, and less repairable, than that of low-LET radiations.

We have designed and constructed a target assembly for production of ultrasoft X-rays on our Cockcroft-Walton accelerator. Preliminary tests to date indicate that we can produce carbon X-rays to give at least an order of magnitude greater dose-rate (using our conventional mammalian cell irradiation geometry) and without the previous limitations on target life (which have previously constrained experiments using the ultrasoft X-ray discharge tube). This should greatly increase our scope for future biologically relevant experiments with carbon X-rays as well as providing the possibility of extending the experiments to X-rays of even lower, as well as intermediate, energies.

With the assistance of Dr. W. Wilson (Battelle Pacific Northwest Laboratories) we have now installed on the A.E.R.E. computer a highly sophisticated Monte-Carlo track structure code for intermediate energy protons, deuterons, α-particles and some heavier ions. This code has been developed by Dr. W. Wilson and Dr. H. Paretzke (GSF, Neuherberg) and will be available for defined collaborative use by us, as well as others. We are establishing a complex sampling routine to apply this code, as well as that for electrons, to study the properties of energy deposition in small cylindrical volumes directly related to possible target structures in mammalian cells. We expect to compute physical data for a wide variety of radiation types including heavy ions, ultrasoft X-rays, hard X-rays and radionuclides, for comparison with existing and future observations of the RBEs of these radiations with the aims of identifying more closely the radiation properties of critical importance and of constraining discussion of proposed mechanisms of action.

Our assessment of proposed models of radiation action has continued with the accumulation of further pertinent biological and physical data as described above. We have also shown that early 'track-segment' calculations, as used in various models of radiation action from the late 1950's until the present, compare unfavourably with the results of modern track structure codes for α-particles. In addition, we have made a personal assessment of the evolving role of microdosimetry, especially as incorporated in the theory of dual radiation action, in radiobiology. In this we have attempted to emphasize the concensus which is now being reached on the critical biological importance of patterns of energy deposition over very small (nanometre) distances and to identify important areas of current disagreement such as the relative importance of long-ranged 'interaction of sublesions' or 'saturable repair'. Further critical experimental evidence to establish the roles of these two processes is needed to further understand the slopes of dose-response curves and to allow reliable extrapolation to the low doses and dose-rates of practical importance in assessing the hazards of radiation in human populations.

Construction of our new plutonium-238 irradiation facility has been completed but it was found to be inadvisable to transfer our existing ^{238}Pu source plate (10 years old) to the new chamber due to deterioration of the source. A new source is now on order for early 1983 after which the facility will be fully calibrated and tested. Thereafter it will be used, inter alia, to compare the effectiveness of very low (as well as high) dose-rate α-particle irradiations with the results of ongoing experiments on the effectiveness of very low dose-rate γ-ray irradiations (see also Project 3).

Title of Project nr 2: Development and characterisation of mutation assays in cultured mammalian cells

Head of project and scientific staff: Dr. J. Thacker (head), Dr. R. Cox, Dr. P. Debenham

Cloned viral and bacterial genes have been used for mutation studies after they were transferred into suitable mammalian cells. We have established that these genes may be integrated as stable single copies so that the frequency with which their activiity is spontaneously lost is relatively low under non-selective conditions. Mutation of these integrated genes by radiation or chemical mutagens has been studied quantitatively by selecting for specific drug resistance after a suitable expression time. We have investigated conditions of drug selection in respect of the most appropriate drug to use, the drug concentration which will exclusively select the mutant phenotype, and the optimal time after mutagen treatment for detecting the maximum mutant frequency. Using these methods reproducible and significant increases in mutant frequency were found after mutagen treatment.

Mutants selected by these methods were isolated and the DNA extracted from their cells. This DNA was analysed by restriction enzyme cutting, gel separation of fragments, Southern blotting and hybridization to 'probes' of radioactively-labelled copies of the viral or bacterial genes. To date all of the mutants selected for zero gene activity have been found to have lost sequences hybridizing to the 'probe' DNA, except for one mutant which retained the gene sequences in a rearranged form.

We have also continued the characterisation of a large series of independently-isolated HGPRT-deficient mammalian cell mutants by studying their abilities to revert to enzyme-proficiency. The majority of γ-ray induced HGPRT-deficient mutants did not show detectable reversion, either spontaneously or after chemical mutagen treatment, while all EMS-induced mutants tested so far have reverted.

Title of Project nr 3: Studies on radiosensitive cultured mammalian cells

Head of project and scientific staff: Dr. R. Cox (head), Dr. J. Thacker, Dr. P. Debenham, Dr. D.T. Goodhead

Quantitatively reliable methods for the DNA-mediated transfer of plasmid encoded genes into diploid and SV40 transformed strains of normal and ataxia telangiectasia (AT) human cells have been developed. Under optimal conditions transfer frequencies for the pSV2gpt DNA vector are between 10^{-4} and 10^{-5} for diploid cells and 10^{-3} and 10^{-4} for SV40 transformed lines. In some transferants that were examined integration of a single copy of pSV2gpt DNA into chromosomal DNA was observed by Southern blotting techniques. Biochemical characterisation of gpt^+ transferants by in vitro enzyme assay show no difference in gpt activity between normal and AT strains. However, under non-selective culture conditions AT strains tend to show greater instability of the acquired gpt^+ phenotype.

Recombinant plasmid DNA species have also been used in the development of molecular assays for DNA repair and recombination. Preliminary results provide evidence of a specific DNA-repair deficiency in the one AT strain so far examined but no evidence of changes in the efficiency of DNA recombination.

As a preliminary to attempting molecular cloning of the gene defective in AT we have used reconstruction experiments to assay the efficiency of selection of radio-normal phenotypes amongst a large excess of radiosensitive AT cells. A degree of selection compatible with expected gene transfer frequencies has been obtained and a variety of approaches are being employed in order to achieve reproducible DNA-mediated transfer of the putative DNA-repair gene into AT cell lines.

In a parallel series of experiments we have isolated a novel X-ray sensitive/UV-normal mutant of the bacterium Escherichia coli, a phenotype that mimics human AT. This mutant has been successfully used as a recipient for the molecular cloning of the complementary gene (xrr) from an E. coli gene library in plasmid pBR322. The plasmid encoded gene has no influence on recA, recB or recC phenotypes and only a small effect on the rorA phenotype; maxi-cell techniques show that the xrr gene product has a M.W. of 16 kdaltons. After more comprehensive characterisation we intend to introduce the xrr gene (with appropriate SV40 DNA sequences) into AT cells with a view to assessing complementation.

Recent radiation mutagenesis studies with plateau phase Chinese hamster cells clearly show that cellular repair processes that act during and after low dose rate γ-ray exposure do so in an error-free manner i.e. cell inactivation and mutation are decreased to the same extent. In contrast, acute γ-ray exposure followed by a post-irradiation holding period resulted in the repair of potentially lethal lesions but no such effect on potentially mutagenic lesions was observed.

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings

COX R., Mechanism of mutagenesis in cultured mammalian cells. In: Environmental Mutagens and Carcinogens, eds T. Sugimura, S. Kondo and H. Takebe (University of Tokyo Press, Tokyo) pp. 157-166 (1982).

THACKER J., STRETCH A and BROWN R., A simple autoradiographic method for checking HGPRT-deficiency in colonies of mammalian cells. Mutation Res., 103, 371-378 (1982).

GOODHEAD D.T., Deductions from cellular studies of inactivation, mutagenesis and transformation. In: Radiation Carcinogenesis: Epidemiological and Biological Significance. Eds. J.D. Boice and J. Fraumeni (Raven Press, New York), (In press).

GOODHEAD D.T., An assessment of the role of microdosimetry in radiobiology. Radiat. Res., 91, 45-76 (1982).

THACKER J., STRETCH A. and GOODHEAD D.T., The mutagenicity of α-particles from plutonium-238. Radiat. Res., 92, 343-352 (1982).

PRITCHARD J., SANDLAND M.R., BREATNACH F.B., PINCOTT J.R., COX R. and HUSBAND P., The effects of radiation therapy for Hodgkins Disease in a child with ataxia-telangiectasia. Cancer Res., 50, 877-886 (1982).

GOODHEAD D.T. and BRENNER D.J., Estimation of a single property of low LET radiations which correlates with biological effectiveness. Phys. Med. Biol. (In press).

GOODHEAD D.T. and BRENNER D.J., The mechanism of radiation action and the physical nature of biological lesions. Eighth Symposium on Microdosimetry, Jülich, Sept. 1982. (In press).

THACKER J., GOODHEAD D.T. and WILKINSON R.E., The rôle of localized single-track events in the formation of chromosome aberrations in cultured mammalian cells. Eighth Symp. on Microdosimetry, Jülich, Sept. 1982. (In press).

COX R., In vivo and in vitro radiosensitivity in ataxia-telangiectasia. In: The Biological Basis of Radiotherapy, Eds. G.E. Adams, M.J. Peckham, G.G. Steel., (Matthew Arnold, London). (In press).

GOODHEAD D.T., Cellular effects of ultrasoft X-radiation and implications for mechanisms of radiation action. In: The Biological Basis of Radiotherapy, Eds. G.E.Adams, M.J. Peckham, G.G. Steel., (Matthew Arnold, London). (In press).

THACKER J. and COX R., The relationship between specific chromosome aberrations and radiation-induced mutations in cultured mammalian cells. In: Radiation-induced Chromosome Damage In Man, (Eds. T. Ishihara and M.S. Sasaki) Vol. IV in the series Progress and Topics in Cytogenetics, A.R. Liss, New York. (In press).

II. Short Communications

GOODHEAD D.T., Microdosimetry in radiobiology. Radiat. Res., 87, 379-380 (1982). (Abstract).

GOODHEAD D.T., THACKER J. and WILKINSON R.E., Comparison of chromosome aberrations and cell inactivation: energy and spatial requirements. Brit. J. Radiol., 50, 535 (1982). (Abstract).

COX R., THACKER J., GOODHEAD D.T., Post-irradiation recovery and repair processes in cultured mammalian cells. Mutation Res., 96, 129 (1982). (Abstract).

GOODHEAD D.T., Reply to Rossi and Kellerer's comments on "An assessment of the role of microdosimetry in radiobiology". Radiat. Res. (In press).

Progress Report

1982

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Contract no.: BIO-E-453-81-UK

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General subject of the contract:

Factors affecting the yield of mutations from spermatogonial stem cells of mammals.

List of projects:

1. Mutation studies in spermatogonial stem cells of the mouse.

*From 1 November 1982: Professor G E Adams

Title of project nr 1: Mutation studies in spermatogonial stem cells of the mouse.

Head of project and scientific staff: Dr. B.M. Cattanach

Studies with the chemical mutagen, ethylnitrosourea, carried out at Harwell have shown that the compound causes very long periods of sterility and even permanent sterility in inbred 101/H male mice at doses which cause far smaller sterilising effects in F_1 hybrid (C3H/HeH x 101/H) mice. This could mean that the spermatogonial stem cells of 101/H mice are more sensitive to killing. Studies upon 101/H mice might therefore provide ways of understanding the mechanisms underlying the cell killing and genetic responses of stem cell populations to mutagens.

Dose response data for stem cell killing caused by X-rays have been obtained, the criteria used as a measure being duration of sterile periods and testis weight following recovery of fertility.

The following findings have been made:

1) Whereas the lowest dose to cause a sterile period attributable to stem cell killing in F_1 hybrid mice is about 3 Gy, clearly-defined sterile periods could be induced in 101/H males with doses as low as 0.5 Gy.

2) The increased sensitivity to killing of the stem cells of 101/H males was demonstrable throughout the dose-range studied (0.5-8 Gy). At the higher doses a proportion of the animals were permanently sterilised and inspection of the gonads of such males showed them to be greatly reduced in size and extensively damaged.

3) The plateau to the response obtained with F_1 hybrid mice and taken as evidence of a heterogeneity in sensitivity to killing among their stem cells was also evident with 101/H mice though less clearly so. However, it occurred over the 3-5 Gy dose-range, rather than over the 6-8 Gy range as in the hybrid.

4) With a view to comparing the dose-response curve for genetic damage with that from other strains of less sensitivity to cell killing, meiotic chromosome preparations are now being made from the testes of the 101/H mice given doses of 2-8 Gy X-rays and these will be screened for translocations induced in their stem cells.

Progress Report
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General subject of the contract:

Non-disjunction studies with Robertsonian translocations in mice.

List of projects:

1. Non-disjunction studies with Robertsonian translocations in mice.

*From 1 November 1982: Professor G E Adams

Title of project nr 1: Non-disjunction studies with Robertsonian translocations in the mouse.

Head of project and scientific staff: Dr. B.M. Cattanach, Dr. A.G. Searle

1) Marking both arms of the tobacco mouse metacentric Rb4Bnr with gene tags has now been achieved although considerable difficulty was achieved. Studies upon the non-disjunction rates associated with this metacentric using the gene marker method are now in progress. The results to date suggest that very high non-disjunction rates will be obtained.

2) Two stocks carrying four metacentrics have been constructed. One carried Rb1Ct, Rb2Ct, Rb6Rma and Rb2H and the other carries Rb2Ct, Rb3Rma, Rb6Rma and Rb2H. They will be combined to make a five metacentric stock.

3) The age effect upon non-disjunction observed with Rb6Rma heterozygotes using the gene marker method has been investigated and found to be attributable solely to the females.

4) Pilot experiments to investigate the possibility of using metacentric heterozygotes as tester animals to facilitate the detection of mutagen-induced non-disjunction in chromosomally normal mice have been conducted. These were of two types. One screened for chromosome 1 losses, the chromosome 1 marker being *ln*. The other screened for chromosome 1 gains, *fz* and *ln* being the markers. In both experiments chromosomally normal females were X-irradiated and placed immediately with Rb1Bnr heterozygotes, these producing gametes about 15% of which have a missing or an extra chromosome 1. Progeny conceived within the first week of maternal exposure were screened for the markers. The results (see Table) indicate that losses can readily be detected and suggest that these increase linearly with dose. The recovery of chromosome gains was, as expected, much lower.

ient ought	Treated parent	Tester parent	Dose (Gy)	Total no. progeny	Marked progeny No.	Marked progeny Frequency (x 10^{-3})
sses	++/++ ♀♀	R1B *ln*/+*ln* ♂♂	0	3634	0	0
			1	2168	1	0.5
			2	2038	3	1.4
			4	1836	5	2.7
ains	+*fz ln*/+*fz ln* ♀♀	R1B++/+++ ♂♂	4	2611	1	0.4

5) Three metacentrics with monobrachial homologies, Rb(4.6)2Bnr, Rb(4.15)4Rma and Rb(6.15)1Ald have now been combined with genetic markers. Intercrosses of the male-fertile compound Rb1Ald/Rb4Rma in which one parent is homozygous for the chromosome 15 marker Ca^d and the other is wild type have confirmed the complementation of gametes nullosomic and disomic for chromosome 15 and the high frequency of non-disjunction in this compound. Attempts to combine all three metacentrics into a compound with tribrachial homology, which could be used as a tester stock if viable and fertile, have now begun. These involve crosses of each of the three compounds of two metacentrics with males carrying the third metacentric. In order to succeed, the male gamete must be nullosomic for the chromosome carried by both metacentrics in the compound; radiation and heterozygosity for the metacentric Rb(5.15)3Bnr are being used to try and achieve this.

Progress Report
1982

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Contract no.: BIO-E-454-81-UK

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General subject of the contract:

A study of the induction, transmission and consequences of chromosomal aberrations in the Syrian hamster (Mesocricetus auratus).

List of projects:

1. Study of the induction, transmission and consequences of chromosomal aberrations in the Syrian hamster (Mesocricetus auratus).

*From 1 November 1982: Professor G E Adams

Title of project nr. 1: Study of the induction, transmission and consequences of chromosomal aberrations in the Syrian hamster (*Mesocricetus auratus*)

Head of project and scientific staff: Dr. J.R.K. Savage
Dr. A.H. Cawood

For several years we have been maintaining a colony of Syrian hamsters with various chromosome anomalies (See Savage, Breckon, Goy and Bigger, Cytogenet.Cell.Genet., 27, 88-97, 1980) outcrossing either as males or females to normal animals. All progeny are examined chromosomally at weaning, so we have a complete cytogenetic record of this colony respecting the transmission of these anomalies to weaned offspring.

We have now begun a study designed to investigate meiosis and embryonic cytology and thus follow the chain of events which leads to the transmission frequencies which we have observed in progeny.

Two translocations have been chosen for this study. 2SH which was originally a translocation between chromosome 7S and 19L but which has lost the der 19 and so has only 43 chromosomes. These animals have reduced fertility, no unbalanced types being found. The other translocation, 3SH, is a balanced reciprocal translocation between chromosomes 2S and 6L (2n = 44) and this produces, when outcrossed to normal animals, Normal, balanced 2;6, and very frequently the unbalanced Dp(2S)Df(6L)3SH/G. Very rarely, the other unbalanced form (Dp(6L)Df(2S)3SH/H) occurs in weanlings.

Chromosome behaviour in heterozygotes has been studied at pachytene using silver staining of the synaptonemal complex coupled with light and electron microscopy and at MI and MII with conventional and fluorescence staining.

For 2SH, the maximum pairing association is 20 II and III but the frequency with which this trivalent is observed is greater at pachytene than at MI. For 3SH balanced heterozygote and 3SH/G, expected pairing is 20 II and IV and the observed quadrivalent frequencies at pachytene and MI are approximately equal for the balanced heterozygote but in 3SH/G, fewer quadrivalents are found, and again pachytene frequency exceeds that

at MI.

These variable discrepancies between multivalent detection at pachytene and MI obviously have an important bearing upon the detection and estimation of radiation-induced translocations which is conventionally done at MI in spermatocytes. Experiments are now in progress to investigate this point further.

The karyotypes of progeny *in utero* are also being examined using the facility of inducing super-pregnancy by hormonal treatments in Syrian hamsters. With this method, females carry on average 18 embryos. This helps greatly in the search for rarer chromosome constitutions. In 3SH balanced heterozygotes, the theoretical expectation is that the four possible types of gametes will be produced in equal numbers. *In utero,* between fertilization and mid-gestation, all four heterozygotes are found, but there is a deficiency of the balanced and especially the unbalanced forms. Between mid-gestation and birth, again all four types can be found, but in these particular tests, no 3SH/H animals were found at weaning.

Previous experience has shown that when such animals are born they do not show any external morphological changes which would distinguish them from normal animals (Savage, Breckon and Goy, Int.J.Obes., 4, 249-251, 1980) and therefore the reasons which underlie this dramatic *in utero* reduction are worthy of more detailed study.

List of publications in 1982

I. Breckon, G. and Savage, J.R.K. Homozygous deficiency: Syrian hamsters with only 42 chromosomes.
Cytogenet. Cell. Genet., 23, 285-294 (1982).

Breckon, G. A modified hypotonic treatment for increasing the frequency and quality of meiotic metaphases from spermatocytes of the Syrian hamster. Stain Tech., 56, (1983) In press.

Breckon, G. and Cawood, A.H. Transmission of translocations in the Syrian hamster. Proceedings of 2nd Kew Chromosome Conference, 1982. In press.

Progress Report
1982

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Contract no.: BIO-E-452-81-UK

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General subject of the contract:

Development and use of methods for detection and analysis of deletions in the mouse, and of somatic mutations at the cellular level.

List of projects:

1. Development and use of methods for detection and analysis of deletions in the mouse, and of somatic mutations at the cellular level.

*From 1 November 1982: Professor G E Adams

Title of project nr 1:

Development and use of methods of detection and analysis of deletions in the mouse, and of somatic mutations at the cellular level.

Head of project and scientific staff:

Dr. A.G. Searle

A. Detection and analysis of deletions in the mouse.

1. Attempt to induce X-chromosomal deletions

The mutant cream (Crm), distal on the X-chromosome, has been used, with crosses of cc CrmCrm females to cc (albino) males and vice versa, from which cc Crm+ daughters (with mosaic pattern visible by UV fluorescence) would be expected. Anomalous CrmCrm or + daughters are tested genetically and cytologically. The following irradiation regimes have given information:- (i) 100 rad acute X at pronuclear stage (known to be especially sensitive) of fertilised egg gave 3/155 confirmed XO, (ii) 100 rad X to maturing oocytes gave 0/152 confirmed XO, (iii) 400 rad X to maturing oocytes gave 5/184 confirmed XO, (iv) 400 rad to post-meiotic male stages gave 2/231 confirmed XO and one anomalous XX with CrmCrm phenotype which is still under test. This last anomaly is the only possible deficiency which has been found, contrasting with 10 confirmed XO. This seems surprising if (as studies on human X-chromosomal anomalies might suggest) any X-chromosomal deficiency led to non-random inactivation of the chromosome concerned.

2. Autosomal deletions

Two deletion-testing stocks (a bp/a bp, d se/d se, ru ep/ru ep and p ru-2/p ru-2) have been constructed and attempts to combine them are now being made. After various preliminary experiments, the following radiation regimes have been decided upon:- (i) 600 rad acute X-irradiation to wild type females, (ii) two doses of 500 rad acute X-rays 24 h apart to wild type males. Male and female mice irradiated in this way are now being crossed to members of the tester stocks. Both radiation regimes are known to induce high frequencies of specific locus mutations. Frequencies of d-se deficiencies are known to be high

after irradiation of maturing oocytes (to be sampled in the present experiment) and of spermatogonia if the proposed fractionation regime is used.

B. Somatic mutations at the cellular level

Attention has been focussed on development of a test system for forward mutations, since these are more relevant for radiation protection. For work at the cellular level, the dilute (d) and leaden (ln) mutants have been found of particular value because of the marked change in melanocyte morphology when they are homozygous (see publication). A third similar mutant, ashen, (ash) has now been imported from U.S.A. and will be incorporated into the test system if suitable. Tested separately, the d and ln loci have both proved to be mutable in our test system. Attempts to find the most suitable genetic background on which to score skin mounts for mutant clones suggest that a combination of two albino alleles, c^{ch} and c^{e} best enhances the difference between mutant and wild type cells. It has also been discovered that melanocytes of the mouse tail remain clearly visible and very numerous in adult life, which gives the possibility of extending the test system to allow mutation detection from radiation exposures long after birth. This possibility is being explored further.

The possibility of developing a somatic mutation test at the subcellular level is also being explored, by use of the fact that particular coat colour mutants produce pigment granules of different shapes and sizes. Suitable methods for extracting these from hair and retina have been determined, as well as quantitative differences between five of the mutants, using the Quantimet. Tester stocks are being constructed which incorporate some of the mutants concerned.

List of publications in 1982

I. Searle, A.G. and Stephenson, D.A.: An in vivo method for the detection of somatic mutations at the cellular level in mice. Mutation Res. 92, 205-215 (1982).

Progress Report
1982

Contractor:
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Contract no.: BIO-E-417-81-DK

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General subject of the contract:
Chromosome pairing and disjunction in human meiosis.

List of projects:

1. Chromosome pairing and disjunction in human meiosis.

The assessment of the effects of radiation and radiomimetic agents on human male and female meiosis, i.e., on genetic recombination and chromosome disjunction requires a detailed knowledge of chromosome pairing and chiasma formation at the ultrastructural level.

Such information is obtained by three-dimensional reconstructions from electron micrographs of serial sections through human spermatocytes and oocytes at various meiotic stages.

The long-term effects of radiation is studied on biopsies from patients with testis cancer using material obtained before and after treatment.

Short-term effects of radiation on chromosome pairing and chiasma formation cannot be studied in human material and are therefore analysed in Bombyx.

Supporting studies on the relationship of homologous and homoeologous pairing with timing of crossing-over are carried out in hexaploid wheat, triploid Coprinus, triploid and tetraploid Bombyx.

Title of project nr 1:

Chromosome pairing and disjunction in human meiosis

Head of project and scientific staff:

Professor Diter von Wettstein
Dr. scient. Preben B. Holm
Dr. scient. Søren W. Rasmussen
Cand.med. Jørgen G. Berthelsen
Stud. scient. Maja Bojko

The investigation of meiosis in the normal human male by three dimensional reconstructions from electron micrographs is about to be completed. The last part of the work includes the analysis of the morphological, numerical and distributional changes of recombination nodules during pachytene as well as the formation and fate of chiasmata at the diplotene-metaphase I interval. In both cases detailed investigations of the temporal changes in nuclear architecture were necessary. Based on morphological criteria the pachytene stage was divided into seven substages and the very short diplotene-diakinesis period into six.

Recombination nodules attach at random to the synaptonemal complex already at zygotene in numbers which exceed that of chiasmata by a factor two. By pachytene stage 1 the number of nodules is reduced to a mean of 75 per nucleus, the distribution among and along the bivalents still being at random. At pachytene stage two, 16 nodules per nucleus (mean value) have attained a more spindle-like shape and are referred to as recombination bars. At this stage the distribution of nodules and bars are no longer entirely at random. Nuclei at stage 3 and 4 possess a mean of 35 bars and 10 nodules and both structures are now distributed nonrandomly. Only one percent of the autosomal bivalents are devoid of nodules or bars compared to 16 percent expected for a random distribution among the bivalents. Likewise nodules and bars are not distributed at random between the arms of the bivalents, each bivalent arm in most cases having at least one nodule or bar. Nodules and bars are furthermore in excess in subterminal regions of all bivalents and at medial to proximal positions in the longer bivalent arms, whereas a deficit is observed in regions flanking the centromeric heterochromatin. This distribution is basically in agreement with the distribution of chiasmata. At pachytene stage 5 a mean of 25 bars and 7 nodules remain and are distributed similarly to those at stages 3-4. By stage 6 most nodules and bars have disappeared and at stage 7 very few remain. Possible derivatives of bars in the form of chromatin nodules and circular structures as described for Bombyx and Coprinus were not identified.

Degradation of the synaptonemal complex is initiated at early diplotene, but in contrast to the situation in most other organisms the elimination is arrested at mid diplotene. A mean number of 60 synaptonemal complex fragments remain between the homologues. Their distribution is similar to that of nodules and bars at stages 3-5, strongly suggesting that the synaptonemal complex fragments are involved in the stabilization of regions where crossing over has occurred.

By the end of mid diakinesis the fragments are replaced by chromatin connections and the released fragments often fuse into small polycomplexes which are finally shed from the bivalents at prometaphase I.

The disappearance of the synaptonemal complex between the homologous segments of the sex chromosomes during mid pachytene is only temporal and a synaptonemal complex reappears by the end of pachytene in 85 percent of the nuclei. The short complex segment is maintained until mid diakinesis and supports the contention that the X and Y chromosomes are associated by a chiasma.

The analysis of the recombination nodules and bars have thus revealed that a minimum of one crossover per bivalents which is required for its regular disjunction is ensured by a series of distributional changes similar to those reported for the male silkworm, Bombyx mori, and the basidiomycete Coprinus cinereus and that the frequency of bivalents without recombination structures is about 1% as in Bombyx and Coprinus. As all bivalents at mid diplotene to mid diakinesis possess chiasmata in the form of synaptonemal complex fragments the chiasma organization in the human male appears to be as efficient as that of Bombyx and Coprinus. A high frequency of primary nondisjunction in the human male is thus most likely due to events occurring at metaphase - anaphase I or have entirely different explanations such as increased frequency of bi- and multinucleate cells, increased frequency of malfunctioning spindle apparatus or kinetochores etc.

The investigation of meiosis in the human female has been continued and to date 2 leptotene, 8 zygotene 11 pachytene and 6 diplotene nuclei have been reconstructed. Chromosome pairing and synaptonemal complex formation occur as in the male although interlocking of chromosomes and bivalents is more frequent, a result which may be attributed to the length of the chromosome complement which exceeds that of the male by more than a factor two. As in the male, interlockings are resolved by breakage and reunion of chromosomes and bivalents by the end of zygotene. The number of recombination nodules and bars are during pachytene of the same order of magnitude as in the male and an analysis of their distribution along and among the bivalents is in progress. At early diplotene the synaptonemal complex is rapidly eliminated and intact remnants are not preserved during diplotene as in the male.

The effect of cytostatica on meiotic cells has been analyzed on 22 completely reconstructed zygotene and pachytene nuclei from boys undergoing combination chemotherapy for acute lymphatic leukemia. Most of the nuclei appeared nearly normal and only one zygotene nucleus showed pronounced abnormalities in pairing and synaptonemal complex formation. Some pachytene nuclei exhibited abnormal nucleolar morphology while in other nuclei atypical condensation patterns of eu- and heterochromatin were apparent and the ratio between recombination nodules and bars differed from that of control nuclei. These observations indicate that combination chemotherapy for acute lymphatic leukemia does not lead to severe distortion of spermatogenesis but that minor effects on the fertility of the patient are to be expected.

The analysis of the long term effect of radiation in the human male has continued. Today 60 biopsies taken before treatment are available

while difficulties have been encountered in obtaining biopsies after restoration of spermatogenesis and so far only three biopsies have been taken. Thirteen pachytene nuclei from these biopsies have been serially sectioned and photographed but have not revealed gross alterations of nuclear morphology. Recently, two biopsies were obtained during radiation treatment. The analysis of this material will be given high priority.

In order to gain more insight into the two phase pairing system originally described in tri- and tetraploid Bombyx females and later demonstrated in a translocation heterozygote of Homo, the investigation of chromosome pairing in allohexaploid wheat has been continued. New procedures have been developed yielding a good and reproducible fixation of wheat microsporocytes. The reconstruction of the nuclei has been facilitated by selectively staining the lateral components of the synaptonemal complex with phosphotungstic acid. Until now 15 nuclei covering the entire zygotene period have been photographed and reconstructions of these nuclei are in progress.

Finally cultures of silkworms have been established and large numbers of larvae are now reared routinely. The larvae will later be used for studies of short term effects of radiation and for biochemical studies of meiosis.

I. Publications in Scientific Journals, Monographs, Proceedings

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Rasmussen, S.W. and P.B. Holm: Chromosome pairing and synaptonemal complex formation. Proc. XIV Int. Congr. Genet. Vol III. MIR. Publishers, Moscow pp 331-334 (1981)

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S.W. Rasmussen and P.B Holm: The meiotic prophase in Bombyx mori. In: Insect Ultrastructure, Vol. 1, Eds. R.C. King and H. Akai. Plenum Press, New York pp 61-85 (1982)

P. Hobolth : Three dimensional reconstructions of synaptonemal complexes at zygotene and pachytene in allohexaploid wheat var. Chinese spring. In: Proc. 2. Kew Chromosome Conference (1982) (in press)

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Progress Report
1982

Contractor:
University of Aarhus
Department of Molecular Biology
C.F. Møllers Allé 130
DK-8000 Aarhus C

Contract no.: BIO-E-418-81-DK

Head(s) of research team(s):
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Dep. of Molecular Biology
Aarhus University
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Prof. O. F. Nielsen
Dept. of Molecular Biology
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General subject of the contract:
Molecular events on the biologically active chromatin form of a single eukaryotic gene in response to radiation.

List of projects:

1. Molecular events on the biologically active chromatin form of a single eukaryotic gene in response to radiation.

Title of project: Molecular Events of Ionizing Radiation on Specific Genes

Head of project: Drs. Ole F.Nielsen and Ole Westergaard

Scientific staff: B.Bonven, P.Brams, E.Gocke, C.Herskind, K.-D.Jentsch, O.F.Nielsen, D.Tiryaki, E.Østergaard, and O.Westergaard

I. A prerequisite for the study of molecular events in chromatin in response to environmental hazards is the capability of isolation of specific genes in the chromatin form. Due to the organization of genes of higher cells in a number of DNA molecules corresponding to the number of chromosomes of the species, this has been an impossible task. However, the presence of the highly amplified ribosomal RNA genes as extrachromosomal chromatin in nucleoli of the protozoa Tetrahymena has allow the isolation of this gene in its functionally active chromatin form. Structural similarities between chromatin of this organism and higher eukaryotes allow the the use of this chromatin as a model system for higher eukaryotic cells.

The endogenous RNA polymerase molecules on the nucleolar chromatin are used to monitore DNA damage due to ionizing radiation by measurement of the decrease in incorporation of XTP into rRNA during in vitro transcription. The effect of ionizing radiation was found to depend upon the complexity of the chromatin and on chemical parameters with $D_{\frac{1}{2}}$ varying in the range 50-2500 Gy. The gene is mainly inactivated by indirect processes when nucleoli suspended in a dilute buffer are irradiated. Various radical scavengers have been used to analyse the relative efficiency of the aqueous radiolytical radicals. Sulphhydryl containing compounds protect under hypoxic conditions the gene to a larger extent than expected. This may be explained as the results of molecular repair processes leading a to lower radio-sensitivity under hypoxic than under oxygenated conditions. A similar sensitivity of the gene is observed when the gene is irradiated in nuclei in the absence of exogenous SH-scavengers. This indicates that compounds with a similar scavenger effect are present in the nuclei but get dissociated during isolation of the nucleoli. The addition of radical scavengers may reduce the sensitivity with an order of magnitude. The result depends, however, strongly on the chemical composition of the solute and is presently subjected to further investigation.

The nucleolar chromatin has turned out to be an excellent system for detection and localization of induced single and double strand breaks of DNA. A novel nuclease activity has been found intimately associated with the nucleolar chromatin in the spacer region flanking the 5'-end of the ribosomal RNA gene. This endogenous nuclease is in analogy with DNA topoisomerases activated by strong denaturants to cleave DNA at specific sites. Three specific single strand cleavage sites have been mapped all of which are associated

with micrococcal nuclease hypersensitive sites and DNase I hypersensitive regions. A double strand break is located near the centre of the molecule. The location of the cleavage sites is compatible with models involving topoisomerases in the regulation of transcription, replication and recombination. The intimate association of the putative topoisomerase with the nucleolar chromatin makes it possible to isolate the enzyme with nucleoli and thereby characterize the enzyme and to study the function of topoisomerases in processes on chromatin of eukaryotic genes.

We have already reported the use of the isolated nucleolar chromatin as a system for studying the interaction of drugs with chromatin and the influence of these drugs on various regulation processes in gene expression. Thus, a number of intercalating compounds have been detected to cause a specific deletion of the normal termination properties of the endogenous RNA polymerases on the chromatin.

II. In order to investigate the cellular mechanism behind the induction of repair enzymes in eukaryotic cells, we have raised monoclonal antibodies against a radiation induced DNA polymerase from Tetrahymena. The polymerase is located in the mitochondria but encoded for by the nucleolar genome. The specific acitivty of the enzyme increases up to 50X on induction, with a linear response in the lower dose range. The enzyme has been purified extensively. The antibodies have been used to immunoprecipitate the DNA polymerase. Analyses of the precipitate on denaturing protein gels show that the antigen has a molecular weight of approximately 46 k daltons. Using the monoclonal antibodies it will now be possible to: (i) study the cellular induction phenomenon in details and (ii) clone the gene coding for the polymerase. In addition it should be possible by use of the antibodies to develope a screening assay for DNA damaging agents, as the DNA polymerase is also induced by various mutagens. The antibodies will be used for studies of cross-reaction with corresponding enzymes from higher eukaryotes. Such studies seem plausible as: (i) a similar induction phenomenon seems to occur in certain insects , (ii) the DNA polymerase in chicken has been found to have a similar subunit structure as the one described for Tetrahymena, and (iii) cross-reactivity has been detected between certain other proteins from Tetrahymena and corresponding proteins from higher cells incl. human cells.

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Progress Report
1982

Contractor:

Commission of the European
Communities DG XII-F
Biology, Radiation Protection
and Medical Research
Biology Group - Ispra
I-21020 Ispra (Varese)

Contract no.: CEC-DG XII-F-ISPRA

Head(s) of research team(s):

Dr. M. Devreux
DG XII-F
Biology, Radiation Protection
and Medical Research
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I-21020 Ispra (Varese)

General subject of the contract:

Genetic and biochemical analyses of radiosensitivity and DNA repair.

List of projects:

1. Genetic and biochemical analyses of radiosensitivity and DNA repair.

Biology Group Ispra - Italy

Direct participation of the Commission in its established programme

Head of research team : M. DEVREUX

General subject of the research : Genetical and biochemical analysis of the radiosensitivity of eukaryotic cells.

Results

A. The genetic analysis of radiation effects on eukaryotic cells cultured in vitro

Scientific staff: R. Cavalloro, M. Devreux, E. Magnien

The studies were carried out with model systems devised in great part in our laboratory: protoplasts of haploid and diploid Nicotiana plumbaginifolia, continuously growing embryogenic cell suspensions of haploid and diploid Daucus carota, and proliferating med-fly cell lines.

A.1. - Protoplast sub-populations

The mother-plant culture conditions strongly determine the extent of cytological heterogeneities and the related complexity of protoplast dose-responses to ionizing radiations.

Density fractionation of protoplast populations was performed on an iso-osmotic step gradient prepared by successively layering one upon the other of various Percoll solutions in KMC minerals. (A mineral solution of chlorides of K, Mg and Ca, with the same osmolarity as the culture medium but with a very low specific density).

Distribution of protoplasts along the gradient was analyzed by a computerized image analysis for cytological parameters (VIDEOPLAN). It showed that, whereas large protoplasts were only present in the highest phases, the correlation with volume was poor for the category of smaller protoplasts which accumulate in any of the gradient phases. On the other

hand, there was a clear-cut discrimination of haploids from diploids based on the mere protoplast volume in all phases.

A.2. - Dose-effect studies

The irradiated protoplast population often shows a biphasic dose-response curve, due to a small proportion of more sensitive sub-populations.

A pre-dose of 200 rad simply eliminates these minor sub-populations while the density gradient fractionation separates these hypersensitive components, distinct from the sensitivity of the most abundant layer, each one being homogeneous in its radiation response.

It is therefore possible to perform a successful selection and characterization of defined sub-populations through density fractionation and to allow reliable dose-effect studies.

An experiment was carried out to compare the effects of γ-ray and fast neutron irradiations on haploid and diploid protoplasts maintained for 0, 1 or 2 days at density of 20, 100 and 500.10^3 protoplasts/ml. The purpose was an attempt to understand the relative influence of the population density at various times on the irradiation response, particularly on recovery processes.

A.3. - Synchronization of nuclear DNA synthesis by aphidicolin

A transient treatment of carrot cell suspension culture with aphidicolin, a specific and reversible inhibitor of nuclear DNA replication, causes an accumulation of cycling cells at the border G_1-S. Such treatment for 22 hours has no deleterious effect on cell metabolism, because when the drug is removed from the culture medium, the whole cell population resumes promptly DNA-synthesis entering synchronously in the S-phase.

Evidence of this has been obtained both from data on tritiated thymidine incorporation and from autoradiographic analysis of labelled nuclei. The S-phase or M-phase synchronous population will be of interest

for a number of experimental purposes (work in collaboration with Pavia CNR laboratory).

A.4. - Mutagenesis

- An experimental system to study mutagenesis during embryogenesis was investigated employing cell suspension cultures of an haploid and a diploid strain of Daucus carota capable, under appropriate conditions, of giving rise to thousands of free floating somatic embryos which can be grown to adult plants. This system represents an unique tool for studying the degree of mutability during the various stages of differentiation.

- With N. plumbaginifolia protoplasts, a few valine resistant calli have been obtained from irradiated diploid protoplasts and are now regenerating shoots and leaves.

The other experimental system now being used, is that of mutagenesis and selection for nitrate reductase deficient mutant cell lines. Such a recessive mutant has a very high frequency of appearance (about 5×10^{-4}) in an haploid but not in a diploid cell population. Therefore, the experiments of mutagenesis with low doses of ionizing radiations must be set up with protoplasts from leaves of haploid N. plumbaginifolia plantlets.

A.5. - Insect cell line

Research was continued with the stabilized cellular lines of Ceratitis capitata Wied. The cytophotometric analysis of the cells, showed a satisfactory caryologic stability with time, which confirms the excellent quality of this material for studies of dose-effect type.

Preliminary studies on the incorporation of tritium were carried out. In particular, ^{3}H-thymidine added to the culture medium (15 uCi/ml) showed a slower incorporation with respect to what was noted in the cells of mammals. The maximum incorporation was obtained at the 24-hour reading.

The radioresistance characteristic of these cells proved to be 4 to 8 time superior to what was found for the mammalian cells.

Further investigations of ionizing radiation effects (γ) distributed either in a continuous dose or in fractionated doses (with intervals between O' and 480') were carried out.

The most evident effect found for the fractionated doses, confirm the fact that there is a greater repair when the time interval between the different doses is longer.

The study of repair in cells of Ceratitis were carried out with ionizing radiations and in the presence of aphidicolin (this compound impedes cell mitosis). It was noted that when aphidicolin, at a concentration inferior to 4 uM was removed from the medium, there was a recovery of the synthesis of DNA and the relative cell divided within 24 hours, in a manner similar to the controls.

A.6. - DNA polymerases in insect cells

The study made in collaboration with Drs. U. Bertazzoni and A.I. Scovassi of the CNR laboratory of Pavia on DNA polymerases in Med-fly cells and embryos, has permitted to establish the structure of the enzyme activities and to verify their peculiar resistance to aphidicolin, the specific inhibitor of polymerase α .

The level of DNA polymerase α , measured during the life cycle of the insect embryos, increased in parallel with the rate of embryonic cell proliferation, whereas DNA polymerase γ increased at much later fertilization time, when cell differentiation is already taking place.

The presence of an activity responding to the β -assay was also evident and its variations were similar to that observed for polymerase-α .

We have investigated the nature of the β-like activity and found that it did not correspond to a true DNA polymerase β upon subsequent sedimentation and purification procedures.

DNA polymerase α, purified 100 folds from Med-fly embryos, was 10 times more resistant to aphidicolin than the mammalian DNA polymerase α, and presented a non linear reciprocal plot suggesting a partially competitive mode of inhibition. This is reflected also by a much enhanced resistance to aphidicolin of the DNA synthesis and cellular growth of cultured Med-fly cells.

The drug, when added at low or high concentrations to fertilized eggs, did not induce any modification to the embryo development and to the larval and adult differentiation.

Work is in progress to analyze the response of Med-fly to ionizing radiations (continuous and fractionated doses) in the presence of aphidicolin, which is known to inhibit DNA replication but not DNA repair in vertebrate cells.

B. Biochemistry of DNA damage and repair

Scientific Staff: F. Campagnari, L. Clerici, M. Talpaert-Borlé

B.1. - Studies of mammalian enzymes acting on DNA

a) DNA polymerases. The study of the specific inhibitors of mammalian DNA polymerases with the aim to differentiate the reactions catalyzed by the replicative deoxynucleotidyltransferase α and by the reparative DNA polymerase β was continued.

The investigation of the inhibition mechanism exerted on DNA polymerase α by β-lapachone, a natural quinone-like substance, was completed.

Aphidicolin, a drug considered a selective antagonist of DNA replication, was tested with the most purified fraction of DNA polymerase α from bovine thymocyte nuclei. In the presence of optimal concentrations of deoxynucleotide substrates, a 2 mM concentration of aphidicolin did not affect adversely the polymerizing reactions promoted by the enzyme on functional primers, such as DNaseI-activated DNA and $polydA_{2000}$:poly dT_{200}. However, the lowering of substrate concentration allowed the drug

to inhibit progressively the DNA polymerase activity. When dCTP in the DNA primed reaction amounted only to 2.5 uM, 200 uM aphidicolin was able to abolish completely the DNA synthesizing reaction.

b) DNases. Two 3'→ 5' exonucleolytic activities were detected in the G-100 fraction of the partially purified DNA polymerase α from the nuclei of calf thymus cells. A sucrose gradient analysis of this fraction revealed that the exonucleolytic activity was associated to 2 proteins with sedimentation constants of about 5.5 S and 3.5 S. It was found that 2 uM aphidicolin did not inhibit the combined DNA polymerase and "proof-reading" 3'→ 5' exonuclease reactions when the mismatched DNA primer $polydA_{1100}$:poly dT_{200}-$\left[^{14}C\right]dC_{1.5}$ was used in the enzymatic assay of DNA replication.

c) Uracil-DNA glycosylase. The distribution of the enzyme in cytosolic and nuclear fractions of the cell homogenates from a number of mammalian tissues was studied. The results were consistent with the retention of significant amounts of enzyme in the nuclei even after disruption of the tissues in order to isolate the subcellular fractions. This is consonant with the expected physiological location of uracil-DNA glycosylase in the nuclei of eukaryotic cells.

Alternative methods for the purification of the enzyme from nuclei and mitochondria of mammalian cells were standardized and successfully used.

d) DNA-glycosylase acting on γ-irradiated DNA. The existence of DNA glycosylases specifically active on altered base residues induced in DNA by ionizing radiations was investigated. The $\left[^{14}C\right]$methoxyamine labeling method (see below) was used to count AP-sites that had been introduced in DNA by physicochemical and enzymatic methods.

The DNA exposed to high doses of γ-rays contained a large number of sites that combined with $\left[^{14}C\right]$methoxyamine (presumably AP-residues) and undefined groups coupling with the reagent and then undergoing time-

dependent release of the adduct by chemical hydrolysis. Preliminary experiments with extracts from calf thymocyte nuclei did not reveal any activity of glycosylases producing additional AP-sites in γ-irradiated DNA.

e) Terminal deoxynucleotidyl transferase. The collaborative study of the distribution of terminal deoxynucleotidyl transferase, TdT, in malignant cells from human lymphomas and leukemias was pursued.

The methods for measuring the enzyme activity in tissue samples were modified to assay properly the TdT present in the mononuclear cells of human blood.

The cytoimmunofluorescence tests and the biochemical assays for detection of TdT were integrated with the determination of the specific immunological markers in the lymphoid cells. This led to identify different populations of immature lymphoblasts expressing the TdT enzyme function in a number of hematological disorders.

The series lymphoid malignances analyzed for TdT was brought to about 200 cases.

B.2. - Preparation of polynucleotides and measurements of AP-sites

a) The large scale synthesis of polydeoxynucleotides that mimicked specifically modified DNAs was continued.

The reaction catalyzed by the purified uracil-DNA glycosylase on DNA and polydeoxynucleotides containing uracil was exploited to prepared DNA-like molecules with a known amount of AP-sites. These apyrimidinic DNAs were used as suitable substrates either to measure the activity of specific AP-endonucleases or to calibrate the selectivity of aldehyde group reagents for the base-free deoxyriboses in DNA.

b) The methodological investigation on the specific reduction of the aldehyde groups in the AP-sites of DNA by tritiated sodium borohydride $[^3H]NaBH_4$, was completed. Under mild reduction conditions with 5mM $[^3H]$

$NaBH_4$ for 30 min at room temperature and pH 8.4, the incorporation of tritium into alkylated-depurinated DNA displayed a negligeable background and was proportional to the number of AP-sites. Thus the reaction was suitable for a direct counting of the AP-residues in DNA. Nevertheless, the instability of $[^3H]NaBH_4$ in aqueous solutions prevented a routine use of the method.

Tritiated sodium cyanoborohydride $[^3H]NaBH_3CN$ was tested as a more reliable reducing agent then $[^3H]NaBH_4$. The electronegative function -CN should hinder the release of the hydride group and would result in a greater stability of the reagent. It was found that the ^3H-labeling of alkylated-depurinated DNA by $[^3H]NaBH_3CN$ was slow, incomplete and not related to the AP-sites.

Finally $[^{14}C]$methoxyamine, $[^{14}C]CH_3ONH_2$ appeared to be the best reagent to count radioactively the AP-sites of DNA. The $[^{14}C]CH_3ONH_2$ was enough stable and could label DNA proportionally to the number of aldehyde groups from the AP-residues without causing breaks in the DNA strands. In 30 min at 37°C and pH 7.2, 5mM $[^{14}C]CH_3ONH_2$ reacted with 90% of either the apurinic sites induced by heating alkylated DNA or the apyrimidinic groups introduced by uracil-DNA glycosylase in synthetic polydeoxynucleotides containing uracil.

The test with $[^{14}C]CH_3ONH_2$ affords a simple and direct counting of AP-sites either intact or associated with strand breaks in DNA. It will be preferred to the two-step methods involving differential measurements of DNA strand scissions before and after chemical or enzymatic cleavage of the labile phosphodiester bonds adjacent to the AP-sites.

B.3. - Cultured mouse embryos

a) Fertilized mouse embryos recovered from the maternal oviducts at the 2 cell stage were grown in vitro until the cells escaped from the zona pellucida (hatching, 4 days) and were used to determine the $L.D._{50}$ of various tritiated compounds incorporable into the nuclear chromatin such as arginine, lysine, histidine, aspartic acid, tryptophan, adenosine

and thymidine. The measurements of lethality were carried out on the 3rd day when the blastocysts expanded.

The $L.D._{50}$ of the tritiated substances ranged from 30 nCi/ml for $[^3H]$ lysine to 400 nCi/ml for $[^3H]$ aspartic acid. Biochemical analyses supported the assumption that the death of the embryos was due to the decay of tritium atoms in the chemical constituents of chromatin. The metabolic uptake of the tritiated precursor molecules by the nuclear material occurred with different kinetics depending on the nature of the tested compound. However, a tritium incorporation equivalent to 0.2 c.p.m. per cell nucleus was sufficient to kill 50% of the embryos.

b) A series of experiments were carried out with Dr. M.J. Carrol of the Dept. of Biochemistry from Trinity College of Dublin in order to syncronize the embryo developing in culture. Aphidicolin and α-amanitin were able to block temporarily the embryo growth without damaging viability and subsequent growth potential. On the contrary, very low doses of actinomycin D arrested the development of embryos in an irreversible manner.

Dr. M. Merlini from the Division of Physics of the Joint Research Centre Establishment in Ispra collaborated to the studies with the mouse embryos.

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Talpaert-Borlé M., Campagnari F. and Creissen D.B. Properties of purified uracil-DNA glyscosylase from calf thymus. An in vitro study using synthetic DNA-like substrates.
J. Biol. Chem., 257, 1208-1214, 1982.

Vezzoni P., Clerici L., Lucchini R., Vezzoni M.A. and Giardini R. Studi sulla poli(A) sintetasi in tessuti linfoidi normali e neoplastici.
Communication at "28° Congresso Nazionale della Società Italiana di Biochimica", September 26-29, 1982.

III F

ABSCHÄTZUNG DES STRAHLENRISIKOS

EVALUATION OF RADIATION RISKS

EVALUATION DES RISQUES D'IRRADIATION

Weitere Forschungarbeiten zu diesen Themen werden auch in folgenden Tätigkeitsberichten beschrieben :

Further research work on these subjects is also described in the following progress reports :

D'autres travaux sur ces thèmes de recherche sont également décrits dans les rapports suivants :

III A.	Jacobi, W./Burger, G.	GSF Neuherberg	BIO 287 D
III A.	Kellerer, A.M.	Univ. Würzburg	BIO 286 D
III B.	Bächmann, K.	Tech. Hochsch. Darmstadt	BIO 487 D
III B.	Fontaine, R.	CEA, CEN Fontenay-aux-R	BIO 464 F
III B.	Madelaine, G.	CEA, CEN Fontenay-aux-R	BIO 436 F
III B.	Maisin, J.R.	CEN, SCK Mol	BIO 330 B
III B.	Peirson, D.H.	UKAEA Harwell	BIO 332 UK
III C.	Dumont, J.E.	Univ. Bruxelles	BIO 360 B
III C.	Taaffe, J.K./Malone, J.F.	Coll. Technol. Dublin	BIO 364 EIR
III D.	Chalabreysse, J.	CEA, CEN Pierrelatte	BIO 372 F
III D.	Gössner, W./Kellerer, A.M./ Spiess, H.	GSF Neuherberg/Univ. Würzburg/ Univ. München	BIO 461 D

Progress Report
1982

Contractor:

Associazione dei Comuni di
Ferrara Poggio Renatico,
Vigarano Mainarda e Bondeno
Via Arturo Cassoli 30
I-44100 Ferrara

Contract no.: BIO-F-446-81-I

Università di Ferrara
Via Paradiso 12
I-44100 Ferrara

Head(s) of research team(s):

Dr. B. Bagni
Serv. di Fisica Sanitaria
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I-44100 Ferrara

Prof. O. Rimondi
Istituto di Fisica
Via Paradiso 12
I-44100 Ferrara

General subject of the contract:

Reduction of patient exposure preserving image quality in diagnostic radiology.

List of projects:

1. Reduction of patient exposure preserving image quality in mammography.

Reduction of patient exposure preserving image quality in mammography.

O. Rimondi, B. Bagni, R. Boccafogli, G. Candini, P. Carraro, M. Gambaccini, R. Tamarozzi

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The program for breast dose reduction and image quality optimization in each facility is devided into various steps:
-collection of the values of the operative parameters, by means of a questionnaire and through measurements carried out in each facility.
-analysis of the gathered data in order to work out the organ dose and the physical image quality. Suggestions of the possible improvements.
-development of a quality assurance activity for mammography, equipment and training support for the operators.

a) - Data collected by means of a questionnaire.

The questionnaire was mailed only to public mammography units. The data collected till now refer to 46 answers.

The most commonly used X-ray unit is the C.G.R. Senographe with Mo anode, Be window and 0.03 mm Mo filter.

In addition to the specially-designed X-ray units for mammography, a certain number of general purpose units with minor modifications are used.

As expected, the answers to the questionnaire are not sufficient to evaluate organ dose and image quality; it is necessary to perform measurements of the film-screen characteristics in our Laboratory, and measurements of the focal spot, H.V.L., and skin exposure in each unit. To this purpose two instruments (exposimeter and star-pattern), simple enough to be used by the operators, were prepared and calibrated.

We underline that these simple and cheap devices will enable each unit to begin a quality assurance activity for mammography too.

b) - Exposimeter calibration.

The exposimeter has to be irradiated like the breast in a usual examination, and calibration is referred to this purpose.

The Senographe (CGR) X-ray unit was employed for measurements.

The results must be tested for the other types of X-ray units too.

Calibration involved the following measurements:
- kVp and voltage ripple of X-ray apparatus. We measured the ripple and tested that it does not affects significantly H.V.L.; this result is important in the context of other

measurements.

- TLD-100 energy dependence Vs. kVp, and Vs. H.V.L.
  The measurements were carried out in the range 20-40 kVp.
- H.V.L. Vs. detectors out-puts ratio.
  The ratio between the readings of the two groups of TLD-100 detectors, contained in the exposimeter, were correlated to the following H.V.L. values: 0.22, 0.28, 0.31, 0.34, 0.35, 0.37, 0.39, mmAl.

c) - Star-pattern calibration.

The star pattern is made by etching a Cu coated board for printed circuit; the very cheap cost of the pattern makes its diffusion easier.

A star pattern support defines the geometrical conditions of exposure, and consequently the M value **(Magnification).**

The registration system of the star-pattern image is a sandwich of three films and three filters instead of a single film without filter.

The film sandwich is preferred to the single film for a practical reason, in fact the operators can obtain at least one suitable image also with the exposure value of a usual mammography.

Publications

I - (1) C. Maroni, R. Zamboni, M. Gambaccini, O. Rimondi "Measurement of the detection quantum efficiency of an X-ray screen-film system" To be published on "Optic Communication", 1982.

II - (1) O. Rimondi et al. "Reduction of patient exposure pre= serving image quality in mammography. Recent results" Internal Report, Presented at the "Annual meeting of Contractors", 23-24 Sept. 1982, Paris.

- (2) M. Gambaccini, "Controlli di qualità in mammografia" Lectures presented at the "III Corso nazionale di Fi= sica delle radiazioni e tecnologie biomediche", 9-12 Nov. 1982, Istituto Superiore di Sanità, Rome.
- (3) R. Forti, "Progetto e taratura di dispositivi per la misura della esposizione, del H.V.L, del fuoco", Thesis, Istituto di Fisica, Università di Ferrara, 15 Dicembre 1982.

Progress Report
1982

Contractor:

Contract no.: BIO-F-516-82-I

Unita Sanitaria Locale
N° 7 Udinese
Via Colugna 50
I-33100 Udine

Head(s) of research team(s):

Dott. V. Barbina
Serv. Fis. San.
Ospedale "S.Maria Miseric."
Via Pieri
I-33100 Udine

General subject of the contract:
Radiation exposure in medical diagnostic procedures in Friuli Venezia Giulia region.

List of projects:

1. Radiation exposure in medical diagnostic procedures in Friuli Venezia Giulia region.

Project BIO-F-516-82-I

Radiation exposure in medical diagnostic procedures in Friuli Venezia Giulia region.

Head of project: V. Barbina, Servizio di Fisica Sanitaria, Ospedale "S. Maria della Misericordia" I-33100 Udine
Scientific staff: R. Padovani, G. Contento, M.R. Malisan, C. Omet

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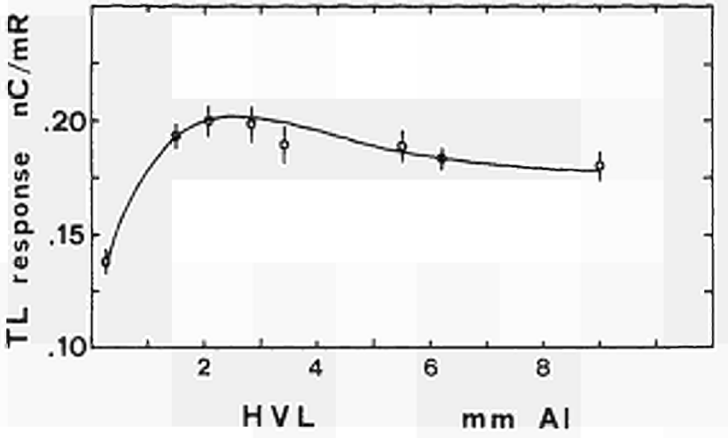
I - A survey of number and types of radiological examinations carried out in the hospitals and medical centers of FVG area in the years 1979 and 1980 has been completed. The data are based on the annual returns of all radiological departments. These included some detailed information which allowed the calculation of the 1980 percent frequencies of the different types of examinations. However, dental radiography outside NHS premises is excluded. The results are the following:

Year	Examinations (thousands)	Annual per capita rate
1979	1233	0.99
1980	1263	0.98

Examination type	Percent frequency (1980)
Chest, heart and lungs	33.0
Cervical, dorsal and lumbar spine	20.9
Limbs	16.9
Head	5.7
Abdomen	3.9
Barium meal	3.9
Dental	2.9
Pelvis	2.2
Intravenous pielography	2.1
Cholecystography	1.9
Tomographies	1.7
Barium enema	1.1
Ribs	1.0
Mammography	0.8
Cholangiography	0.7
Angiographies	0.6
Others	0.7

II - The TLD system for the measurements of patient skin doses has been checked. It consists of a set of LiF ribbons (TLD 100) in black polythene sachets. The response in total backscattering from tissue equivalent material vs. beam quality has been measured, as shown in the figure:



The sensitivity is relative to exposures free in air measured by the secondary standard of the ENEA laboratory, Bologna, for energy-calibrated X-ray beams of 10 cm diameter.

III - A booklet introducing the purpose and methods of the project has been published. It was mailed to radiologists, physicists and technicians of FVG, as well as all the people who could share a concern about the population exposure in radiological procedures.

List of publications in 1982

I. --

II. L'Esposizione alle Radiazioni in Radiologia Medica nel Friuli Venezia Giulia; a short introduction to the project program.

Progress Report
1982

Contractor:

Nuclear Energy Board
Lower Hatch Street 20-22
IRL-Dublin 2

Contract no.: BIO-F-449-81-EIR

Head(s) of research team(s):

Dr. J.D. Cunningham
Nuclear Energy Board
Lower Hatch Street 20-22
IRL-Dublin 2

General subject of the contract:

Analysis of radiation doses to patients in diagnostic radiology.

List of projects:

1. Analysis of radiation doses received by patient, undergoing diagnostic X-ray examinations in Ireland.

TITLE OF PROJECT: Analysis of radiation dose to patients in Diagnostic radiology.

HEAD OF PROJECT: John D. Cunningham

SCIENTIFIC STAFF: D. Howett
A. Brennan
J. O'Grady

TECHNICAL STAFF: Miss C. Mulholland.

The programme of work during 1982 included the completion of a survey of diagnostic radiological examinations carried out in Irish hospitals to determine their nature and frequency. Data has been collected for four one week periods and the information obtained for each examination included th examination type, the sex and age of the patient, the number of exposures and the duration of screening procedures. The overall response rate has been excellent and a preliminary check indicates that it will exceed ninety per cent. The checking of the returned survey forms and the coding of the radiological examinations has almost been completed and transfer of the data for computer analysis has been commenced.

The second phase of the project involves the measurement of the radiation doses, particularly the gonad doses with a view to determining the geneticall significent doses being screened by patients. Doses delivered by those examinations making a significant contribution to the GSD will be measured by means of lithium borate thermoluminescent powder attached directly to patients Difficulties were experienced with the TLD system during the year which resul in a protracted commissioning period. These difficulties have been resolved and some pilot studies satisfactorily undertaken. The preliminary results of the frequency survey are being examined to assist in the determination of the number and type of radiological departments to be visited and the number of measurements to be made of the different examinatio

Several radiological departments were visited to assess the performance characteristics of the X-ray units and the general protection levels of the rooms. Protection features checked included the design and arrangement of

rooms, safety features and measures taken for the protection of staff. The performance characteristics included the kilovoltage accuracy, with dial kilovoltage and with variation of the current, filtration, focal spot, collimation, beam alignment, exposure time and radiation output. Checks of the latter included consistenay and magnitude and variation with changes in kilovoltage, current and time. Additional tests for fluoroscopic units included resolution and maximum exposure rates. The performance of the equipment checked to date has been good.

Progress Report
1982

Contractor:

Contract no.: BIO-F-373-81-I

Università di Pisa
Dipartimento di Medicina
Lungarno Pacinotti
I-56100 Pisa

Head(s) of research team(s):

Prof. Dr. L. Donato
Istituto di Patologia Medica
Università di Pisa
Via Roma 2
I-56100 Pisa

General subject of the contract:

Radiation protection in medical diagnostic procedures.

List of projects:

1. Reduction of radiation dose to young subjects in medical investigation.
2. Improvement of current methods for assessing radiation exposure in medical diagnostic procedures.
3. Assessment of radiation cost versus diagnostic benefit of radiological and radioisotopic procedures.

The present contract is aimed at

1) the improvement of methods for assessing radiation exposure in various types of medical application;
2) the assessment of the radiation burden vs. diagnostic benefit of various radiological and radioisotopic procedures;
3) the study of ways and means for reduction of the radiation burden both to patients and operator in diagnostic procedures, particularly in the young age.

In 1982 the activity developed has been orientated as follows:

For Line 1: Study of the safety conditions in the installation of compact medical cyclotrons;
For Line 2: further development of computerized radiology in non cardiovascular applications;
For Line 3: definition of a diagnostic protocol for thyroid diseases minimizing patient exposure.

Title of project nr. 1

Reduction of radiation dose to young subjects in medical investigation

Head of project and scientific staff :
L. Donato, G. Valli, M. Franchi, M. Baroni, G. Coppini, G. Perri

The development of computerized radiology, and its application to cardiovascular angiography, have resulted in a very substantial reduction both in patient and operator exposure, and in trauma to patient, without loss of diagnostic accuracy. Apart from the cardiovascular field its application in general diagnostic radiology has the potentiality to reduce drastically the radiation burden in the radiological department, at the expenses of a non negligible change in organization and expertise. The high cost of the equipment appearing on the market, on the other hand, in times in which health costs have become so high, poses serious problems of planning in this field.

The activity developed in 1982 has been devoted to the development and initial application of a low cost "add-on" system for digital radiology which has been installed in parallel in the departments of general radiology and of cardiovascular radiology of this hospital. The equipment has been shown to be easily connected to X-ray generators, and to be of simple use by the hospital radiologist and X ray technician. A first software package includes, in particular, the acquisition procedures of single and serial images both in fluoroscopy and fluorography modes with a special regard to the optimization of the acquisition

timing and spatial resolution requirements. These programs have been shown to held great promise of substantial reduction of both patient exposure and general work up in gastroenterology and urology.

List of publications in 1982

G. Valli, M. Baroni, G. Coppini: "Radiologia numerica riduzione del rischio per il paziente: situazione e prospettive".
La Radioprotezione del paziente - Pisa 1982.
Baroni M., Coppini G., Valli G.: "Digital cardiovascular fluoroscopy: a Low cost system for development of computer analysis techniques".
Computer in Cardiology, IEEE Comput. Sci. Soc., Seattle 1982.

Title of project nr. 2

Improvement of current methods for assessing radiation exposure in medical diagnostic procedures.

Head of project and scientific staff :
R. Guzzardi, C.R. Bellina, U. Bottigli

The production and use of short-lived radioisotopes associated with sophisticated methodologies of tomographic imaging, are of great value for both diagnosis and investigation of patho-physiological mechanisms.

Recently, the requirement of production of short-lived Positron Emitters (C-11, O-15, N-13, F-18) in large quantities and close to the patient, have forced the development of a new generation of cyclotrons characterized by:

a) fixed energy (16 MeV protons and 8 MeV deuterons)
b) compactness
c) easy to use.

A radiochemistry facility is always associated to the system for the production of simple precursors or more complex organic or non-organic labelled compounds for the in vivo use.

The installation of the described system in a usually high populated hospital area, poses serious problems both of safety and efficient organization. Consequently, as a preparatory investigation to the installation of one such cyclotron in this unit, a design study has been undertaken in order to define the specifications and the design

criteria of the installation taking into account the following aspects:

a) localization and shielding of the cyclotron
b) transport system of the irradiated gases
c) shielding and control of the Gas-Processing system and Radiochemistry
d) automatization of the production of labelled compounds using dedicated microprocessors in order to obtain reliable, safe, fast and reproducible conditions.

The project specification are presently in the realization phase and this installation is presently assessed as a model for similar projects planned both in Italy and in the rest of Europe.

List of publications in 1982

P. Salvatori, U. Bottigli, R. Guzzardi, C. Crouzel, D. Comar: "Cyclotrons for Medical Use: Characteristics and Installation Aspects".
J. Nucl. Med. All. Sci., Jan 1982, 21, 41-54.

Title of project nr. 3

Assessment of radiation cost vs. diagnostic benefit of radiological and radioisotopic procedures

Head of the project and scientific staff:
R. Bianchi, G. Mariani, A. Carpi, M.G. Toni, G. Iervasi, A. Nicolini, N. Molea, Mr. G. Lazzerini

A new diagnostic protocol for thyroid disorders has been designed, in order to reduce patient exposure to ionizing radiations.

As a result of this diagnostic protocol application of thyroid scanning and uptake for thyroid disorders has been drastically reduced. In fact, the determination of thyroid uptake (^{131}I or ^{99m}Tc-pertechnetate) in the evaluation of thyroid function (with relation or not with iodine deficiency) has been replaced by the combined determination of the serum levels of total thyroxine and 3,5,3'-triiodothyronine and of the free thyroxine fraction; it is currently under evaluation whether or not the association of the free 3,5,3'-triiodothyronine fraction adds some relevant diagnostic information not otherwise available. The above determinations supply the basic functional data for the differential diagnosis of hypothyroidism (with the association of the TSH serum levels) and hyperthyroidism.

As concerns the morphological evaluation the thyroid gland, once some abnormality is suspected on clinical ground (thyroid nodules, etc.) an echotomographic study usually allows the differential diagnosis between fluid and solid content. At this point of the diagnostic protocol, our experience indicates that the most suitable tool is the

technique of thyroid needle-biopsy; this procedure (which is performed as a simple modification of aspirative cytology) is characterized by an extremely high degree of diagnostic accuracy, as it has been validated in these last two years of application.

The combined evaluation of tumor markers (both thyroid-specific and non specific markers) and of anti-thyroid antibodies also proved useful in increasing the diagnostic accuracy in the area of nodular thyroid diseases.

The experience cumulated so far on this diagnostic protocol is based on a total of 478 new patients seen over a one-year period. All of them had the routine in vitro function tests performed in basal conditions (total serum T_4 and T_3, free T_4); free serum T_3 was measured in 182 patients. Serum TSH was measured in 95 patients with suspected hypothyroidism, and a TRH stimulation test in 23 borderline patients. Thyroid needle-biopsy was performed in 178 patients.

Progress Report
1982

Contractor:

Contract no.: BIO-F-458-81-D

Gesellschaft für Strahlen- und Umweltforschung mbH.
GSF
Ingolstädter Landstrasse 1
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Head(s) of research team(s):

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Dr. G. Drexler
Institut für Strahlenschutz
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

General subject of the contract:

Analysis of occupational exposure and exposure in medical diagnosis.

List of projects:

1. Assessment of occupational exposure.
2. Radiation protection in medical diagnosis.

Title of project nr. 1
Assessment of occupational exposure

Head of project and scientific staff:
Dr. G. Drexler, Dr.G. Burger, Dipl.-Phys. J. David,
Dipl.-Phys. H. Eckerl, Dipl.-Kfm. G. Haid, Dr.D. Regulla

Data of 1981 were evaluated in the same way as the 1980 data in the previous year to enable a direct intercomparison. The main results are:

1) Basic data of the surveyed institutions.
   Only minor changes occurred in the Sasic data of the institutions. The total number of institutions increased by approx 2%. The distribution of the institutions among the different activities (medical, industrial etc.) remained practically unchanged. Investigation of the number of employees per institution showed a small increase in the number of institutions with up to two employees.

2) Basic data of the surveyed personnel.
   The total number of surveyed persons increased by nearly 4%. Much more persons are now recorded with their sex and age as in the last year. The sex-distribution of the surveyed personnel was in 1981 the following (values of 1980 in brackets):
   Male 52,0% (46,0%), Female 42,1% (39,6%) and unknown 5,9% (14,4%).
   The age-distribution remained nearly unchanged, as the distribution of the personnel among the most important professions did.

3) Dosimetrie data
   A summary of the 1981 dosimetrie results is given in the following table. For intercomparison also the 1980 results are given in brackets.

Total number of surveyed persons	57 979	(55 814)	
Number of persons with annual dosis > 0	10 745	(14 067)	
Number of surveyed males	30 159	(25 741)	
Number of surveyed females	24 412	(22 081)	
Total collective dose	3926	(4879)	man-rem
Collective dose with annual doses up to 1,5 rem per person	2434	(3065)	man-rem
Collective dose with annual doses above 1,5 rem per person	1492	(1814)	man-rem
Collective dose males	3212	(3327)	man-rem
Collective dose females	604	(713)	man-rem
Mean annual dose per person	0,068	(0,087)	rem
Mean annual dose per person with D > 0	0,365	(0,347)	rem
Mean annual dose males	0,107	(0,129)	rem
Mean annual dose females	0,025	(0,032)	rem

Comparing the 1981 dosimetric results with the 1980 figures, one can find as the most remarkable difference a decrease of the total collective dose and, consequentely, a decrease in the mean annual dose per person. On the other hand, there is also a decrease in the number of persons with annual doses above the recording level resulting in the fact that the mean annual dose of these persons increased by nearly 5%. A similar trend can be seen in the occupational subgroups, as far as those subgroups can be identified. An identification of occupational subgroups is up to now only possible in the medical field.Only persons with a medical profession are stoved together with a code, which allocates them to a occupational subroup (as f.e. Radiologists, Nuclear medicine and so on). In order to allocate also the occupational exposure within the industrial field to those occupational subgroups, a similar code was developped for industrial workers and will be explored in 1983.

Title of project Nr. 2

Radiation protection in medical diagnosis

Head of project and scientific staff:

Dr. G. Drexler, Dr. G. Burger, Dipl.Phys. W. Panzer,
Dr. D. Regulla, Dr. G. Williams, Dipl.Math. M. Zankl

Assessment of exposure in medical diagnosis

1. By means of a test device developed in 1981, a field study was performed, including 171 dental X-ray facilities. The participants were selected from dentists regularly monitored by the GSF Personal Dosimetry Service. The first findigs regarding field-size and radiation quality of the beam and surface dose to the patient are:

   91% of the X-ray machines were in agreement with the German standards prescribing a maximum field size of 6 cm diameter for circular fields or 6 cm diagonal for rectangular fields at the entrance plane.

   85% turned out to have a filtration of at least 2.0 mm AL for 70 kV or 1.5 mm AL for lower tube voltages.

   The distribution of surface doses is shown in fig.1 where the number of participants (N) is plotted versus the dose values.

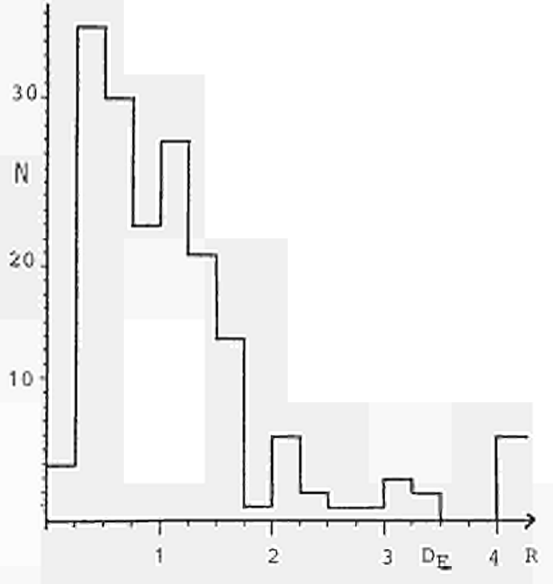

Fig.1: Distribution of surface doses

Based on measurements with a phantom containing a real mandible embedded in perspex the corresponding dose to the dental film was determined (fig.2). The most surprising that these values do not depend significantly on the speed of the different types of film used by the participants.

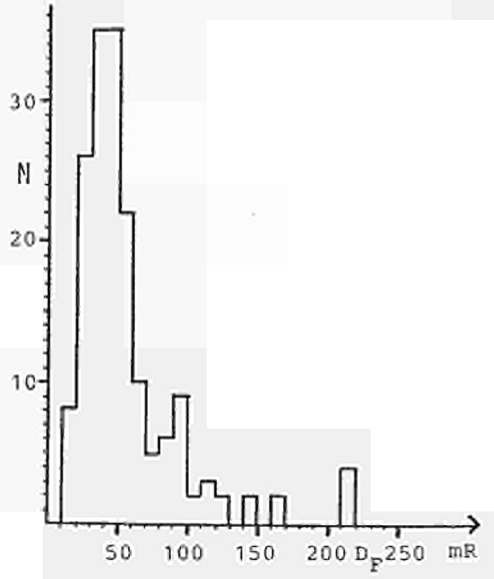

Fig. 2: Distribution of film doses

2. For Mammography a similear system has been developed and tested under laboratory and routineconditions. It consists out of a 4 cm high plastic box (diameter: 10 cm) and two cards containing each of them three thin Ca SO_4: Tm dosemeters. The box is to be filled with water and exposed under respective standard conditions by the participants. The card will be sent back and allow the evaluation of entrance and exit dose. The dosimetric properties of the Ca SO_4: Tm dosimeters (accuracy, reproducibility and energy dependence) proofed to be fully sufficient for such a survey, when they are treated as individual dosemeters with individual calibration and correction factors. In the energy range covered by Mammography they can be considered as practically energy independent.
3. By modifying existing equipment a macro TV Image Analysis System has been installed. In preliminary experiments its ability was checked to measure physical image parameters which are correlated with image quality. For describing the acuity of imaging systems the images of edges and wires were evaluated.

List of publications in 1982

I. Eckerl, H. Thomasz, E., Drexler, G.: Ermittlung der effektiven Äquivalentdosis bei der gynäkologischen Radiumtherapie. "Strahlentherapie" 158, S.422-426,(1982)

Eckerl, H. Drexler, G.: Ergebnisse der Personendosimetrie und deren Interpretation. In: Strahlenschutz für Patienten und Personal in Diagnostik und Therapie mit offenen Radionukliden. Strahlenschutz in Forschung und Praxis, Band XXIV.(Hsg.: Wilhelm Börner et al.) Stuttgart: Georg Thieme Verlag, S.173-176 (1982).

II. Drexler, G., Eckerl, H., Haid, G., Scheibe, D.: Statistische Ergebnisse aus der amtlichen Personendosisüberwachung 1981. GSF-Bericht S-878, ISSN 0721-1694

Progress Report
1982

Contractor:

Medical Research Council
Cyclotron Unit
Hammersmith Hospital
Ducane Road
GB-London W12 OHS

Contract no.: BIO-F-389-81-UK

Head(s) of research team(s):

Dr. T. Jones
Cyclotron Unit, MRC
Hammersmith Hospital
Ducane Road
GB-London W12 OHS

General subject of the contract:

The use of cyclotron produced radioisotopes and tomographic recording procedures for studying regional tissue function in man.

List of projects:

1. Value and radiation load cost of tomographic radioisotopic studies in clinical medecine and research.

Title of project nr 1

Head of project and scientific staff :

T Jones
A A Lammertsma
C G Rhodes
K R Butler

Using short lived cyclotron produced isotopes and positron emission tomography certain parameters of regional tissue functions can be measured in man non-invasively. These procedures are being used as tools for clinical research at the MRC Cyclotron Unit, Hammersmith Hospital. This report itemises the new clinical scientific information that has emerged from this programme in 1982. This places in context the value of these studies in relation to the imposed associated radiation body burdens.

The programme of research is subdivided into the clinical specialities:

Neurology

Measurement of regional cerebral blood flow, oxygen utilisation, glucose utilisation and blood volume have shown:

In patients with carotid artery disease, and who are at risk of having a stroke, the brain blood vessels are dilated. Initial studies on the effect of preventive surgery indicate that this condition can be reversed. In dementia patients, studies have been initiated to observe the actual effect that drugs, prescribed for the treatment of this disease, are actively having on the pathophysiology. Focal physiological disturbances have been observed with the brain of epileptic patients who are continuously fitting. These data give more specific information on the localisation and nature of the underlying brain lesions.

Cancer

In both breast cancers and lymphomas, it has been shown that these neoplasms have a more than adequate blood supply relative to their use of oxygen. This has direct implications as to the rationale that is behind certain therapeutic regimes. Studies have been initiated on how the physiology of tumours and their surrounding normal tissues change during and following treatment.

Cardiology

In angina patients, focal depressions of the heart muscles' function has been observed. These have been seen to occur spontaneously and often without associated pain. This finding draws attention to the fact that the heart tissues in these patients are in a continuous state of disturbance.

Lung

A new technique has been developed to quantitate, tomographically, regional lung ventilation. For this, use is made of Neon-19 which has an 18 second half life. Preliminary data with this method indicates disturbance of the distribution of ventilation in smokers.

Peripheral Vascular Disease

In patients with leg ulcers which persistently refuse to heal, results indicate that the lesions are well supplied with blood flow relative to their use of oxygen. This data substantiates the hypothesis that a diffusion barrier exists that inhibits gas exchange between the blood vessels and the parenchyma tissue.

As this specialised use of radio-isotopes in medicine advances it is becoming clear that its full investigative strength is realised when combinations of tracers are implemented. This places further emphasis on seeking more efficient tomographic devices in the interest of maintaining the absorbed doses to an acceptable level.

List of publications in 1982

1. M Ito, AA Lammertsma, RJS Wise, S Bernardi, RSJ Frackowiak, JD Heather CG McKenzie, DGT Thomas and T Jones. Measurement of regional cerebral blood flow and oxygen utilisation in patients with cerebral tumours using ^{15}O and positron emission tomography: analytical techniques and preliminary results. Neuroradiol. 23(7), 63-74, 1982.
2. AA Lammertsma, JD Heather, T Jones, RSJ Frackowiak and GL Lenzi. A statistical study of the steady state technique for measuring regional cerebral blood flow and oxygen utilisation using oxygen-15. J Comput Assist Tomogr. 6: 566-573, 1982.
3. P Wollmer, CG Rhodes, VW Pike, DJ Silvester, NB Pride, A Sanders, AJ Palmer and RH Liss. Measurement of pulmonary erythromycin concentration in patients with lobar pneumonia by means of positron tomography. The Lancet, II: 1361-1364, 1982.
4. GL Lenzi, RSJ Frackowiak and T Jones. Cerebral oxygen metabolism and blood flow in human cerebral ischemic infarction. J Cereb Blood Flow & Met, 2: 321-335, 1982.

Progress Report

1982

Contractor:

Contract no.: BIO-F-368-81-D

Univ. Erlangen - Nürnberg
Schlossplatz 4
D-8520 Erlangen

Head(s) of research team(s):

Prof. Dr. H. Pauly
Univ. Erlangen - Nürnberg
Krankenhausstrasse 12
D-8520 Erlangen

General subject of the contract:

Effective dose equivalent due to X-ray diagnostic procedures and dose equivalent resulting from natural radiation exposure including the enhanced technological exposure in a typical urban agglomeration.

List of projects:

1. Population exposure and risk assessment in X-ray diagnostic procedures.
2. Population exposure due to natural radiation, including technologically enhanced natural radiation, as a basis for reference risk assessment.

Titel des Projektes Nr.1: Population exposure and risk assessment in X-ray diagnostik procedures.

Leiter des Projektes und wissenschaftliche Mitarbeiter:

Priv.-Doz.Dr.Th.Schmidt
E.Hofmann
Dr.K.Nopitsch
H.David
W.Niederalt
M.Zumpe

Absorbed Energy

Absorbed energy is used as a basis for the assessment of risk in this research work, and therefore one must know the absorbed energy necessary for the various radiodiagnostic examinations. The method used here to directly ascertain the absorbed energy in the phantom is extremely complicated and time - consuming. For this reason, we tried to correlate the absorbed energy with a more easily accessible value, namely the exposure - area product. The results obtained for some standard radiographic examinations are summarized in Table 1

Table 1

Orgam	absorbierte Energie (Messung im Phantom) (mJ)	absorbierte Energie, berechnet aus dem Flächendosisprodukt (mJ)	
		nach Carlson (1)	nach Shrimpton (2)
Schädel seitlich	4	5	6.5
HWS ap	4.4	5	7
BWS ap	7	5	6.5
LWS ap	8.2	6.2	8.2
LWS seitlich	12	13	17
oberes Abdomen (Magen)	4.5	4	5.5
unteres Abdomen	22.5	20	27
Beckenübersicht	14.3	14	19
Unterarm	0.5	2.5	3

Both the method of Carlson (1) and that of Shrimpton (2) were used for the calculation of the absorbed energy from the exposure - area product.

In general, the directly measured values range between those that are calculated according to the method of Carlson and Shrimpton.

As Table 1 shows the method to calculate the absorbed energy from the exposure - area product seems to be unsuitable for radiograms made in the skull and extremities. At present, also uncertainty exist in the calculation of absorbed energy from the exposure - area product in the examinations of the lungs. However, it can be concluded that the exposure - area product is appropriate as an output variable for the calculation of the absorbed energy in examinations of the body trunk. The inaccuracy in determining the absorbed energy is under 30 % in this case.

Variation of the Absorbed Energy

Knowledge of the variance of exposures for the different radiodiagnostic examinations is necessary to estimate the average absorbed energy in the population. For that purpose the exposure - area product of the same examinations was measured in the radiodiagnostic practice of 60 different physicians. As example, Figure 1 shows the results obtained for the exposure of the lungs. The differences are surprisingly high, even if the differing diameters of the patients examined are taken into consideration. Nor can the differences in the dose be explained by varying image quality as studies have shown (e.g. 3, 4).

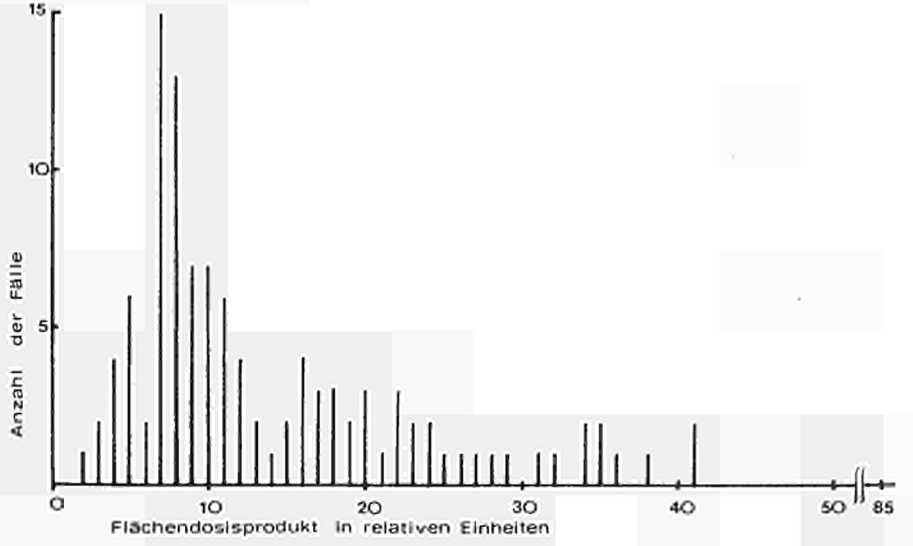

Figure 1

The results of the field study on the variance of X-ray exposure in comparable radiograms coincides in its form of distribution quite well with the results obtained, for example, in the USA (5).

One of the next goals of this research study will be to draw a conclusion on the actual absorbed average energy in the population taking into consideration the variance occuring in practice.

References:

1 Carlson,C.:
Acta Radiol. 1, 433-458 (1963)

2 Shrimpton, P.C., D.C.Jones and B.F.Wall:
Proceedings: Patient Exposure to Radiation in Medical X-Ray Diagnosis. Commission at the European Communities, Munich-Neuherberg, April 27, 1981

3 Lissner,J.:
Röntgenpraxis 35, 36D (1982)

4 Ärztliches Mitteilungsblatt Mittelfranken, Heft 12 (1982), 3 - 6

5 Environmental Protection Agency Washington, EPA 52D/4-76-P12 (1976)

List of publications in 1982

I. Schmidt,Th., H.Pauly, G.Hasl:
Die Integraldosis bei der Computer-Tomographie des Schädels. Fortschr.Röntgenstr. 136, 157-165 (1982)

II. Hofmann,E.:
Die Bestimmung der absorbierten Energie mit Thermolumineszenz-Dosimetern bei Röntgenaufnahmen des Unterarms.
Dissertation, Medizinische Fakultät der Universität Erlangen - Nürnberg, 1982

Bregulla, Ch.:
Der Beitrag der Röntgendurchleuchtung zur Strahlenexposition des Patienten.
Dissertation. Medizinische Fakultät der Universität Erlangen - Nürnberg, 1982

Titel des Projektes Nr.2:

Population exposure due to natural radiation, including technologically enhanced natural radiation, as a basis for reference risk assessment.

Leiter des Projektes und wissenschaftliche Mitarbeiter:

Dr.H.Pfister, H.Wolf, A.Rauh, R.Spitz

Based on the evaluation of population structural data and the built-up of a statistical significant random sample performed in 1981 for the urban agglomeration under study, the field studies were started in April 1982. 348 CaF_2 - TL - dosemeters and 120 radon diffusion chambers were distributed to a first of three portions of the random sample together with precise instructions for wearing the TL - dosemeters and for positioning the radon chambers in the dwellings. After an exposition time of 3 to 4 months and after extensive interviewing of the test persons, according to a questionaire which asked for all relevant exposition data or facts, the CaF_2 - dosemeters and the radon chambers were recollected and the personal doses were determined. Distribution and recollection of the dosemeters and the interviews were performed by the Gesellschaft für Konsum-, Markt- und Absatzforschung e.V., Nürnberg. The evaluation of the polycarbonate track etch detector foils from the radon diffusion chambers developed by URBAN and PIESCH is done at the Health Physics Division, Karlsruhe Nuclear Research Center.

Only 180 out of 348 CaF_2 - dosemeters could be taken for personal dose determination because of loss or incomplete wearing the dosemeters by test persons. The preliminary results are given in the computer histogram below (Figure 1). The total personal doses per unit exposition time are expressed in terms of mean exposure rates in µR/h. No correction is made for the cosmic radiation contribution.

The radon concentration determinations are not yet completed.

In October 1982 the next part of the field studies has been started consisting of the second portion of the random sample.

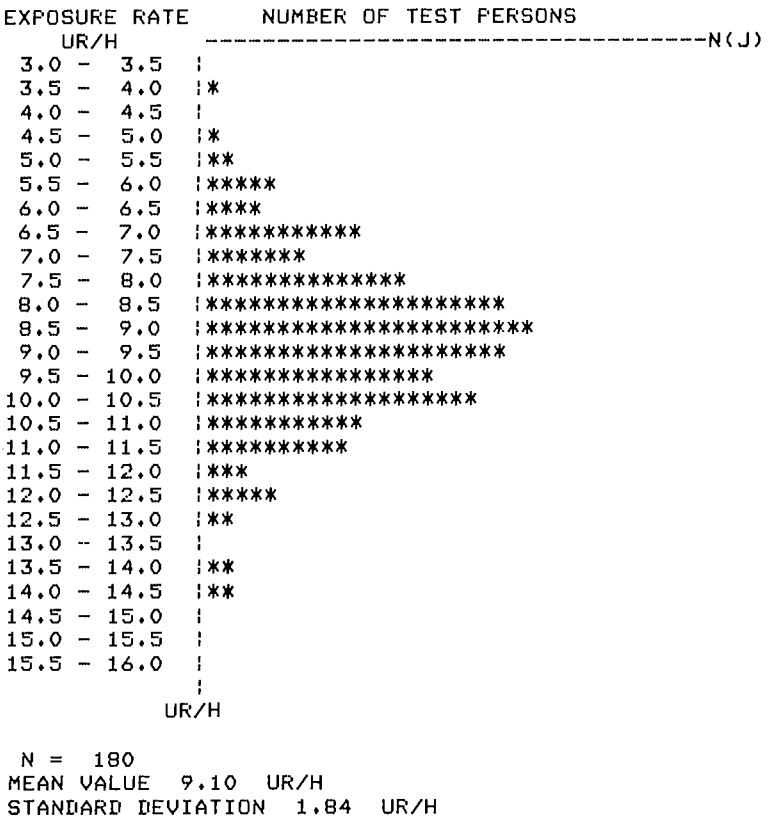

Abb.1 Frequency distribution of the personal doses per unit exposition time expressed as mean exposure rates in µR/h.
First portion of the random sample of the population of the urban agglomeration of Nürnberg - Fürth - Erlangen.
Exposition time: 3 to 4 months between April and September 1982.

Publications in 1982:

I. ----

II. Wolf,H.:

Die natürliche Strahlenexposition der Bevölkerung Erlangens aus Personendosismessungen mit CaF_2-Dosimetern.

Dissertation,
Medizinische Fakultät der Universität Erlangen - Nürnberg, 1982

Progress Report
1982

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

Contract no.: BIO-F-387-80-UK

Head(s) of research team(s):

Dr. J. A. Reissland
Physics Department
NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

General subject of the contract:

Measurement of doses to patients from the medical uses of radiation.

List of projects:

1. Measurement of somatic doses arising from diagnostic X-ray examinations.

Title of project no. 1: Measurement of somatic doses arising from diagnostic X-ray examinations

Head of project and scientific staff: B.F. Wall
P.C. Shrimpton
D.G. Jones
S.F. Barry

The main areas of endeavour this year have been the consolidation of our calculations relating energy imparted and exposure-area product (Rcm^2), the carrying out of a pilot survey of patient measurements at a local hospital and the planning and commencement of a national somatic dose survey.

(1) Calibration of Diamentor in terms of energy imparted

The previously reported calculations of the factors relating the energy imparted to the patient and a Diamentor reading of Rcm^2 for the X-ray beam have been extended to include further conditions of irradiation. Using as before the theoretical X-ray spectral data of Birch et al (1979), calculations indicate that relative to the results for constant generating potential and 17^o target angle, the energy fluence per R may be up to 15% less for a completely unsmoothed full-wave rectified waveform, and may change by -5% to +10% in varying the target angle from 22^o to 10^o. In addition, further Monte Carlo simulations involving smaller elliptical cross-section cylinders than those used originally show that the fractional energy absorption for children may be up to 30% less than that for an adult trunk under similar conditions.

These and further calculations will enable our method for the derivation of energy imparted to cope with the wide range of conditions likely to be encountered during the forthcoming national survey of patient doses.

The results of our investigations into the accuracy of the Diamentor in indicating Rcm^2 have been published recently (Shrimpton and Wall, 1982).

(2) Preliminary patient measurements

Following the development and verification of a technique for deriving energy imparted from a measurement of Rcm^2 during an examination, a pilot survey of 160 patients has been conducted at Princess Margaret Hospital, Swindon. In addition to a Diamentor measurement, sachets containing lithium borate TLD powder were attached to either breast in order to assess mean dose to breast tissue. Details of these measurements, together with the important parameters of technique (kV, projection, size and number of films, etc.) were recorded on a separate form for each patient. Measurements necessary for the interpretation of the patient measurements were also carried out on the X-ray equipment in each of the two rooms monitored. These procedures included the calibration of each Diamentor system after installation and an assessment of total beam filtrations and fidelity of kV settings.

The Table below summarises preliminary results for the four most common of the complex examinations: Intra-Venous Pyelography, barium enema, barium meal and cholecystography/cholangiography.

Examination	No. of Patients	Energy Imparted (mJ)		Mean Breast Dose (mGy)	
		Mean	Range	Mean	Range
IVP	92	184	51 - 536	3.2	0.2 - 36.4
Ba Enema	17	317	205 - 491	1.9	0.3 - 4.4
Ba Meal	18	231	43 - 466	3.1	0.1 - 17.0
Chole.	7	79	23 - 225	0.2	0.02 - 0.3

(3) UK Somatic Dose Survey

The pilot exercise at Swindon has proved valuable in refining techniques and procedures prior to embarking on the main survey to establish somatic dose values representative of radiological practice in the UK. This will involve measurements on a large number of patients at a carefully selected sample of hospitals and concentrates on the ten radiological examinations that have been identified as probably accounting for the bulk of the collective dose to the population. These comprise the four complex examinations referred to above, and in addition the following six simpler examinations: lumbar spine/lumbo-sacral joint, chest, head, abdomen, thoracic spine and pelvis. Complementary to the Diamentor measurements, the use of TLD's has been extended in this survey to allow the estimation of dose to all the major organs of interest. In the case of the four complex examinations, five TLD's are attached to each patient throughout the entire procedure (two on the breasts, two on the back and one on the throat), whilst for the six simple examinations single TLD's are attached to the patient in turn to measure entrance skin dose for each film. Procedures are being developed to allow organ doses to be deduced from these TLD measurements.

Collection of patient data is being conducted in two stages. The first of these involves measurements at four hospitals and is nearing completion. Analysis of these data will provide the basis for selecting a larger representative sample of hospitals for the second stage of patient measurements.

Reference

Birch, R., Marshall, M and Ardran, G.M., 1979. Catalogue of Spectral Data for Diagnostic X-rays, SRS-30 (HPA, 47 Belgrave Square, London SW1X 8QX).

List of publications in 1982

I. An evaluation of the Diamentor transmission ionization chamber in indicating exposure-area product (Rcm^2) during diagnostic radiological examinations.

P.C. Shrimpton and B.F. Wall

Phys. Med. Biol., 27 (6), 871-878, 1982

A possible method for estimating somatic doses from diagnostic radiology and nuclear medicine

B.F. Wall

In Proc. of BIR, Brit. Jour. Radiol., 55, 658, 1982.

II. The assessment of somatic doses from diagnostic radiology.

P.C. Shrimpton, D.G. Jones, B.F. Wall and E.S. Fisher

Research and Development Report 1979-1981, NRPB R & D 4 (In Press).

Progress Report
1982

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB-Oxon OX 11 ORQ

Contract no.: BIO-F-497-82-UK

Head(s) of research team(s):

Dr. J.A. Reissland
Physics Department
NRPB
Chilton, Didcot
GB-Oxon OX 11 ORQ

General subject of the contract:

Assessment of radiation doses to the population of Great Britain from nuclear medicine.

List of projects:

1. Assessment of radiation doses to the population of Great Britain from nuclear medicine.

Title of project no. 1: Assessment of radiation doses to the population of Great Britain from Nuclear Medicine

Head of project and scientific staff: B.F. Wall
G.M. Kendall
S.F. Barry

The strategy to be employed in gathering information on the numbers and types of nuclear medicine procedures undertaken in Great Britain has been discussed with a number of experts and the techniques adopted in other countries have been studied. Assistance from the Hospital Physicists' Association was sought and their Radionuclide Topic Group is actively collaborating in the survey.

Central records are not available in Britain listing all hospitals where radiopharmaceuticals are administered to patients. This information has been obtained by reference to applications to the Department of Health for authorization to administer radioisotopes and by correspondence with hospital physicists who act as Radiological Protection Advisors for hospitals with nuclear medicine facilities. A complete list was compiled of about 320 hospitals including private clinics and Ministry of Defence establishments.

At the end of 1982 a questionnaire was sent to each of these hospitals via the Radiological Protection Advisors asking for information on their nuclear medicine imaging facilities (if any) and on their work-load for the year. The questionnaire listed 93 diagnostic and 6 therapeutic procedures employing radiopharmaceuticals and requested the total number of patients undergoing each procedure in 1982, together with the typical administered activities for adults. The basis on which activities are calculated for children was also requested. Information will be required on the age distribution of patients but it was considered to be so difficult to extract from routine records that it could not reasonably be asked for at this stage of the survey. The favourable replies received to the earlier correspondence with hospital physicists suggest that a very high response rate should be achieved.

A computer program has been written to analyse the returned data. It will list the radiopharmaceutical procedures with the number of patients and the typical activities administered for each hospital. Total frequencies and activities will be calculated and the procedures will be rearranged in order of their frequency of use throughout the country, together with the national average for the typical administered activity.

The quantities to be calculated in assessing the doses and risks to the British population have been carefully considered. It is hoped that the ICRP Task Group, that is presently evaluating the methods for calculating dose equivalents to organs from radiopharmaceuticals, will publish its recommendations in time for us to use them. Suitable risk and weighting factors will be combined with these organ doses to calculate the detriment associated with the major nuclear medicine procedures and the somatic and genetic effective dose equivalents. Age and sex variations in the probability of the realization of somatic and genetic effects will be taken into account in calculating population related risk quantities like the GSD and SSD (somatically significant dose).

List of publications in 1982

I.

II. A survey to estimate the dose to the population from nuclear medicine. B.F. Wall, G.M. Kendall and S.C. Darby. Research and Development Report 1979-1981, NRPB/R & D 4 (In Press).

Progress Report
1982

Contractor:

Contract no.: BIO-F-495-82-I

Centro Informazioni Studi
Esperienze Spa, CISE
Sede Legale
Via Carducci, 14
I-Milano

Head(s) of research team(s):

Dr.ssa. Elsa Bazzano
Sezione Ambiente
CISE
Via Reggio Emilia, 39
I-Segrate (MI)

General subject of the contract:

Assessment of indoor dose from γ emitters and radon daughters in Milano (Italy).

List of projects:

1. Assessment of indoor dose from γ emitters and radon daughers in Milano (Italy).

BIO-F-495-82-I
Assessment of indoor dose from γ emitters and radon daughters in Milano (Italy)

Dr.ssa Elsa Bazzano
Sezione Ambiente
CISE
Via Reggio Emilia, 39
I-Segrate (MI)

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1. Dwellings choice and classification

Total number of dwellings included in the 1982 program for examination of γ exposure is 100; they had been classified according to location in the town, age, building materials of outside wells, building materials of the floors, level of the floor, heating, air exchange, occupancy factor, domestic use of water, etc. We hope that the house classification according theese characteristics will be useful to explain TLD and track etch data that will be recorded.

2. Indoor γ exposure rate

Assessment of indoor γ dose rate had been made with duplicate exposure of dosimetry for two periods of one month eachone. TLD had been calibrated by ENEA Laboratory of Dosimetry of Bologna. There are at present available ultimate results of all 100 dwellings: they are well classified into a strict interval of γ dose rate and they range from 8,7 μR/h to 15,1 μR/h, with a medium value of 12,15 ± 1,00 μR/h.
We grouped the 22 districts of the town in 4 sets to examine the recorded data according their location into the town: we found no relation to exist between γ exposure level and location.
We examinated indoor γ dose according the age of the buildings and we found slightly higher levels in more aged with respect of the more recent houses. The data grouped by kind of building material show the higher levels exposure rate in dwellings with full bricks in their external walls, slightly lower levels with hollow tiles walls and the lower levels in prefabricated houses. We looked for recognizing the influence of the floor level on γ exposure and we found at the first two floors higher γ dose rates than at other

floors.
One can nevertheless observe that the dwellings located at the first floors are often old houses and full bricks walls made, too.

3. Indoor radon and radon daughters long term determination

Radon and radon daughters concentration levels had been determined by CR-39 track etch passive detectors realized and calibrated by ENEA laboratories of the Casaccia, Roma. The passive detectors had been placed into the dwellings for a two months period; radon gas concentration ranges from 1,35 pCi/l to 3,3 pCi/l with a medium value of 2,17±0,42 pCi/l. No correlation seems to be exists between radon gas concentration and γ exposure, the parameter that only seems taken into account of indoor radon gas concentration is the occupancy factor. Experimental exposures of open detectors had been carried out for radon daughters concentration levels determination; measurements are in progress.

4. Radiometric analysis of building materials

Various kind of building materials had been investigated by CISE low level γ ray spectrometry equipment realized with high resolution Ge(Li) detectors. Radioactive concentration data had been obtained with the aid of a minicomputer; the used code (IANUS) had been purposely studied for environmental investigations. The measured average concentration levels of natural radioactive elements recorded in the building materials are: (pCi/kg ± 1 σ)

Material	^{40}K	U	Th
Cement (8 samples)	6590±91	414±4	388±4
Sand (18 samples)	14420±144	448±3	617±4
Bricks (3 samples)	17400±550	1250±21	1400±24

One can observe the low level radioactivity content of cement samples with respect to sand and bricks samples; bricks reveal a typical radioactivity content of clay minerals of Lombardia.

Progress Report
1982

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

Contract no.: BIO-F-424-80-UK

Head(s) of research team(s):

Dr. R. H. Clarke
Nuclear Assessments Dep.
NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

General subject of the contract:

The development of methodologies for evaluating and controlling the risks associated with planned and accidental releases of radioactivity.

List of projects:

1. Methodologies for the estimation of the radiological consequences of accidents and their application.
2. Methodologies for the evaluation and control of exposure from effluents during normal operation.

Title of project no 1: A methodology to evaluate the economic cost of land contamination following accidental releases of radionuclides

Head of project and scientific staff: M J Clark and J Dionian

The total impact of an accidental release to atmosphere of radioactive material from a nuclear reactor would include the health, economic and social consequences of that release. The health consequences due to radiation exposure can be assessed in an established manner, drawing on our accumulated knowledge of dispersion processes, concentration mechanisms pathways to man, radiation dosimetry and radiobiology. The economic and social consequences of an accident, and of any emergency countermeasures taken to reduce radiation exposure, are more difficult to quantify and they are not simply related to the scale of the accident. These other consequences are nonetheless real and should have an influence on pre-emergency planning, and on decision making after an accident. A method has therefore been developed to estimate the cost of applying countermeasures after an accident. The countermeasures would be applied in order to control radiation exposure due to the contamination of land with radionuclides and may involve the imposition of restrictions on the use of, and access to, contaminated areas. The social effects of countermeasures are less amenable to quantification than the economic effects, and have not been considered here; however, given their likely overriding importance, such effects would need to be considered separately in the decision making process.

Principles and Method

In the event of a nuclear accident, the economic consequences will depend to a great extent on what countermeasures are used. If areas of land become contaminated there are four main countermeasures which could reduce radiation exposure but have a significant cost; a) a food ban, b) a temporary evacuation of people from their homes and places of work, c) a more permanent entry ban (interdiction) involving the permanent relocation of people, and d) decontamination. All have the same objective, to reduce radiation exposures after an accident, and decontamination also aims to restore the environment to that existing before the accident.

An important input to decision making should be a comparison of the costs of different countermeasures with the expected reductions in radiation exposure. The economic definition of a 'cost' is not simply something which may have a bill of sale or receipt, it is a benefit forgone. On this basis, the cost of a countermeasure will include the lost opportunity to use contaminated land for agricultural, commercial, industrial and residential purposes. This would be reflected in the contribution made to the nation's Gross Domestic Product in the area prior to the accident, thereby indicating the potential loss of goods and services which could result from the imposition of countermeasures. Other more direct costs, such as the cost of disposal of farm produce and

the provision of transport and emergency services for evacuation, would need to be assessed separately.

Assessments of Costs

Methods for assessing costs have therefore been developed, assuming that the cost of a countermeasure is a function of the contribution made to Gross Domestic Product (GDP) in affected areas prior to an accident. This contribution is assumed lost if the area is evacuated or when a food ban is imposed, and the loss can be compared with the expected reduction in radiation detriment, as shown in Figure 1.

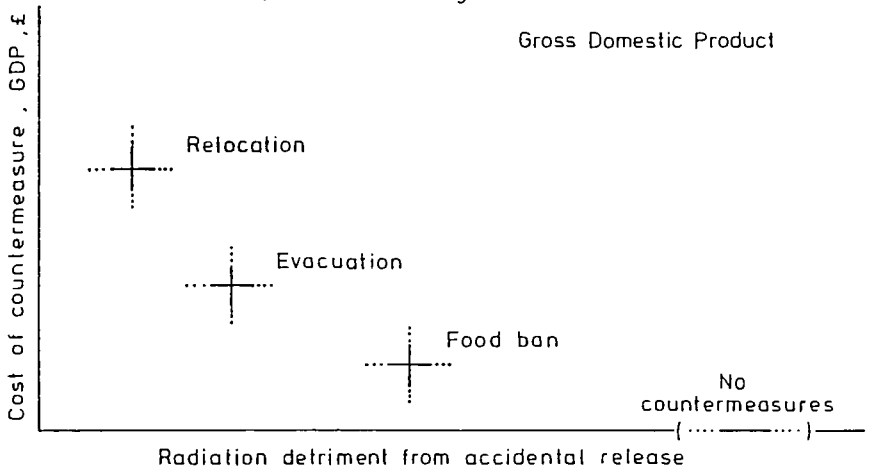

Figure 1 Schematic diagram of the cost-effectiveness of emergency countermeasures

Two methods for assessing the cost of countermeasures have been developed; one using the Second Land Utilisation Survey of Britain to estimate contributions made to GDP per unit area of the country, the other assigning a contribution to GDP per person. The cost can therefore be assessed either from a knowledge of the area of land affected by a contamination incident or from estimates of the number of people requiring evacuation and relocation. The two methods have been extensively described and their results compared in NRPB-M85, the associated publication listed below.

The main application of the methods is likely to be in emergency planning, and the effectiveness of remedial actions after an accident. More generally the methods could be used to estimate the relative impact of countermeasures at different sites, as an input to siting policy decisions, and also as an aid to the design of nuclear installations by providing a basis for comparing the cost of safety design features with the cost of emergency countermeasures.

Associated Publications

M J Clark and J Dionian. ECONO-MARC. A method for assessing the cost of emergency countermeasures after an accident. Chilton, NRPB-M85 (1982). To be issued.

Title of Project No. 2: The relative significance of critical group and collective doses from airborne effluents

Head of project and scientific staff: G.N. Kelly and J.A. Jones

In assessing the significance of discharges of radionuclides to the atmosphere two radiological quantities are of particular importance; the dose to the critical group and the collective dose from the practice. Two aspects, relevant to the evaluation of these two quantities, have been investigated for airborne discharges of radionuclides[1]. First, consideration was given to the distance over which collective dose is accumulated for nuclides with a range of half lives. Second, the influence of controling critical group doses in limiting the magnitude of collective doses was investigated.

In assessing collective dose it is important to ensure that the summation should continue until further addition would not significantly increase the estimate. The distance over which the summation needs to be carried out will vary with, among other parameters, the location of the discharge, the physical and chemical form of the discharged material and radioactive half-life. In general the distance over which the summation is required will increase with increasing radioactive half-life and residence time of the nuclide in the atmosphere. The assessment of collective doses from airborne discharges of radionuclides has, for computational convenience, commonly been evaluated as the sum of two components, that arising from the first pass of the material and that from its global circulation, where applicable. Global circulation is only important for a few radionuclides (e.g. krypton-85, carbon-14, tritium and iodine-129) which have long radioactive half-lives and long residence times in the environment. Adequate models exist for the estimation of the global component of the collective dose and consideration is limited here to the evaluation of the first pass component. The variation in the first pass collective dose with the distance over which it is summed has been evaluated and the results are summarised below. A range of nuclides, with differing characteristics, has been considered and the results are applicable to discharges from locations typical of Western Europe. For those nuclides which are deposited from the atmosphere to the ground summation over about 1000 km will, for most practical purposes, provide an adequate estimate of the first pass collective dose. For noble gases, which are not deposited from the atmosphere, the summation needs to be extended to greater distances to achieve the same precision. Summation over 3000 km will result in about 90% and 70%, respectively, of the total first pass collective dose from xenon-133 and krypton-85 being evaluated. For krypton-85 integration of the first pass dose over such a distance will often be sufficient as the global component will be the major contributor to the total collective dose from the discharge of this nuclide.

Nuclide	Deposition velocity m s^{-1}	Fraction of the first pass collective dose received within a given distance of the release			
		100 km	300 km	1000 km	3000 km
I-129	10^{-2}	0.34	0.64	0.95	1.0
Cs-137	10^{-3}	0.14	0.45	0.84	0.95
I-129	10^{-5}	0.16	0.41	0.84	0.95
Xe-133	0	0.10	0.35	0.73	0.92
Kr-85	0	0.07	0.24	0.50	0.70

In many countries regulatory controls are placed on critical group doses from airborne discharges of radionuclides effectively resulting in a limit on the collective dose, the magnitude of which will vary with the location and radionuclide composition of the discharge and the habits of the critical group. It is useful to examine the relationship between the collective and critical group dose for a range of conditions of practical interest. Such relationships will provide a simple means of estimating the potential collective dose corresponding to a limit placed on critical group doses.

Collective doses have been estimated for the discharge of a number of nuclides at levels where the critical group dose from each is 1/20th of the appropriate dose limit for members of the public, a level sometimes adopted in regulatory practice. Values have been derived for a number of assumed release locations and habits of the critical group; those tabulated below are for a discharge at a height of 15 m from a location typical of conditions in Western Europe, and for a critical group that lives and obtains its diet within 100-500 m of the release. For these conditions the collective dose, corresponding to a critical group dose of 1/20th of the dose limit, is in general about 1 man Sv. Given the range of monetary values typically assigned to unit collective dose there is unlikely to be much scope for further cost effective reduction of doses below these levels for discharge from nuclear installations. Results are given in the main report[1] for other assumed release locations and habits of the critical group.

Nuclide	Collective effective dose equivalent commitment (man-Sv)
Kr-85	1.6
Xe-133	1.4
Cs-137	0.8
Pu-239	0.27

References

1. J.A. Jones and G.N. Kelly. The estimation of collective dose from airborne effluents and its relationship to critical group doses. NRPB, to be published.

Progress Report
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Contractor: Contract no.: BIO-F-480-81-I

Com.Naz.per la Ricerca e
per lo Sviluppo dell'Energia Nucl.
e delle Energie Alternative, ENEA
Viale Regina Margherita 125
I-00198 Roma

Head(s) of research team(s):

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Divisione Scienze Ambientali
CSN della Casaccia, ENEA
C.P. 2400
I-00100 Roma

General subject of the contract:

Assessment of the indoor dose in Italy.

List of projects:

1. Assessment of indoor exposure in Italy.

Title of project nr 1 - "Assessment of indoor dose in Italy"

Head of project and scientific staff :
G. BUSUOLI, G. SCIOCCHETTI,
F. SCACCO, G.F. CLEMENTE,
G. INGRAO

Radon and radon daughter exposure

Special apparatus and devices were developed during 1982, employing passive integrating detectors to be used for the nation-wide survey of the indoor exposure. In particular an apparatus was set up which allows the simultaneous chemical and electrochemical process of several track detectors. Calibration tests for different environmental conditions of a newly developed prototype of a passive environmental dosimeter were also performed.

All the experimental methods employed for the measurement of the radon and radon daughter air concentration and integrated exposure have been tested on the course two intercomparison exercises organized by the CEC during 1982. Measurements by means of both passive and active detectors have been performed in test conditions inside the radon chamber of the NRPB.

A sample of 50 buildings located in the Lazio region and constructed of vulcanic tuff and peperino blocks was surveyed by grab sampling and active measuring devices. In Table 1 are summarized all the experimental data. The survey showed that significant thoron daughter concentrations were present in several buildings, thus indicating the need of further investigation on the role played by thoron on the whole indoor exposure in such area.

External gamma dose

About 200 buildings located in different regions of Italy have been surveyed during 1982 and the related experimental data are reported in Table 2. The measurements in the Milano area have been performed in collaboration with CISE. The results given in Table 2 show that a much larger variability exists in the Roma area as compared to the other considered areas. This may be due to the well known high natural radiation environment which is characteristic of the Lazio region.

Further studies have been performed to test other TL dosimeters to be used for nationwide survey of the indoor external gamma dose. The experimental data have evidentiate the possibility of using the LiF-TL dosemeters on the basis of a measurement time of 2 months.

Tab. 1 - Radon, radon daughter concentrations and equilibrium degrees for a sample of 50 buildings in Alto Lazio

Measured value	Range	Average
Radon conc. (Bq m^{-3})	18 - 518	162
Pot. alfa energy conc. (μ j m^{-3})	0416 - 2.43	0.56
Equil. degree (%)	15 - 90	57

Tab. 2 - Indoor gamma dose in some Italian areas

Measurement Area	Number of considered buildings	Average exposure indoor	Average exposure outdoor	Exposure Range
Roma (Lazio)	20	28.8	-	13.0 - 52.9
Milano (Lombardia)	100	12.3	-	10.3 - 15.1
Bologna (Emilia Romagna)	50	13.1	11.1	11.2 - 16.1
Rimini (Emilia Romagna)	10	10.2	-	8.4 - 12.1
Pesaro (Emilia Romagna)	9	11.5	-	10.1 - 13.5
Area Rural in the Alps.	10	17.6	12.2	14.8 - 20.9

List of publications in 1982

1. Publications in Scientific Journals, Monographs, Proceedings.

G. SCIOCCHETTI, F. SCACCO and G.F. CLEMENTE, 1982 : Evaluation of the radon and radon daughter exposure of the italian population. Proc. of the specialist meeting of the assessment of radon and daughter exposure and related biological effects. Rome, Italy, March 3-8, 1980, RD Press, University of Utah Salt Lake City, Utah 84112.

G. SCIOCCHETTI, G.F. CLEMENTE, G. INGRAO and F. SCACCO : Results of a survey on radioactivity of building materials in Italy. Health Physics (in Press).

Progress Report
1982

Contractor:

Centre de Développement des Etudes
et Applications en Hygiène
et Sécurité, CEDHYS
Avenue Fontcouverte 18
F-84000 Avignon

Contract no.: BIO-F-320-81-F

Head(s) of research team(s):

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F-92260 Fontenay-aux-Roses

General subject of the contract:

Comparative study of the impact of conventional and nuclear industries on the environment and the public from the twofold standpoint of radioactive and chemical releases.

List of projects:

1. Comparative study of the radioactive and chemical impact on the population of atmospheric releases of conventional and nuclear electricity generating industries.

Projet 1 - Etude comparative de l'impact sur la population des rejets d'effluents radioactifs et chimiques des centrales conventionnelles et nucléaires.

Chef de Projet : R.COULON

Participants : J.AIGUEPERSE - F.ANGUENOT - M.ARCHIMBAUD - A.BOUVILLE J.HUGON - J.M.QUINAULT - C.ROUSSEL - S.ROUSSEL

Ce projet s'inscrit dans le cadre d'une étude plus large qui a pour but d'apprécier et de situer de façon objective les risques liés à des activités industrielles nucléaires et non nucléaires, parmi l'ensemble des risques auxquels est soumise la population d'une région donnée, en l'occurrence celle du Sud-Est de la France (Grand Delta du Rhône).

1.LES TERMES SOURCES

Après l'étude des installations entrant dans les filières énergétiques (charbon, fuel, nucléaire) réalisée en 1981, l'intérêt s'est porté en 1982 vers les autres installations industrielles considérées comme sources principales de pollution et de nuisances tant pour les travailleurs que pour les membres du public. Une image de la structure industrielle du sud-est de la France a été commencée en regroupant les établissements industriels de plus de 99 salariés, soit environ 4000 établissements, par activité et taille de l'établissement. Ce travail se poursuivra en 1983.

2.LES REJETS

Pour connaître les quantités de polluants émises par les installations industrielles, trois démarches ont été utilisées :

2.1. Recherche de données dans la littérature générale, démarche qui ne peut être que complémentaire car les valeurs trouvées peuvent ne pas être représentatives de l'installation étudiée.

2.2. Obtention de données auprès des responsables des installations ou des services ministériels. Cette approche a été utilisée car elle est la moins contraignante mais elle se heurte soit à l'absence de certaines valeurs, soit à des problèmes de confidentialité. Elle a été mise en oeuvre pour les rejets des installations du cycle du combustible nucléaire.

2.3. Mesure au niveau des émissions : en 1982, trois campagnes de mesures (1) ont été effectuées.

La première a porté sur une centrale au charbon (lignite) où des prélèvements isocinétiques de poussières ont été effectués dans la cheminée (150 m) de la tranche de 250 MW, cette centrale possédant par ailleurs trois autres tranches de 55 MW (h : 65 m) de conception plus ancienne. Sur les poussières prélevées dans la cheminée ont été mesurés des radionucléides naturels (K-40, U-238, Ra-226, Th-232). Cette campagne a permis de préciser des résultats obtenus en 1981 sur cette même centrale. Par ailleurs, la détermination des quantités de quelques métaux-traces contenus dans le lignite nous a permis d'estimer la valeur de rejets.

La seconde campagne s'est déroulée sur le site d'une centrale au fuel qui comporte deux tranches de 700 MW et une cheminée de 250 m pourvue de dépoussiéreurs de type cyclone. Des prélèvements isocinétiques de SO_2, NO_2, vapeurs organiques et poussières ont été effectués. Ces prélèvements de poussières,dont la gravimétrie et la granulométrie ont été déterminées, ont conduit à des mesures spécifiques d'hydrocarbures polycycliques aromatiques tel que le benzo-a-pyrène, de métaux traces (As, Be, Cd, Cr, Ni, Pb, V) et de radionucléides naturels (K-40, U-238, Ra-226 et Th-232).

La troisième campagne de mesures s'est déroulée sur le site d'une autre centrale au fuel, de conception plus ancienne que la précédente et qui comporte quatre tranches de 250 MW et quatre cheminées de 150 m, toutes dépourvues de dépoussiéreurs. Dans l'une des cheminées ont été répétés les prélèvements de gaz et de poussières de manière identique à ceux effectués dans la cheminée de la précédente centrale. Les mêmes produits chimiques et radioactifs ont été déterminés.

Les rejets calculés à partir des résultats obtenus au cours de ces campagnes sont rassemblés pour quelques polluants dans les tableaux ci-après.

Enfin, la synthèse de l'ensemble de ces résultats est en cours sous des formes permettant d'établir des comparaisons entre les divers types de centrales.

3.LES EXPOSITIONS

a) D'une façon générale, les concentrations dans l'environnement et les expositions ont été évaluées à partir des émissions, ce qui a nécessité :

3.1. l'utilisation de modèles de dispersion atmosphérique du type

Quantité $(kg.(GW)^{-1}$ ou $(k\ Bq(GW)^{-1})$ de polluant rejetés par les centrales thermiques

. Gaz

	Charbon	Fuel	
	250 MW	250 MW	700 MW
SO_2	1.10^4	$1.6\ 10^4$	$3.3\ 10^4$
NO_2	200	-	$3.3\ 10^3$
HF	-	-	<0.1

. Poussières

	Charbon	Fuel	
	250 MW	250 MW	700 MW
Ø<5,5µm	800	)	272
Ø<5,5µm	800	) 383)	116

. Métaux-traces (x 10^{-3})

	Charbon	Fuel	
	250 MW	250 MW	700 MW
As	8	3	2
Be		0,3	0,08
Cd	32	2,5	2
Cr	160	3,5	54
Ni	200	2000	4300
Pb	24		80
V	400	10800	12000

. Vapeurs organiques

	Fuel	
	250 MW	700 MW
Acroleine	0,11	non dosable
Benzène	5.10^{-4}	0.06
Formoldehyde	0.58	0.11
Nitrobenzène	0.19	0.44
Styrène	1.10^{-4}	< 0.28
Toluène	2.10^{-4}	0.25

. Radionucléides naturels

	Charbon	Fuel	
	250 MW	250 MW	700 MW
K-40	224	228	30
Ra-226	640	50	15
U-238	720	10	15
Th-232	86	6	10

panache et gaussien pour évaluer l'exposition locale due à chaque source.

3.2. La mise au point d'un modèle de dispersion atmosphérique multi-sources pour évaluer l'exposition à l'échelle régionale due à l'émission simultanée par plusieurs sources : c'est un modèle de type "bouffées" qui tient compte des transformations chimiques et des dépôts.

3.3. L'utilisation, dans le cas de la campagne de mesure effectuée sur une centrale au fuel, d'une méthode de simulation par injection continue dans la cheminée d'un gaz traceur (SF_6) et le suivi de la concentration résultante dans l'atmosphère par plusieurs détecteurs mobiles (2).

3.4. Le rassemblement des données d'environnement nécessaires, à savoir:

. données sur l'hydrologie (débits, vitesses, largeurs, charges MES), qui en 1982, ont concerné le Rhône.

. données sur l'utilisation des eaux pour l'irrigation et la fourniture d'eau de boisson.

. données sur l'agriculture (cultures et superficies correspondantes, cheptel vif ...)

. données sur la climatologie (répartition des vents en fréquence et direction selon la présence ou non des précipitations, températures, humidités relatives, hauteurs et durées de précipitation).

Les travaux effectués en 1982 ont porté sur la recherche des sources d'information, le choix des données à retenir ainsi que leur forme, la préparation d'un système informatique permettant de les stocker et de les utiliser sous des formes et des localisations différentes (3).

3.5. Le rassemblement des connaissances sur le transfert dans l'environnement des polluants concernés. Une étude particulière a été effectuée sur le comportement de ^{226}Ra en milieu terrestre et aquatique (4).

b) Une autre façon d'évaluer l'exposition a consisté en l'utilisation des mesures des polluants dans l'environnement. En 1982, on a :

. effectué des mesures dans l'environnement, couplées à certaines campagnes de mesure des émissions. Ces mesures ont été effectuées en des points situés sous le panache et ont porté sur la gravimétrie, la granulométrie, les teneurs en SO_2, SO_4 et métaux traces. Des mesures témoins ont également été réalisées aux mêmes points pendant l'arrêt de l'installation.

. commencé à rassembler les mesures existantes, notamment celles qui sont effectuées dans le cadre des réseaux de contrôle et de surveillance.

Ces résultats seront stockés selon la même codification informatique que les données d'environnement.

4.LES NUISANCES

En dehors des travaux indiqués ci-dessus, on a continué, en 1982, à suivre des travaux réalisés par ailleurs qui sont susceptibles de compléter ceux qui sont effectués dans le cadre du présent contrat, notamment :

. un bilan de l'irradiation médicale due aux examens de radiodiagnostic.

. un bilan de l'irradiation naturelle (irradiation externe à l'extérieur et à l'intérieur des habitations, irradiation interne due au radon et ses descendants à l'intérieur des habitations).

5.BILAN DES EFFETS

Nous avons continué de tenir à jour une bibliographie portant sur les principaux aspects de l'étude et plus particulièrement sur les effets sur la santé de l'homme, des différents polluants. Comme nouvelle relation exposition effet on retiendra celle présentée pour le cadmium qui propose 0,03 cancers pour 10^6 personnes exposées quotidiennement pendant un an à 1 ng/m^3 de Cd et ce par la voie de l'inhalation.

6.PERCEPTION DES RISQUES

Une pré-enquête psychosociale a déjà été réalisée. Elle a permis de préciser certaines attitudes de la population et d'affiner le questionnaire qui lui sera proposé dans l'étude finale.

PUBLICATIONS

A. Rapports internes concernant le projet

(1) Etude expérimentale de l'impact d'industries conventionnelles sur l'environnement.
Rapports n° RP/82/278 DPr-SHI - Sept.82 et RP/82/295 - DPr-SHI - Déc.82

(2) Etude du transfert atmosphérique sur le site d'une centrale au fuel
Rapport n° RG/82/245 - DPr-SHI - Déc.82

(3) Bilan des données d'environnement du Grand Delta
Rapport SERE/LRA - Nov.82

(4) Comportement et transfert du Ra 226 dans les milieux aquatiques et terrestres.
Rapport SERE/LRA - Juin 82.

B. Communications présentées au Congrès d'Avignon "sur la comparaison des risques associés aux grandes activités humaines 18-22 octobre 1982.

Ces communications sont le résultat d'études financées ou non par la CEE mais intéressent notre projet.

R.COULON, A.BOUVILLE
Intérêt de la comparaison des risques dans le développement économique et propositions pour une approche au niveau régional : un exemple d'application.

J.P.PAGES, G.MORLAT, E.STEMMELEN
Variété des points de vue et perception des risques.

J.BRENOT, Ph.HUBERT
Analyse critique des relations dose-effet en pollution atmosphérique ambiante.

M.ARCHIMBAUD, J.CHALABREYSSE, C.MARES, G.BOURGINEAU
Comparaison du pouvoir mutagène des aérosols générés par différentes formes de production d'énergie.

C.ROUSSEL, R.BERTRAND, G.GARNIER, Ph.BERARD, M.ARCHIMBAUD
Evaluation des conséquences sur l'environnement des rejets des centrales thermiques.

F.ANGUENOT, J.AIGUEPERSE, A.BOUVILLE, A.DESPRES, R.COULON
Impact radiologique des rejets atmosphériques d'une centrale au charbon.

Progress Report
1982

Contractor:

Centre d'Etudes sur l'Eval.
de la Prot. dans le Domaine
Nucléaire, CEPN
Fondation Curie
F-75005 Paris

Contract no.: BIO-F-444-81-F

Head(s) of research team(s):

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General subject of the contract:

Analysis of occupational exposure and diagnostic medical irradiations.

List of projects:

1. Evaluation of occupational irradiation in pressurized water reactor (PWR) plants.
2. Evaluation of diagnostic medical irradiations.

Title of project nr BIO-F-444-81-F
"Evaluation of occupational irradiation in pressurized water reactor (PWR) plants".

Head of project and scientific staff :
Pierre PAGES - Jacques LOCHARD

The main goal of the study is to provide pertinent data to the assessment of occupational radiation exposures at PWRs. These data could hopefully be used as part of an overall analysis for dose reduction actions to assure that occupational exposures are as low as reasonably achievable.

The basic data collected for this investigation come from French PWRs now in operation (12 units) and consist of systematic records of collective doses incurred during each job associated with normal power plant operation and maintenance. In order to get meaningful interpretation of dosimetric results and to identify possible actions for reducing occupational exposures, complementary data were collected on reactors operation history, operating procedures (chemistry, purification), dose rate at main components and radiation protection management.

The main results are as follows :

1. The largest part of the total annual doses is to be attributed to the planned outage for maintenance and refueling (81 % in average over all units).

2. The total collective dose associated with normal refueling outage is decreasing during the first cycles and then reaches a constant level (Fig. 1) in spite of the evolution of the mean irradiation level of circuits due to deposition of activated corrosion products (85 % of total doses are due to Co 60 and Co 58) (Fig. 2). The observed slope of the outage collective dose is primarily due to the first outage including a general check up (decennal visit) of the reactor components and for the following cycles, it accounts for the benefits of personnal training and experience combined with a levelling off of components activation.

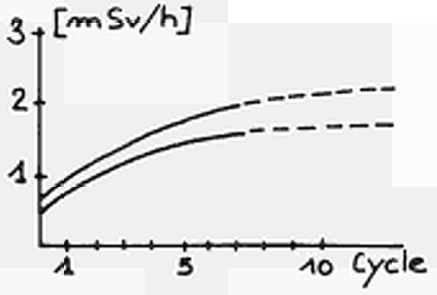

Fig. 1 : Evolution of mean dose rate at primary loop components

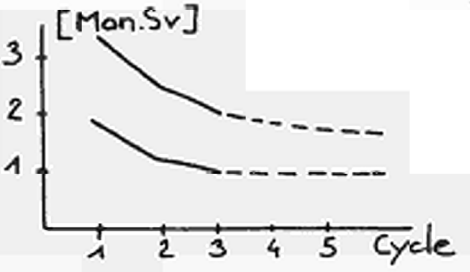

Fig. 2 : Evolution of the range of values of total collective doses for normal refueling outages

3. The analysis of collective doses related to jobs during planned outage for refuelling leads to the following break down (average values).

1.	Reactor vessel opening and closing	10 %
2.	Refueling and core instrumentation	6 %
3.	Steam generator maintenance	20 %
4.	Inspections	3 %
5.	Maintenance on valves	22 %
6.	Cleaning, decontamination, job preparation	25 %
7.	All others	14 %
		100 %

In so far as the French nuclear program is developped on a highly standardized basis for design and operating procedures as well as for radiation protection management, a limited plant-visit survey has been carried out at a few foreign PWRs in order to confort these results. The main conclusion which can be drawn is that collective doses are rather independant from the size of units, but can be sensitive to plant design.

Title of project nr BIO-F-444-81-F
"Evaluation of diagnostic medical irradiation".

Head of project and scientific staff :
F. FAGNANI, C. MACCIA, J.P. THIERRY.

The analysis has been structured through a methodology including two complementary approaches :

- a field survey aiming at a detailed description of medical activity in radiology at the national scale,
- a preliminary experimental dosimetry analysis.

National survey on radiology activity

The survey is relative to a national sample (1/10) of radiological units, representative of the general situation (in diagnostic radiology, public and private sectors, hospital and ambulatory services). The case of dental and mass screening examinations and of CT scanners has been excluded. The survey was organized in two successive phases. A first sample of 476 units was selected corresponding to 1 372 radiological tables. These units were described in terms of : radiological equipment, manpower, general activity and management.

The second phase concerned a stratified sub-sample of 549 tables with a detailed investigation of the activity during a week (in june 1982). The response rate of this second phase was 64 % leading to an effective observation of 354 tables. A total number of almost 13 000 X-rays examinations were observed (half of them in public hospitals, 20 % in private clinics and 30 % in ambulatory services).

The description included :

- caracteristics of patients,
- motivations and results of the examinations,
- physical parameters (mAs, kv, filtration SFD),
- number and size of films (by incidence)
- fluoroscopy times.

Experimental dosimetry study

This study was conceived in order to provide reference values for the 28 types of examinations considered in the field survey. First, a validation analysis was performed to analyse the variability of the quality and X-Ray beam associated with different combinations of equipment (tubes and generators).

The dosimetry itself was conducted on a RANDO phantom, and, reference values of organ doses (lens, thyroïd, lung, gonads, skin) for all possible combinations of films sizes, incidences and anatomic targets were obtained.

The case of red bone marrow was not completed for practical reason and documented through international data. This set of results are combined with data from the national survey to provide a preliminary evaluation of the collective effective dose equivalent and of the genetically significant dose associated with the national, annual activity in diagnostic radiology.

Progress Report
1982

Contractor:

Imperial College of Science
and Technology
GB-London SW7 2AZ

Contract no.: BIO-F-499-82-UK

Head(s) of research team(s):

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Pure & Applied Botany Dept.
Imp. Coll.of Sc.and Techn.
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GB-London SW7 2BX

General subject of the contract:

Developments in methodology for estimating collective and individual detriment arising from accidental releases to the atmosphere.

List of projects:

1. Developments in methodology for estimating collective and individual detriment arising from accidental releases to the atmosphere.

Title of Project Nr BIO-F-499-82-UK

Developments in Methodology for Estimating Collective and Individual Detriment Arising from Accidental Releases to the Atmopshere

Head of project and scientific staff:

Dr A.J.H. Goddard, Dr J.N.B. Bell,
Dr H.M. ApSimon, Miss M.J. Minski,
Mr J. Wilson, Mr N. Mitchell

Existing versions of the MESOS model for dispersal of atmospheric releases of activity over Western Europe allow the direct calculation of exposure and contamination at a radial grid of discrete points centred on a source location. This grid was adequate for collective dose calculations for operational releases, for which average levels of air concentrations and ground contamination vary slowly spatially, and for statistics of individual exposure at any selected points in the grid for short term, unplanned, releases. However it is inadequate for calculating collective doses from unplanned releases, where contaminated air could pass between grid points; in this context it is necessary to consider in more detail the area which lies below the model contamination as it passes across the region of interest.

A method has therefore been devised which uses the trajectories of individual releases, initiated at three hour intervals, to assess the particular areas contaminated by each three hour release, during their successive travel time steps. These areas are related to the grid established by the Association EURATOM-CEA to specify population data and food production data for Community countries. If a fraction of the area of a cell is exposed then it is assumed that the corresponding fraction of the population or food produced in that cell is involved. Thus by examining the different levels of contamination of air or ground in the successive time steps as each three hour release is followed, it is possible not only to calculate the total collective dose resulting from each three hour release but also how this collective dose is built up from individuals exposure to different orders of magnitude of contamination. These methods of extending MESOS to collective dose calculations from unplanned releases are currently being programmed and tested.

The complementary experimental work at the Silwood Park field station has begun during 1982. A study has been made of the principal wheat growing areas of the European Community and the major soil types within these areas have been identified. Currently an intensive literature survey is being made to determine the most important soil characteristics which will influence transfer factors for radionuclides from these soils to wheat. On this basis of this study a limited number of soil types characteristics of wheat growing areas of the Community will be selected for field experiments. A system of 24 outdoor lysimeters, each with a volume of 0.40 m^3, is being renovated for the experimental work. These lysimeters will be filled with the appropriate soils and sown with wheat in Spring 1983, after surface application of radionuclides. Time dependent transfer factors for wheat grain will be determined and the contribution of resuspended soil particles will be estimated by using titanium as a soil tracer. In addition to the principal soil types, a wider range of Community soils has been identified; small scale subsidiary experiments will be conducted to determine transfer factors to wheat from these, using pots alongside the lysimeters, in order to obtain a better understanding of variability arising from differences in soil characteristics within the Community.

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Contractor:

Contract no.: BIO-F-445-81-F

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Head(s) of research team(s):

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General subject of the contract:

Radiodiagnostic irradiation of men and reproduction.

List of projects:

1. Feasability study on the frequency of preconceptual X-ray examinations and their consequences.

RAPPORT PRELIMINAIRE

Titre du projet n° BIO-F-445-81-F :
Etude de faisabilité concernant la fréquence
des examens radiologiques préconceptionnels
et leurs conséquences.

Chef du projet et chercheurs associés :
Denis HEMON, Albert HIRSCH, Philippe LAZAR, Sylvia RICHARDSON

I- INFORMATIONS RECUEILLIES

Les informations recueillies de Mai à Décembre 1981 ont été complétées par 12 mois de recueil en 1982. Le fichier ainsi constitué comprend :

1)- L'ensemble des demandes de remboursement d'actes radiologiques effectués de Mai 1981 à 1982 sur des hommes âgés de 20 à 45 ans au moment de l'acte, soit 431 851 demandes de remboursement.

2)- L'ensemble des demandes de remboursement de frais d'accouchements effectués de Mai 1981 à Décembre 1982 sous un numéro de sécurité sociale "masculin" (soit 11 799 demandes de remboursement).

3)- Les deux types d'informations sont appariés par le numéro de sécurité sociale, pour 2 147 sujets.

II- ANALYSES STATISTIQUES

Celles-ci sont en cours de réalisation elles permettront :

- d'établir la répartition statistique du nombre d'examens radiodiagnostiques effectués sur des hommes en âge de procréer.
- d'établir la fréquence avec laquelle un examen radiodiagnostic effectué sur un homme en âge de procréer est suivi dans les trois mois d'une conception donnant lieu à un accouchement.

- d'établir la fréquence avec laquelle un accouchement correspond à une conception précédée dans les trois mois d'un examen radiodiagnostic de l'époux.

III- RENSEIGNEMENTS CONCERNANT LA NATURE DES EXAMENS RADIOLOGIQUES

En relation avec les contractants français responsable d'une enquête sur la consommation radiologique en France, il a été convenu d'analyser la relation existante entre la codification administrative (code "Z") des demandes de remboursement d'actes radiologiques et leur nature exacte.

Cette étude permettra de préciser la proportion d'actes radiologiques présentant un risque d'exposition au niveau des gonades.

Progress Report
1982

Contractor:

Gesellschaft für Strahlen- und Umweltforschung mbH.
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

Contract no.: BIO-F-422-80-D

Head(s) of research team(s):

Prof. Dr. W. Jacobi
Institut für Strahlenschutz
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

Dr. H.G. Paretzke
Institut für Strahlenschutz
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

General subject of the contract:

Quantification of radiation risks.

List of projects:

1. Quantification of radiation risks.

Title of Project No. 1 : Quantification of Radiation Risks

Head of Project and scientific staff : H.G. Paretzke and W. Jacobi,
P.Messerer, F.Schindel, M.Matthies, K.-J.Godt, P.Jacob, W.Merkle,
E. Wirth.

1. The loss of healthy life span due to the existence of naturally occuring cancers as causes of death might be considered as an useful, additional quantity in risk intercomparisons beyond e.g. the quantity "mortality", which is of major interest presently. In addition to the expectation value of this loss of life span for one particulat person, also the variationsof this value with age, sex, location, secular time periods, etc. might be other quantities worth consideration in risk intercomparisons. As a starting point for this project, we have analysed relevant mortality data from 195o - 198o and for various federal states in Germany.

   As regards cancers as causes of death, in view of the available registries, data for lung cancer, breast cancer, thyroid cancer and leukemia (here the data did not permit a differentiation between the different types) and all cancers could be considered. From these data the respective losses of life span were calculated by removing each time that particular cause of death from the list of competing risks. The results are presently being documented in a report.

2. The work on improvements of dose-conversion factors for incorporated radionuclides concentrated on cancerogenic effects of radiation on the human respiratory tract , e.g. by radon and its daughter products. A comprehensive manuscript on this topic could be finished and submitted for publication in an Encyclopedia by Elsevier Publ.

   For radiocarbon C-14, a nuclide which is of potential importance in the evaluation of future energy strategies, the applicability of a specific activity model has been tested and found useful /1/.

3. The computer program SIRIS for the simulation of epidemiologic data sets has been improved and extended. It now can take secular changes in the mortaility spectra into account as well as the so-called "healthy worker effect". The code has been successfully applied to several performance tests of various statistical data analysis techniques (e.g. SMR, person-year, CMD) with special emphasis on the identification of possible sensitivities, artefacts, etc. of those techniques.

   One result of this work was the identification of the possible reason for some conclusions reached by Mancucso.Stuart and Kneale when analysing the Hanford-worker data set; a detailed report is in preparation. In collaboration with the NRPB, Chilton, a study,using SIRIS, for the actual data of the data set mentioned above has been started; this work is still in progress.

Since chromosome aberrations in human lymphocytes might be considered a sensitive, quantitative biological reaction to radiation exposure, work has been done on the improvement of the statistical regression of calibration data as well as on the derivation of dose values from measured aberration data. It was found that many publications in this field use inappropriate statistical methods for derivation of regression or calibration curves; therefore, in two publications improved methods were proposed and their effect on actual data sets demonstrated./2,3/.

Because of high public interest in this topic, a large set of epidemiological data on malformations of newborn and infants was made available to us for statistical analysis. Particular problems in this analysis were due to the existence of confounding variables and secular trends. The analysis disproved claims of others that this data set clearly showed effects influenced by the coming into operation of a nuclear power station./6/.

4. Irrespectively of the problems of quantification if this quantity, the possible operational usefulness of "healthy life span lost" due to the existence of an additional health risk (e.g. radiation exposure) has been analysed. However, the generally still unsoved and well-known questions related to the lack of axiomatic risk definitions and measures again became apparent,and thus this work is still in progress.

Publications in Scientific Journals,etc.

/1/ E. Wirth : The applicability of the C-14 Activity Model, Health Physics , in press

/2/ W. Merkle: Poisson Goodness-of-Fit Tests for Radiation Induced Chromosome Aberrations, Int. J. Rad. Biol. 4o, 865-692 (1981)

/3/ W..Merkle: Statistical Methods in Regression and Calibration Analysis of Chromosome Aberration Data, Rad.Environ. Biophysics, in press

Internal Reports, etc.

/4/ P. Messerer : Maßzahlen zur vergleichenden Beurteilung der Mortalität : Altersstandardisierung und SMR-Berechnung, Arbeitsbericht ISAR 4/82 , GSF-Institut für Strahlenschutz, 1982

/5/ H.G. Paretzke : Dose-Effect-Time Relationships for Late Somatic Effects, In: J.J. Broerse, G.B. Gerber,Eds. :"Neutron Carcinogenesis", EUR-8o84, p.419-436 (1982)

/6/ H.G. Paretzke, W.Merkle, F. Schindel : Epidemiologische Analyse von Meldungen über Fehlbildungen bei Geborenen in Baden-Württemberg, GSF- Bericht S 87o

Progress Report
1982

Contractor:

Rijksuniversiteit Gent
St. Pietersnieuwstraat 25
B-9000 Gent

Contract no.: BIO-F-496-82-B

Head(s) of research team(s):

Dr. A. Janssens
Laboratorium voor Kernfysica
Proeftuinstraat 86
B-9000 Gent

General subject of the contract:

Study of the radiation protection problem caused by the presence of ^{226}Ra in construction materials.

List of projects:

Study of the radiation protection problem caused by the presence of ^{226}Ra in construction materials.

Title of project nr 1 : Study of the radiation protection problem caused bij the presence of ^{226}Rn in construction materials.

Head of project and scientific staff :
Dr. A. Janssens, Dr. J. Uyttenhove, Dr. R. Jacobs, F. Raes, H. Vanmarcke, E. Cottens.

The passive track-etch radon detection system developed in Karlsruhe was introduced in our institute. The bath and power supply for electrochemical etching have been constructed in our workshop. Five small modules each feed four track-etch foils. Each module has a current indication and a fast electronic overcurrent protection. The modular design also has the advantage of using standard components. Stability and reliability of the modules are excellent.

We measured radon in a number of non-representative houses in order to gain experience with the system. The highest radon concentration was 2 pC/l. We took part in the intercomparison of passive detectors organised by the CEC. Afterwards our system was calibrated with a Pylon source. At the end of the year a preliminary survey over Belgium was started. About hundred dosemeters were distributed over the country in order to obtain a quite representative sample. The exposure time will be one year.

A CaSO4-Dy TLD system is in the process of testing and calibration. This system will be used to measure the indoor gamma radiation background. A Ge(Li) gamma detector is used for the measurement of the specific activities of nuclides in building materials. A number of gypsum samples from different origin have been measured. The Ra-226 activity of phospho-gypsum is typically of the order of 10 pCi/g.

A 1000 l chamber has been installed to carry out the laboratory experiments in our project. Preliminary measurements were performed of the radon exhalation of gypsumboard with the Lucas method and of radon daughter activities with a surface-barrier detector and a multichannel spectrometer. Different aerosol concentrations and size distributions can be generated in this box. Our apparatus for measurement of this size distribution is a mobility aerosol spectrometer (MAS) consisting of a TSI classifier and a

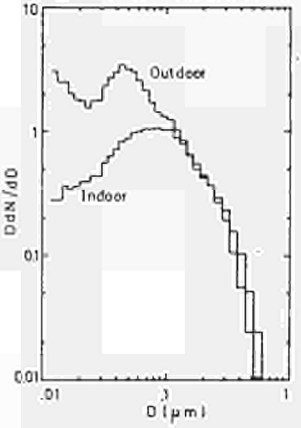


Fig.1. Size distributions

continuous condensation nuclei counter (CNC). Comparative measurements were performed with our MAS-system and with the diffusion battery used in NRPB (K. Cliff and J. Miles). Some tests indicated instabilities or malfunctioning of the latter system, but we had not enough time for a full evaluation of the problem. The operation of our MAS-system is being automated in order to allow future extensive measurements of indoor aerosol size distributions. One such measurement is reproduced as an illustration in Fig. 1.

The future analysis of the laboratory measurements and of the case studies in houses has been prepared by writing the appropriate computer programmes. One programme calculates the attached and unattached radon daughter activities as a function of ventilation, plate-out and attachment rates. A "weighted equivalent activity" has been defined as the sum of the unattached fraction, multiplied with a factor allowing for its higher associated lung cancer risk,and the attached fraction. This quantity has been plotted in Fig.2 for multiplication factors 8 (Jacobi's model) and 36 (James' model cfr ICRP-32). Another computer programme offers a numerical solution to the time-dependent diffusion equation. It was found that disturbancies of the concentration gradient in the wall and thus of the corresponding exhalation rate are restored rather quickly and the effect on the room concentration of such transients is minimal. Such disturbancies can be due to sudden changes in ventilation rate or to a pressure drop across the wall.

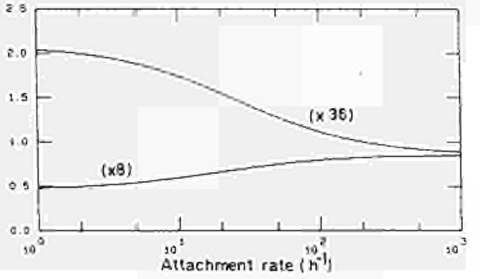


Fig.2: Weighted equivalent activity computed with the indicated factors and normalised to the radon concentration (assuming equilibrium and zero unattached fraction), as a function of attachment rate; plate-out rate is 20/h and ventilation rate 0.2/h.

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

F. Raes and A. Plomp,
Comparison of two condensation nucleous counters TSI model 3020; calibration of the photometric mode.
in Proc. GAeF symposium, Bologna 1982, to be published in J.Aerosol Sc.

II. Short Communications, Theses, Internal Reports, Patents. . .

A. Janssens, F. Raes, J. Uyttenhove, R. Jacobs,
Radon inhalation from indoor air; low activity measurements,
in Annual Report Nucl. Phys. Lab.1981, 81-83

Progress Report
1982

Contractor:

Commissariat à l'Energie Atomique
Rue de la Fédération 29-33
B.P. 510
F-75752 Paris Cédex 15

Contract no.: BIO-F-319-81-F

Head(s) of research team(s):

Dr. J. C. Nenot
Département de Protection
CEA-CEN de Fontenay-aux-Roses
B.P. n° 6
F-92260 Fontenay-aux-Roses

General subject of the contract:

Study of the various components of natural irradiation associated with the dwelling place.

List of projects:

1. Various components of natural irradiation associated with the dwelling place.

ETUDE DES DIFFERENTES COMPOSANTES DE L'IRRADIATION LIEES A L'HABITAT

MADELMONT Claude

- Mesure de l'irradiation externe

Le programme de mesures à l'intérieur et à l'extérieur des habitations s'est poursuivi. Les résultats obtenus durant l'année 1982 sont les suivants :

	DOSES ABSORBEES (mrad.an^{-1})					
	à l'extérieur des habitations (composante terrestre)			à l'intérieur des habitations (ray^{t} cosmique déduit)		
	Mini	Moy	Maxi	Mini	Moy	Maxi
03 Allier	65	106	149	74	115	377
24 Dordogne	23	58	121	22	62	131
37 Indre et Loire	33	56	92	31	69	97
40 Landes	14	33	58	18	50	82
46 Lot	27	55	118	28	61	151
63 Puy-de-Dôme	55	99	166	51	113	298

Les dosimètres de plusieurs autres départements sont également soit en cours d'exposition, soit en cours d'exploitation.

Un incendie du Bâtiment 02 (CEN-FAR) où s'effectuent les lectures des dosimètres a apporté des perturbations notables (perte et retard des résultats de mesures).

- Mesure de radon dans les habitations

En ce qui concerne les mesures de l'énergie potentielle α due aux descendants du radon et du thoron par dosimètres "actifs" deux séries de mesures ont été menées comme prévues en 1981 : l'une dans la région parisienne (19 résultats), l'autre dans le département des Bouches du Rhône (25 résultats).

La valeur moyenne de l'énergie potentielle α totale ainsi mesurée correspond :

- à 48,2 n J.m^{-3} minimale = 2,4 maximale = 206,7 pour la région parisienne
- à 43,8 n J.m^{-3} minimale = 9,4 maximale = 142,8 pour les Bouches-du-Rhône

Ces deux valeurs ne présentent pas de différence statistiquement significative. Il est également intéressant de noter que ces mesures donnent un pourcentage de l'énergie potentielle α totale due aux seuls descendants du radon pouvant varier de 40 à 90. Cette toute première campagne conduit à conclure qu'il faudra :

- multiplier le nombre de points de mesures par département afin d'avoir une meilleure estimation des distributions;
- obtenir des conditions plus homogènes d'exposition dans les maisons;
- passer à la lecture automatique.

L'emploi du détecteur passif (LR 115) dans les habitations a été testé au cours de l'année 1982. La reproductibilité des résultats est correcte, la sensibilité du détecteur est satisfaisante. Il est donc envisagé de l'associer aux mesures effectuées avec le détecteur actif.

Dans le cadre d'une campagne d'intercomparaison organisée par la CCE au NRPB, les détecteurs actifs et passifs ont été exposés dans deux conditions différentes :

- une exposition à faible concentration en radon pendant un temps court (3 heures) : expérience (1)
- une exposition à plus forte concentration pendant un temps plus long (18 heures) : expérience (2).

Les résultats obtenus sont les suivants :

détecteurs actifs (énergie potentielle totale)

expérience (1) = 0,79 μ J.m^{-3}

expérience (2) = 2,4 μ J.m^{-3}

Progress Report
1982

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

Contract no.: BIO-F-498-82-UK

Head(s) of research team(s):

Dr. M.C. O'Riordan
Radiolog. Assessments Dept.
NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

Dr. J.A. Reissland
Physics Department
NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

General subject of the contract:

Determination of dose to the public from natural radiation in houses and assessment of its significance.

List of projects:

1. Determination of dose to the public from natural radiation in houses and assessment of its significance.

Title of project nr B10-F-498-82-UK

Determination of dose to the public from natural radiation in houses and assessment of its significance

Head of project and scientific staff:

M C O'Riordan and J A Reissland. A D Wrixon, K D Cliff, B M R Green, J C H Miles, L Brown, C M H Driscoll

This project is concerned with the determination of the doses to the public from gamma rays and radon decay products in houses. It is part of a Community-wide effort to assess the significance of exposure to natural radiation.

A national survey of indoor gamma ray dose rates and radon gas concentrations is being carried out, the objectives of which are to determine the exposure of the population and how indoor radiation levels vary throughout the country and are influenced by building materials and other factors. This survey is being carried out entirely by post. As a prelude to the survey, a pilot study was carried out to test the efficacy of the passive monitoring devices and the administrative procedures to be used. Regional surveys are also being carried out in areas where exposures might, for geological reasons, be expected to be significantly above average.

For the national survey, approximately 12000 addresses were taken in a random but representative way from the national postal address files. The intention is to survey about 2000 homes and allowance has to be made for refusal by householders to participate in the survey. The national survey started in June 1982 and addresses are being contacted at such a rate that 47 homes are being issued with monitoring devices every fortnight. Each home will be monitored for one year with track etch devices (CR-39 plastic), the devices being replaced after six months; thermoluminescent devices (lithium fluoride chips) will only remain for six months. No measurement results are yet available, but the status of the survey on 1 January 1983 was as follows: 1824 addresses contacted; 50% positive responses from those for which the initial contact programme has been completed; 658 homes issued with monitoring devices. A questionnaire on the dwellings and their management which is to be issued participants is currently being finalised and work is underway to develop procedures for analysing the results and other data.

The houses in the pilot study were all within 40 km of Chilton, the Board headquarters near Oxford. Regional surveys have been carried out in Cornwall and Devon and in part of the Swansea valley in South Wales. The provisional results from these studies are given in the table. Detailed analysis of the results of the pilot study is continuing and a comparison of the passive results given in the table with the results of active measurements in 20 of the Chilton houses is being made. Measurement of the long term average concentrations of radon in the houses in the regional surveys is being made with track etch devices.

SOME PROVISIONAL RESULTS OF REGIONAL SURVEYS IN DWELLINGS

Region	Number of houses	Geology or site	Room in dwelling	Radon concentration		Radon decay product concentration at one air change per hour		Gamma-ray dose rate	
				Geometric mean, $Bq\ m^{-3}$	Geometric standard deviation	Geometric mean, mWL	Geometric standard deviation	Geometric mean, $\mu Gy\ h^{-1}$ in air	Geometric standard deviation
Chilton	138	Chalk/clay	Living	32	9.8			0.029	2.7
			Bedroom	22	10			0.032	2.2
Cornwall	304	Granite	Ground floor			6.8	5.2	0.094	1.4
Devon	143	Granite	Ground floor			3.5	6.5	0.090	1.5
Swansea	8	Slag from copper smelter	Ground floor			0.5	4.2	0.051	1.7

List of publications in 1982

I. Publications in Scientific Journals, Monographs, Proceedings.

Some aspects of human exposure to ^{222}Rn decay products. M C O'Riordan, A C James, K Brown. Radiation Protection Dosimetry 3, 75-82 (1982).

Radon daughter exposure in the UK. K D Cliff, A D Wrixon, B M R Green, J C H Miles. Accepted by Health Physics journal.

The control of exposure to natural radiation. M C O'Riordan, G A M Webb. In: Radiological Protection - Advances in Theory and Practice, Inverness, Scotland, 6-11 June 1982, pp 275-280. The Society of Radiological Protection.

II. Short Communications, Theses, Internal Reports, Patents...

A range of technical and planning memoranda were prepared which will eventually be subsumed in open publications.

Progress Report
1982

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

Contract no.: BIO-F-335-81-UK

Head(s) of research team(s):

Dr. J. A. Reissland
Physics Department
NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

General subject of the contract:

Environmental factors influencing the doses from inhaled radon daughters.

List of projects:

1. Experimental and theoretical assessment of aerosol size distribution in different environments.

Contract No. BIO-F-335-81-UK

Title of Project: Experimental and theoretical assessment of aerosol size distributions in different environments

Head of Project and scientific staff: Mr. K.D. Cliff
J.C.H. Miles

Measurement of aerosol size distributions in the air of offices abutting streets carrying heavy vehicular traffic have been carried out in London. A limited number of measurements at the kerb side of these streets have also been carried out. These measurements were made with the ten stage screen diffusion battery with a dwell time of 25 seconds at each stage giving a total measurement period of 4.5 minutes. A continuous flow condensation nucleus counter was used to determine the condensation nucleus concentration at each sampling port of the diffusion battery. The use of the diffusion battery to determine the size distribution of the sampled aerosol cloud assumes the aerosol cloud to be stable over the period of measurement in both size distribution and in total aerosol concentration. This is not often the case within buildings and rarely the case in the open air. In buildings the perturbations of the aerosol cloud during short time intervals are small and typical aerosol size distributions can be obtained by taking the average of several consecutive measurement runs. The conditions are much more severe in the open air where gusting winds cause rapid variation in the aerosol conditions at the entry to the diffusion battery. This problem has been mitigated by incorporating a 25ℓ aerosol buffer reservoir in series with the diffusion battery. The mean residence time in the reservoir is 6.25 min. and some small particles are lost by coagulation. These losses can be accounted for by calibrating the entire measurement system using a series of monodisperse aerosols.

To ascertain the degree of agreement between the diffusion battery and the electrostatic aerosol classifier used by the Laboratorium Voor Kernfysica, (Ryksuniversiteit-Gent) to measure the size distribution of submicron aerosols, the diffusion battery and associated equipment were taken to Gent and simultaneous measurements carried out with both sets of equipment sampling the same contained aerosol cloud. The agreement was

poor: the electrostatic classifier produced the expected log-normal distribution of aerosol sizes but the distribution determined by the diffusion battery was markedly skew compared with the log-normal distribution. However, the modal size obtained by the two instruments were in reasonable agreement.

Tests of the condensation nucleus counter showed a large discrepancy between the photometric and count modes. This has been rectified by a complete re-calibration of the instrument carried out by the UK agents of the manufacturer.

On occasions, results are obtained with the diffusion battery which cannot be explained by diffusion theory, namely that a higher aerosol concentration is measured after the air has been drawn through the first screen than is observed when sampling the inlet air directly. It has been suggested that large particles (greater than 1.0 μm) might be entrained in the main air flow through the battery and bypass the sampling port for the inlet air hence being recorded only at later stages. To test this hypothesis, an impactor stage is being constructed to precede the diffusion battery and ensure the removal of large particles.

Typical size distributions obtained in laboratory air late in the evening when the aerosol is reasonably stable are bimodal with modal values at 0.015 μm and 0.14 μm. The smaller mode has a peak height of approximately a third that of the major mode. It is felt that for reliable use the diffusion battery requires frequent calibration using a range of monodisperse aerosols. These difficulties have been relieved by the acquisition of an electrostatic aerosol classifier that can be used to generate monodisperse aerosols for the calibration of the diffusion battery.

Progress Report
1982

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

Contract no.: BIO-F-337-80-UK

Head(s) of research team(s):

Dr. J. A. Reissland
Physics Department
NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

General subject of the contract:

Quantification of risks associated with ionizing radiation using data from human studies.

List of projects:

1. Assessment of the findings which emerge from radiation epidemiology.
2. Investigation of relative, absolute and multifactorial risk models.
3. Comparative risk studies of radiation and other industrial factors.

Contract No. BIO-F-337-80-UK

Project No. 1 Quantification of risks associated with ionising radiation using data from human studies

Title of Project Assessment of the findings which emerge from radiation epidemiology

Head of Project and scientific staff: Dr. J.A. Reissland
Dr. G.M. Kendall
Dr. S.C. Darby

The detection of a small number of radiation induced cancers against a large background of cancers which occur for other reasons is very difficult. A discussion of methodological problems and a critical review of the literature has recently been prepared and published (J.A. Reissland, Rad. Prot. Dosimetry 2 pp. 199-207, 1982).

A basic methodology has been proposed and applied to data for workers at the Hanford nuclear establishment in the USA (Darby and Reissland, J.R. Statist. Soc. 144 (3) 1981). This analysis covered data up to 1974. New data have been received going through to 1978. These extended data are being checked and prepared for analysis.

The methodology for analysing epidemiological data is based on the concept of "person years at risk" (PYAR). PYAR are the total number of years which members of the study population contribute to the survey. The total PYAR are stratified into sub-groups which contain only contributions from comparable individuals. This allows confounding factors such as age, sex and length of employment to be taken into account. A general computer program called ARFAR (At Risk For Any Reason) has been written to assist with the calculation of PYAR. At present ARFAR sub-divides according to age, sex, calendar year, years of employment and dose received. This last item distinguishes ARFAR from other generalised person years at risk programs. It is expected that ARFAR will be of wide interest to scientific workers so considerable attention is being given to the user interface.

The input required by ARFAR is of two types. Firstly, there are the data needed to determine the sub-divisions of the person-years at risk (PYAR). Secondly, there are personal data for each individual. The program divides the total PYAR according to four factors: age, calendar year, duration of exposure and dose. The data required are the number of groups for each factor and their limiting values. For instance to split age into 5 year age groups from 20 to 40 the number of groups (4) and the limiting values (20, 25, 30, 35 and 40) are required. At present dates are required in the form day, month and year. Four dates are required for each individual: birth, entry to survey, exit from survey and death. Also required are the number of dose records, the date at which each dose record ends and the dose itself.

Output is in the form of a series of tables containing the number of PYAR. Each table contains all age and calendar year groups. Successive tables are for different duration of exposure groups and different dose groups.

There are several facilities which have yet to be included and which would be very useful. Minor adjustments are needed to allow more flexibility in input and output. For example, catering for dates given by month and year or year only, printing out suitable headings for the tables and allowing the printing of tables where the PYAR have been summed over several groups within a factor or factors. More demanding facilities that it is hoped to include are an extension to more than 4 factors, a facility for using mortality rates to give expected deaths and to allow a function of dose to be

defined. This last facility would not only include some function of the dose received but also allow a "latent period" to be considered by a temporal shift of the dose data.

List of publications

J.A. Reissland, "Epidemiological methods of assessing risks from low level occupational exposure to ionising radiation". Rad. Prot. Dosimetry 2 pp. 199 - 208 (1982).

G.M. Kendall, S.C. Darby and E. Greenslade "Patterns of dose incurred by workers on the NRPB's Dose Record Keeping Service" I Annual Doses" J. SRP 2 (3) pp. 20-25.

Internal Report

G.M. Kendall. The relationship between annual and lifetime annual and lifetime dose quantities for members of a stable population.

Project 2 — Quantification of risks associated with ionizing radiation using data from human studies

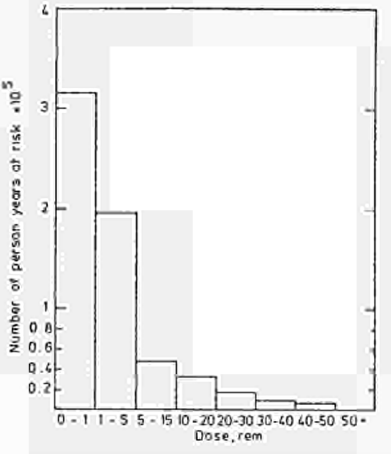
Title of Project: Investigation of relative, absolute and multifactorial risk models

Head of Project and scientific staff:

Dr. J.A. Reissland
Dr. S.C. Darby
Dr. G.M. Kendall
Mr. S.F. Barry
Dr. P.J. Dimbylow

There is, as yet, no theoretical basis for the selection of a particular model to describe the induction of cancer by low doses of radiation at low dose rates. Epidemiological investigations using real data are often limited to the qualitative question "is a radiation-associated effect detectable". Where quantitative estimates are made these are generally within the framework of a specific model. It is not yet possible to use human data to distinguish between possible dose response models. It is likely that a wide variety of approaches to this problem will be necessary (for example, animal experiments and mathematical modelling). Nevertheless, it is generally accepted that the complexity of the issues involved make information derived from human data most important.

In order to help develop techniques for such analyses a joint project between NRPB and GSF, Neuherberg, is underway. The aim is to test analytical techniques using simulated epidemiological data containing a number of "radiation induced cancers" derived from a selected dose-response function. For the exercise to be worthwhile, it is important for the data to be as realistic as possible. The personal details and dose histories are based on real data, though names and other personal identifiers have been removed. Causes of death are assigned by a computer program SIRIS developed by GSF. The analysis of the simulated datafile will be carried out using ARFAR, the person-years at risk computer program. Progress so far covers the agreement of an outline protocol, data transfer in both directions and the first stages of data analysis. The bar chart shows the distribution of person years at risk over dose groups.



Project 3 — Quantification of risks associated with ionizing radiation using data from human studies

Title of Project — Comparative Risk studies of radiation and other risk factors

Head of Project and scientific staff — Dr. J.A. Reissland
Dr. G.M. Kendall
Mrs. J.R. Greenhalgh

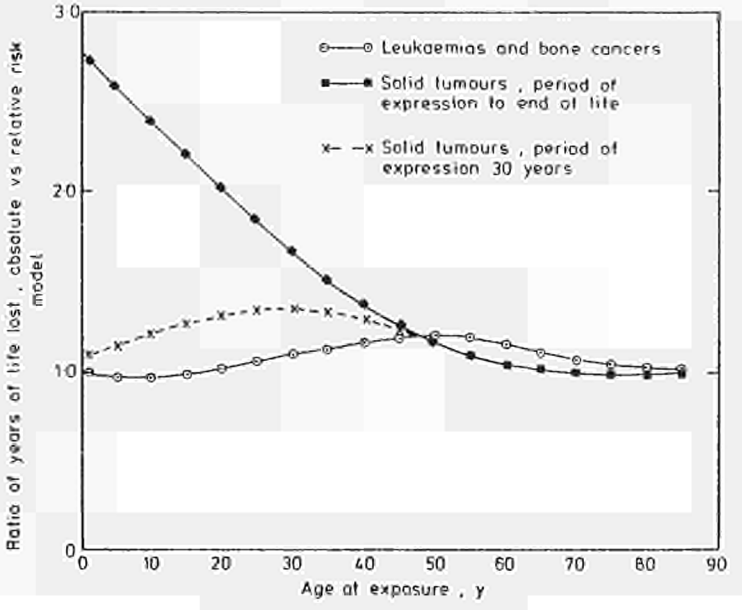
There is a well-recognised problem in deciding the way in which different kinds of detriment should be combined to give an overall picture of the harm arising from any given hazard or practice. In radiation protection it is common to distinguish genetic effects and early and late somatic effects. In most common situations, doses are low and early somatic (non-stochastic) effects do not arise. However, the position is still complex since the population consists of sub groups of different age, sex and level of exposure.

The genetically significant dose (GSD) is a quantity which can be used to summarise the genetic detriment to a population from any pattern of doses. It has been shown that in a population with stable birth and death rates, but an arbitrary pattern of child bearing, that a population genetically significant dose can be defined which is simply related to the ordinary per caput genetically significant dose. This relationship is useful in estimating genetic risk in circumstances in which demographic data is not well known.

Even when attention can be concentrated on fatal cancers as the most important late somatic effect there is no single statistic which completely represents the consequence of population exposures. Nevertheless, such summarising statistics are important tools in understanding and comparing different sources of risk. The total number of deaths caused by an agent is a simple and well-understood example. The number of "years of life lost" is another statistic which has been suggested (notably in ICRP Publication 27, though the basic concept is much older, M. Dempsey "Decline in Tuberculosis", American Review of Tuberculosis 86 ppl57-164, 1947). The methodology suggested in ICRP 27 included some approximations - for example, the use of average values for loss of life from induced cancers. Where sufficient data are available life table techniques allow more accurate values to be used. A worked example of this extended methodology has been given (J.A. Reissland, G.M. Kendall, J.R. Greenhalgh "Quantification of the health hazards associated with different energy sources" in Proceedings of the conference on Health Impacts of Different Sources of Energy, IAEA, Vienna 1982).

The recent report by the BEIR Committee (National Academy of Sciences, Washington, 1980) discusses dose response models which are more general than the generally assumed linear no threshold relationship. Some consideration has been given to the implication of these suggestions. It would seem that the patterns of dose normally incurred by workers do not extend into a range in which non-linear components of a more complex dose

response curve would become important (G.M. Kendall, S.C. Darby, E. Greenslade, J. SRP 2 (3) pp 20-25, 1982). The same would, of course, be true of doses incurred by members of the public. On the other hand, the choice of absolute or relative risk model may affect estimates of years of life lost even where total induced fatalities remain the same. This is because in a relative risk model induced deaths tend to occur later and, other things being equal, lead to fewer years of life being lost. The BEIR Committee describe absolute and relative risk projection models and comment that total induced deaths will be the same once follow up is complete. Nevertheless, the "years of life lost" differ. This is demonstrated in the figure which shows the ratio of years of life lost using BEIR's relative and absolute risk models. While the very large differences for solid tumours in young people are probably a consequence of unrealistic features of the model, it is clear that differences of up to 20% commonly arise.



List of publications

J.A. Reissland, "Epidemiological methods of assessing risks from low level occupational exposure to ionising radiation". Rad. Prot. Dosimetry 2 pp. 199 - 208 (1982).

G.M. Kendall, S.C. Darby and E. Greenslade "Patterns of dose incurred by workers on the NRPB's Dose Record Keeping Service" I Annual Doses" J. SRP 2 (3) pp. 20-25.

Internal Report

G.M. Kendall, The relationship between annual and lifetime annual and lifetime dose quantities for members of a stable population.

Progress Report
1982

Contractor:

Contract no.: BIO-F-500-82-UK

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

Head(s) of research team(s):

Dr. J.A. Reissland
Physics Department
NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

General subject of the contract:

Intercomparison of active and passive measuring devices for radon, thoron and daughter products.

List of projects:

1. Intercomparison of active and passive measuring devices for radon, thoron and daughter products.

Contract No. BIO-F-500-82-UK

Title of Project: Intercomparison of active and passive measuring devices for radon, thoron and daughter products

Head of Project and scientific staff: Mr. K.D. Cliff
Mr.J.C.H. Miles

Intercomparisons of both passive and active techniques of measuring radon and radon decay products have been carried out. Ten centres using fourteen types of dosemeters participated in the intercomparison of passive dosemeters, and eleven centres using eighteen sets of equipment participated in the intercomparison of active measurement techniques. Most of the exposures were carried out in a 43 m^3 steel room at NRPB Chilton.

Closed passive dosemeters were exposed to 190 kBq $m^{-3}h$ and 45 kBq $m^{-3}h$ and a linearity check from 147 to 2720 kBq $m^{-3}h$. The dosemeters showed generally good agreement and linearity.

Open dosemeters were exposed in four runs; 190 kBq $m^{-3}h$ with equilibrium factor F = 0.97, 143 kBq $m^{-3}h$ with F = 0.11, 182 kBq $m^{-3}h$ with F = 0.32, and exposure to approximately 6 WLH during three months in an office. The agreement between the centres was less good in this case.

For the intercomparison of active measurement techniques two runs were carried out; one with a ventilation rate of 1.5 air changes per hour, in which the radon concentration declined to about 80 Bq m^{-3} by the end of the run, and one with virtually zero ventilation rate and a radon concentration of about 800 Bq $m^{-3}h$. The first run is difficult to interpret because of the changing radon concentration.

The results of measurements of radon gas made during the second run cover a range of 718-940 Bq m^{-3} with a mean of 836 Bq m^{-3}, and the results of measurements of radon decay products made during the second run cover a range of 104-173 mWL with a mean of 128 mWL.

A full report has been prepared and will be issued by the Commission of the European Communities.

Progress Report
1982

Contractor:

National Radiological
Protection Board, NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

Contract no.: BIO-F-501-82-UK

Head(s) of research team(s):

Dr. J.A. Reissland
Physics Department
NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

General subject of the contract:

Intercomparison of environmental thermo luminescent dosemeters.

List of projects:

1. Intercomparison of environmental thermo luminescent dosemeters.

Intercomparison of environmental thermoluminescent dosemeters

Contract No. BIO-F-501-82-UK

Head of Project and Scientific Staff: Dr. J.A. Reissland
Dr. A.F. McKinlay

The first stage of the exchange has commenced as per the agreed protocol by the NRPB distributing composite measurement packages containing dosemeters from each of the 6 participating groups. These are now at all the measurement sites. Calibration and cosmic ray exposure of dosemeters will be carried out by NRPB. The measurement period is due to end mid-February when the dosemeters will be returned to NRPB for redistribution to the originating laboratories.

Progress Report
1982

Contractor:

Deutsches Krebsforschungs-
zentrum
Institut für Nuklearmedizin
Im Neuenheimer Feld 280
D-6900 Heidelberg 1

Contract no.: BIO-F-369-81-D

Head(s) of research team(s):

Prof. Dr. med. K. E. Scheer
Institut für Nuklearmedizin
DKFZ
Im Neuenheimer Feld 280
D-6900 Heidelberg 1

General subject of the contract:

Research Project "Thorotrast" : Follow-up study.

List of projects:

1. Investigations to evaluate the long-term effects caused by artificial radiation in man (Thorotrast patients) - Thorotrast follow-up study.

Head of the Project:

Prof.Dr. K.E. Scheer (Contractual Partner)
Prof.Dr. W.J. Lorenz (Assistant Head of Project)
Prof.Dr. G. van Kaick (Coordinator)

Scientific Collaborators of the Institute of Nuclear Medicine:
Dr. H. Lührs, Dr. M. Schweigler

Statistical Evaluation:
Prof.Dr. H. Immich, Dr. H. Wesch

During 1982 we examined 133 patients (84 patients of the Thotrotrast group and 49 patients of the control group) with the biophysical and diagnostic methods described in the previous reports. We detected primary liver tumors in 12 Thorotrast patients. The tumor could be surgically removed in 2 patients. Transplantaton of the liver was carried out in one patient. Two other neoplastic lesions were found in the retroperitoneal space (malignant histiocytoma) and in the kidney (histological confirmation not yet available). Three Thorotrast patients suffered from severe liver cirrhosis.
In one patient of the control group by echography and CT a benigne hemangioma of the liver could be diagnosed.

The causes of death were claryfied of 35 Thorotrast patients, who died in the past year. In 22 patients death was caused by primary liver tumors (9 hemangiosarcomas; others carcinomas) and in 2 patients by liver cirrhosis. Tumors of other organs as cause of death were: bronchogenic carcinoma (2), renal carcinoma (1), pancreatic carcinoma (1),malignant histiocytoma (1). A new case of a histologically confirmed bone sarcoma was registered in the Thorotrast group; the patient was succesfully treated by operation and is still alive.In the control group one patient died by metastases of a melanoma.

Fig. 1 demonstrates the cumulative incidence of liver tumors and leukaemias in the examined and non-examined group. The mean volume of 25 ml Thorotrast corresponds to a tissue dose rate in the liver of 25 rad/y and in the red bone marrow of 9 rad/y. The relationship of cumulative leukaemias to cumulative liver tumors is about 1 : 10.

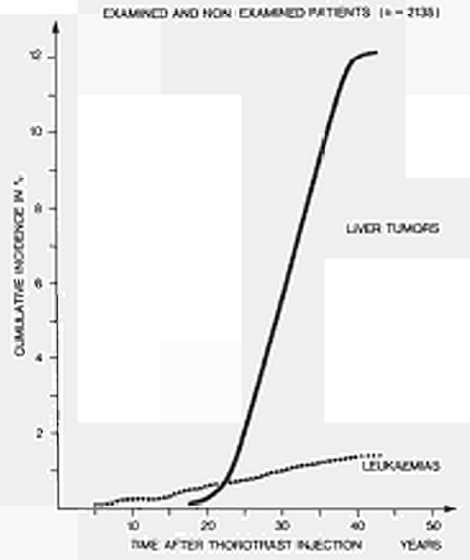


Fig.1 Cumulative incidence of liver tumors and leukaemias in examined patients.

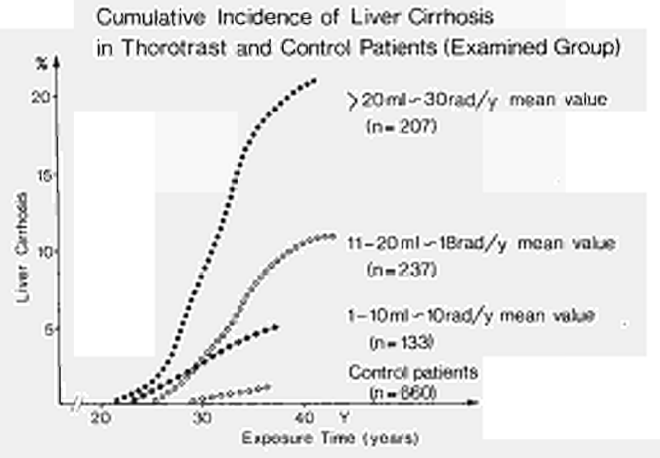


Fig.2 Cumulative incidence of liver cirrhosis in examined Thorotrast patients with different liver dose rates and in patients of the control group.

In Fig. 2 a dose-effect relationship could be proved regarding liver cirrhosis of the examined Thorotrast patient, who have died. The control group is presented for comparison.

Finally it should be mentioned that an important task in the last year was to finish the checking of the patients data stored at the data bank of our institute.

List of publications

G. van Kaick, H. Muth, A. Kaul, H. Immich, D. Liebermann, D. Lorenz, W. J. Lorenz, H. Lührs, K. E. Scheer, G. Wagner, K. Wegener, H. Wesch
Thorotrast Exposures -
Results of the German Thorotrast Study
Presented at the international conference an radiation carcinogenesis - epidemiology and biological significance - National Cancer Institute,
Bethesda, Maryland, May 24 - 26, 1982
(The presentation will be published 1983 by Pergamon Press)

Progress Report
1982

Contractor: Contract no.: BIO-F-423-81-F

Commissariat à l'Energie Atomique
Rue de la Fédération 31-33
F-75752 Paris Cédex 15

Head(s) of research team(s):

Dr. G. Uzzan
Institut de Protection et de Sur. Nucl.
CEA-CEN de Fontenay-aux-Roses
B.P. n°6
F-92260 Fontenay-aux-Roses

General subject of the contract:

Methods for evaluating the consequences of irradiation of the public.

List of projects:

1. Methods for evaluating individual and collective doses resulting from various types of exposure to ionizing radiation.
2. Methods for evaluating the radiological detriment and its cost.

Title of project nr I : METHODS FOR EVALUATING INDIVIDUAL AND COLLECTIVE DOSES RESULTING FROM VARIOUS TYPES OF EXPOSURE TO IONIZING RADIATIONS

Head of project and scientific staff : A. GARNIER
L. ANGELETTI
Dr R. MAXIMILIEN
A. SAUVE

I - MODES OF EXPOSURE RELATED TO ENERGY PRODUCTION

I.1. Study of long-range transport and diffusion models in marine environment

Many documents have been gathered for this study, made in a first step in the frame of a sub-contract (SC-006-F) (LSEES). First, the interest was focused on physical problems in marine environment.

Two classes of models use differential equations. The first one is based on compartments analysis and has already been used by other teams to evaluate global impacts of radioactive releases. The second one, based both on ocean hydrodynamics theory and observation, seems to be more appropriate for predicting trajectories of pollutant in the whole North European waters, determining critical zones, and assessing radiological consequences for european populations.

Conseouently, it is intended to go on with application studies of this second class of models, and, as far as possible, to compare the results with those of " in situ " radioactivity measurements,in the frame of new research contracts with european institutes and laboratories (University of Liège, Marine Radioecological Laboratory of la Hague). These programs are meant to complement each other as well as other existing programs.

I.2. Studies related to gaseous effluents

I.2.1. Regarding the gaseous effluents of nuclear facilities, the final five-year contract report on long-range transport and assessment of collective doses on the european scale has been achieved (Imperial College, SC-001-UK). Papers have been published in scientific journals or congress proceedings.

Presentation and discussion of the model and results to itsapplication also appear in the Association EUR-CEA Report (§ 1.3.). Program and data basis were communicated, on their request, to two other teams (Karlsruhe and Studsvik).

I.2.2. Attention has been paid to the behaviour of atmospheric pollutants, so far as it is related to assessing consequences of planned and unplanned release.

The results of experimental studies on the absorption of airborne molecular iodine onto vegetation in dry and humid air have been reported.

A similar approach could be made of problems related to SO_x and NO_x deposition under different climatic conditions at the time of release.

I.3. Synthesis studies

I.3.1. The methods and results worked out in the frame of this project are exposed in a report, joined as Annex 1 to the consolidated report 1976-1980 of the Association, which contains four chapters :

- methods of evaluating the radioactive contamination of the physical environment
- methods of evaluating the radioactive contamination of food products
- methods of evaluating the doses to populations : socio-economic data and models of exchanges
- examples of applications.

I.3.2. In a congress-paper, it has been shown that the methods designed as well as the data collected in order to assess the radiological impact of nuclear installations may contribute to the assessment of risks related to other (industrial, agricultural, ..) activities. This was illustrated mainly by results acquired in the frame of the program.

II - MODES OF EXPOSURE RELATED TO OTHER HUMAN ACTIVITIES

II.1. A review of information collected on natural sources of exposure to ionizing radiations shows the important contribution of radon and its short-lived daughters to the effective dose equivalent.

A study has been designed to find a method for evaluating domestic exposure to radon and its influencing factors (SHI, sub-contract SC-007 F).

The first step, partly archieved, was aimed at selecting houses with high enough levels to allow a valid study of influencing factors.The results are being examined.

II.2. Another study attempted to evaluate the population exposure due to radiodiagnostic procedures (CEPN, sub-contract SC-002 F). In this field, due to the lack of data it is necessary to proceed by interviews in different institutions and of public health authorities.

. The mass chest screening activity in 1980 (10 million examinations) was analyzed as well as tendencies to reduce this type of examinations among different groups of populations. The radiological impact was evaluated and the results were in good agreement with the data found in the literature.

. A review was also made on the radiological examinations of teeth : types of examinations and associated irradiation. A survey should be made to allow an evaluation of resulting population exposures.

Title of project nr II : METHODS FOR EVALUATING THE RADIOLOGICAL DETRIMENT AND ITS COST

Head of project and scientific staff : Dr R. MAXIMILIEN
L. ANGELETTI
M. DALEBROUX
A. SAUVE

I - EPIDEMIOLOGICAL STUDIES

Two main research lines have been developed :

I.1. Carrying on of the investigations undertaken during the preceding years

. The first investigation consists of the follow-up of the long-term health state of a cohort of about 12 000 British citizens submitted between 1930 and 1960 to radiological treatment for minor head and neck diseases. Results on mortality (1950-1978) and morbidity (1970-1978) prove a significant increase in the frequency of mouth and pharynx cancers within certain subgroups as compared to the general British population. This analysis also suggests an increasing trend in the frequency of other head and neck malignant diseases, particularly during the recent years. Of course, the data were standardized for a number of confounding factors prior to interpretation. An additional study has been set up in order to :

- attempt to compare the trends by extending the follow-up ;
- tackle the problem of assessing the dose-effect relationship on the basis of only rough estimates of the doses delivered (3-4 exposure categories), awaiting more convincing results from the health standpoint.

. A second investigation has been carried out on the feasibility of a European Register for workers exposed to ionizing radiations. The results of this study suggest rather negative general conclusions : given the methodological requirements and constraints as to the availability of health records, it appears that a register of occupational exposure to ionizing radiations can be created only in Great Britain (where such a register already exists), Denmark and Italy. Only these countries have accessible mortality records. It seems that no other Member State has a system allowing individual morbidity follow-up. Inquiries have been started to examine several possibilities of progressing along this line, or even broadening the registration to the whole set of occupational hazards.

I.2. New studies

As far as the assessment of accidental detriment is concerned, a study has been undertaken on the occurrence of cararacts on individuals that had been submitted to ocular radiological treatment for a number of minor eye diseases. For the sake of logic, the study is first restricted

to (i) counting cataract cases among the treated individuals, and comparing the frequencies observed in two or three subgroups that have received different doses, and (ii) preparing a more detailed study (eye examination, acurate dosimetry, ..).

. Synthetic reviews of the literature have been started to estimate the feasibility of new studies as

- comparison of health risks among uranium and coal miners
- assessment of noncarcinogenic effects of ionizing radiations.

II - ESTIMATION OF THE COST OF RADIOLOGICAL HAZARD

- Analyses of, and research on, implicit cost of health detriment in different industrial fields have been developed :

. A first study has been carried out, by the cost-efficiency method, on protection choices allowing a decrease of the present radiation exposure levels of uranium miners. The different options were compared with respect to their performances in terms of actual collective dose avoided and/or health effects (lung cancer mortality). The practical aspect of this method should be pointed out, which should help evaluate the protection devices liable to be utilized in the future in sedimentary uranium mines.

. A second study was devoted to assessing the implicit cost of the public health detriment on the basis of an example from chemical industry : in the case of monovinyl chloride (MVC), there is a rather large among-mills variability in the cost of avoided liver angiosarcoma, which is generally much smaller than the cost for other industries, especially power plants.

- Given the recent interest of the individual dose variability in assessing the Sievert-man cost, a methodological study has been carried out to weigh the different arguments liable to support this type of approach. The different methodological choices have been examined and concrete examples of application proposed.

LIST OF PUBLICATIONS IN 1982

H.M. APSIMON, A.J.H. GODDARD
Atmospheric transport of radioisotopes and the assessment of population doses on a european scale, Final EUR/CEA five years contract report, 1982, Rapport EUR/CEA (à paraître)

H.M. APSIMON, A. DAVISON, A.J.H. GODDARD
The probability distribution of individual exposure due to hypothetical accidental releases of various radionuclides to the atmosphere
BSRP Congress, Inverness, 6-11 Juin 1982

L. ANGELETTI et al.
Effet de l'humidité atmosphérique sur la captation de l'iode élémentaire par les végétaux (à paraître en rapport CEA-R-5202, 1982)

A. GARNIER
Exemples de méthodes et de données générales élaborées dans le domaine de la protection radiologique, utilisables pour l'évaluation des risques d'exposition à divers agents nocifs (communication présentée au Congrès SFRP, Avignon, Octobre 1982)

R. MAXIMILIEN
Les sources naturelles d'exposition aux radiations ionisantes,
Revue d'Epidémiologie et de Santé Publique, 1982, 30, 2, 169-182

E. STEMMELEN
Radiocontamination de l'homme par la chaîne alimentaire - Le cas des viandes dans la CEE en 1977, Rapport CEA-R, 1982 (étude réalisée en sous-contrat)

A. OUDIZ, J.F. ROUBY, G. UZZAN
A comparison of the occupational risks and protection costs in asbestos factories and in nuclear power plants
Third International Symposium on Radiation Protection - Advances in theory and practice, Inverness (Scotland), 6-11 Juin 1982

J. LOMBARD, A. OUDIZ, G. UZZAN
La prise en compte du risque travailleurs dans la mise en oeuvre du principe d'optimisation des doses au public
Revue de Radioprotection, 1982

R. MAXIMILIEN
La radiothérapie de l'acné : Revue bibliographique
Rapport CEA (à paraître)

S.P. ALLWRIGHT, P.A. COLGAN, I.R. McAULAY, E. MULLINS
Natural background radiation and cancer mortality in the Republic of Ireland (à paraître dans : l' " International Journal of Epidemiology "), 1982

J. LOMBARD
La prise en compte de la variabilité des doses individuelles dans l'évaluation du coût de l'homme Sievert
Rapport CEA (à paraître)

Méthodes d'évaluation des doses individuelles et collectives résultant des rejets normaux et des émissions accidentelles - In : Association EUR/CEA, Méthodes d'évaluation des conséquences de l'irradiation des populations, Rapport Final, 1976-1980, EUR/CEA
(à paraître)

J. WRIGLEY
Long-range atmospheric dispersion of radioisotopes, thesis
Imperial College, Fabruary, 1982

J. TELL
Contribution à l'étude de l'irradiation médicale - Les actes radiologiques de médecine dentaire en France
Revue bibliographique (à paraître)

Progress Report

1982

Contractor:

Service d'Hygiène Industrielle
CEA-CEN de Grenoble
B.P. 85X
F-38041 Grenoble Cédex

Contract no.: BIO-F-423-81-F

Sub-contract : SC-007-F

Head(s) of research team(s):

Dr. J. Chalabreysse
CEA-CEN de Grenoble
B.P. 85X
F-38041 Grenoble Cédex

General subject of the contract:

Influence of the way of living on the domestic exposure of populations to radon : methodological approach.

List of projects:

1. Influence of the way of living on the domestic exposure of populations to radon : methodological approach.

Title of project nr SC 007-F

INFLUENCE OF THE WAY OF LIVING ON THE DOMESTIC EXPOSURE OF POPULATIONS TO RADON : METHODOLOGICAL APPROACH

Head of project and scientific staff : J. CHALABREYSSE

OBJECT OF THE STUDY

Refinement of a method for assessing domestic exposure to ionizing radiations, and evaluating the influence of a number of parameters.

This study is being performed in the area of the Rhône Valley ranging between Montelimar and Orange. From the geological standpoint, this area is mostly of sedimentary type. The research consists of two parts :

- Basic selection of houses liable to possess high enough radon contents to allow a detailed investigation of the parameters.

- Thereafter, repeated measurements will be made on the instantaneous radon-content of the air, the air-renewal rate in the rooms, and on several meteorological parameters. Measurements will also be made on radon emanation from the ground as well as on radon-content of water. Seasonal variability will be taken into account.

The first of these two parts has been started and is being accomplished by means of LR 115 Kodak trace detectors placed for 4-6 weeks into individual houses. Up to now, 120 such dosimeters have been placed among which 50 have already been analyzed. Four houses seem to be worth of interest, the radon level being about 2-6 pCi/l. Around ten houses have values ranging between 1 and 2 pCi/l. The rest of the sample shows values smaller than 1 pCi/l.

The answers to the detailed questionnaire related to these measurements (building materials, way of life, etc..) are being treated by computer.

As for the second part, particularly the measurement of air-renewal rate, a special device has been refined using diffusion and measurement of SF6 marker gas. The first trials are satisfactory.

Progress Report
1982

Contractor:

The Medico-Social Research
Board
Lower Baggot Street 73
IRL-Dublin 2

Contract no.: BIO-F-423-81-F

Sub-contract : SC-008-EIR

Head(s) of research team(s):

Dr. G. Dean
The Medico-Social Research
Board
Lower Baggot Street 73
IRL-Dublin 2

General subject of the contract:

A follow-up study on the dose-effect relationship of X-ray therapy to the head and neck area.

List of projects:

1. A follow-up study on the dose-effect relationship of X-ray therapy to the head and neck area.

Title of project nr SC-008-EIR
A FOLLOW-UP STUDY ON THE DOSE-EFFECT RELATIONSHIP OF X-RAY THERAPY TO THE HEAD AND NECK AREA

Head of project and scientific staff : Dr Geoffrey DEAN

Scientific staff : Dr Michael ALDERSON, Dr Peter GOLDBLATT, Dr Michael MORIARTY

Under a previous contract, a study was undertaken on the long-term effects of low dosage radiation to the head and neck area for various skin disorders to ascertain whether they had an increased risk of head and neck cancer. The study showed a significantly higher than expected number of cancers of the buccal cavity and pharynx and a higher than expected number of all head and neck cancers taken together. The present study is to ascertain if there is a radiation dose-effect responsible for this increase in cancer risk.

The radiation received by the patients has now been coded and will be analysed at four levels of radiation. Patients who have died with a diagnosis of cancer of the head and neck area on their death certificates or whose names have been added to the National Cancer Register since mid -1978 at the termination of the previous study and patients who were admitted to the regional cancer registers prior to 1971 when the National Cancer Register began, will also be included.

The study is being undertaken in collaboration with the Office of Population Censuses and Surveys, London.

Punch cards showing the radiation therapy received and the other patient data will be forwarded to the Office of Population Censes and Surveys in January, 1983, for analysis.

Progress Report
1982

Contractor:

The Medico-Social Research
Board
Lower Baggot Street 73
IRL-Dublin 2

Contract no.: BIO-F-423-81-F

Sub-contract : SC-009-EIR

Head(s) of research team(s):

Dr. G. Dean
The Medico-Social Research
Board
Lower Baggot Street 73
IRL-Dublin 2

General subject of the contract:

A study of ascertain whether patients who received X-ray therapy directly to the eyes develop cataract.

List of projects:

1. A study of ascertain whether patients who received X-ray therapy directly to the eyes develop cataract.

Title of project:	A study to ascertain whether patients who received X-ray therapy directly to the eyes develop cataract.
Head of project:	Dr.Geoffrey Dean
Scientific staff:	Dr.Michael Alderson, Dr.Peter Goldblatt, Dr.Michael Moriarty.

Cataract caused by X-ray radiation was noted in cyclotron workers and in the Japanese atomic bomb survivors. Merrian and Focht claimed that an exposure of 750 to 950 rad spread over one to three months would give up to a 50% chance of causing cataract. During the course of an earlier study 509 patients were found to have been treated with direct X-ray therapy to the eye or eyes and 399 of them have been traced. There were 220 alive and 179 dead. The cause of death of those who have died and their pattern of death compared with the general population is being studied from their death certificates.

A research project is now being undertaken, in collaboration with the Office of Population Censuses and Surveys in London, to trace what has happened to the 220 patients who, according to the National Health Service Central Register in Southport, were still alive and to see how many of them developed cataract and of what type. Letters have been sent to the Family Practitioner Committees asking them to forward a letter to the patients' present general practitioners. The letters to the doctors ask them to study their patients' records to ascertain if at any time the patient has been treated for cataract or other eye condition and whether the patient has been seen by an ophthalmologist. For 16 of these patients in Scotland a similar technique has been used through the Common Services Agency, Scottish Health Service, Edinburgh, under the direction of Dr. Michael Heasman.

At the time of this report, 96 replies have been received from the first letter addressed to the doctors in England and 7 out of 16 in Scotland. A second letter is now being sent out to those who have not replied to the first. Some patients recently changed their doctor, too recently for their new doctor to be reported to us by the Family Practitioner Committees. It is already clear that replies will be received about most of the living patients in this study. A personal approach will be made to the doctors who do not reply to letters asking them for their co-operation.

From the study we hope to ascertain:

1. If the patients who received direct radiotherapy to the eyes had an increased risk of cataract and, if so, of what type.

2. Is there a radiation threshold level and at what level is the threshold and what is the dose-time relationship between the radiotherapy treatment and the development of cataract.

Details about the radiation received by each patient has been coded for analysis with the other relevant data.

Progress Report
1982

Contractor:

Centre d'Etudes sur l'Eval.
de la Prot. dans le Domaine
Nucléaire, CEPN
Rue d'Ulm 26
F-75005 Paris

Contract no.: BIO-F-423-81-F

sub-contract : SC-002-F

Head(s) of research team(s):

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CEPN
B.P. n° 48
F-92260 Fontenay-aux-Roses

General subject of the contract:

Contribution to the study of medical irradiation : systematic diagnostic radiology in France.

List of projects:

1. Contribution to the study of medical irradiation : systematic diagnostic radiology in France.

Introduction

The aim of the study was a first attempt to evaluate the public radiation exposure from mass chest radiography for tuberculosis case-finding in France in 1980. Considering the total lack of data on the subject it has been necessary to proceed by interviews in the different institutions involved in mass chest screening. In the case of occupationnel medicine and local public health authorities, which both represents more than 70 % of the total mass screening activity in France, a general survey based on representative samples on the national level has been performed.

The mass chest screening activity in 1980.

With 10 millions examinations in 1980, mass screening chest X-ray examinations remains a quiet important medical activity despite recent regulatory attempts to reduce it. Table I describes this activity according to the different types of examinations (fluoroscopy, conventional radiography and radiophotography).

	Number of examinations in 1980 (10^6)		
	Radiography	Radiophotography	Fluoroscopy
Occupational medicine	0.5	2.7	2.2
Local public health authorities	0.4	1.1	0.2
Others (Army, hospitals,.....)	0.1	2.4	0.2
Total	1.0	6.2	2.6

Table I : Mass chest screening activity in 1980.

The radiological impact

The total effective collective dose equivalent associated with the 1980's activity has been evaluated according to the ICRP concept. Exposures of tissues and organs at risk (skin, lungs, thyroïd, gonades, bone marrow) have been assessed using an Alderson Rando anthropomorphic phantom and TLDs. Measurements have been performed on reprensentative X-ray machines presently in operation. The results have been compared with the data from the literature on the subject. A good agreement has been founded. Table II gives the final results in terms of "per caput" and collective doses equivalents.

	"Per caput" dose equivalent (mSv/examination)	Collective dose equivalent (Sv/year)
Radiography	0.05	50
Radiophotography	0.10	620
Fluoroscopy	0.7	1 820
Total		2 490

Table II : Per caput and collective dose equivalent associated with mass chest screening.

Mass chest screening effectiveness

The informations collected during this study show that the case finding rate of tuberculosis for the general population by the mean of mass screening in between 0.2 and 0.4 °/₀₀. These numbers lead between 2 000 and 4 000 new cases discorved per year representing 15 to 30 % of all new cases. The present mean cost for a case finding is between 37 000 and 75 000 FF (1980).

Progress Report
1982

Contractor:

Centre d'Etudes sur l'Eval.
de la Prot. dans le Domaine
Nucléaire, CEPN
Rue d'Ulm 26
F-75005 Paris

Contract no.: BIO-F-423-81-F

sub-contract : SC-003-F

Head(s) of research team(s):

Dr. F. Fagnani
CEPN
B.P. n° 48
F-92260 Fontenay-aux-Roses

General subject of the contract:
Research to define the implicit values of an occupational health hazard : methodological approach applied to uranium extraction.

List of projects:

1. Research to define the implicit values of an occupational health hazard : methodological approach applied to uranium extraction.

Title of project nr : BIO-F-423-81-F-SC-003-F
"Research of the implicit values of an occupational health hazard : methodological approach applied to uranium extraction".

Head of project and scientific staff.
F. FAGNANI and A. OUDIZ, J. LOMBARD

The various studies about the effective dose equivalent related to inhalation of radon and its decay products suggest that the doses received by uranium miners in non-sedimentary mines might be higher than previously admitted.

This situation has lead to examine new radiological protection options the implementation of which would reduce further the effective dose associated with inhalation of radon and its decay products.

A cost-efficiency analysis has been carried out in order to classify the various sets of available protection options : "turbulators" and/or electrostatic precipitations and/or increase of primary or secondary ventilation rates.

The main difficulty is the calculation of the α energy in various points of the simplified model mine which has been adopted. Then, one has to assess the efficiency of the combined options and not of the options taken solely.

The results are given for a simplified mine were 17 minors are employed during 10 years. They are expressed in terms of implicit cost of man-rem associated with the implementation of every set of options.

	Combination of options				Cost of combination of options	Collective dose	Cost of man-rem	Ranking
DAM	P*	S*	T*	F*	**(10^3F)	Rad + Ext.Irr man.rem, 10 years	10^3 F per man.rem	
N***	20	3	N	N	815	487.91	-	-
Y	20	3	N	N	1 045	358.1	1.8	1
Y	30	3	N	N	1 721	252.17	6.4	2
Y	30	3	Y	N	1 850	240.62	11.2	3
Y	60	3	Y	N	3 217	168.56	19	4
Y	60	5	Y	N	3 553	158.64	33.9	5
Y	120	5	Y	N	6 112	137.66	122	6
Y	120	5	Y	N	7 458	132.16	244.7	7
Y	120	11	Y	Y	11 178	129.56	1 430.56	8

Implicit cost of man-rem associated to various combinations of protection options.

* P : Primary flow rate - S : Secondary flow rate - T : Turbulateur - F : Filter.

** Total Cost (Investment + Operation and Maintenance) of the combination of options, for 10 years operation.

*** N : Not implemented, Y : Implemented.

The current level of protection (ranking n° 2) is consistant with a cost of man-rem of about 6 400 french Francs.

At this level of protection, the maximal individual dose equivalent is included in the range of (1,6 - 3,7) rem/y according to the following equivalence :

(0,6 - 2,5) WLM ≡ 1 rem

The next option consists of introducing a small ventilator called "turbulator" in each working area, and this corresponds to an implicit value of 11 200 french Francs per man-rem.

Progress Report
1982

Contractor:

Centre d'Etudes sur l'Eval.
de la Prot. dans le Domaine
Nucléaire, CEPN
Rue d'Ulm 26
F-75005 Paris

Contract no.: BIO-F-423-81-F

sub-contract : SC-004-F

Head(s) of research team(s):

Dr. F. Fagnani
CEPN
B.P. n° 48
F-92260 Fontenay-aux-Roses

General subject of the contract:

Research to define the implicit values of an occupational health hazard : the case of vinyl-chloride-monomer.

List of projects:

1. Research to define the implicit values of an occupational health hazard : the case of vinyl-chloride-monomer.

Title of project nr : BIO-F-423-81-F-SC-004-F
"Research of the implicit values of a public health hazard : a case study in the chemical industry".

Head of project and scientific staff :

F. FAGNANI and P. JAXEL, A. OUDIZ, J. LOMBARD.

This work has been developped within the general framework of comparative studies devoted to the determination of the implicit value of public health hazards, from which costs of man-Sievert can be derived. The example specifically chosen is the protection against the emissions of vinyl chloride (VC) from manufacturing plants of VC and polyvinyl chloride (PVC).

The carcinogenic properties of VC have lead many countries to regulate the emissions into the environment from VC and PVC plants. Four plants have been studied here, for which the various emission abatement technologies already implemented have been analysed from the point of view of cost and efficiency. This efficiency, expressed in terms of avoided emissions of VC has been then translated in terms of avoided health effects. An atmospheric diffusion model was used in order to calculate a collective exposure indicator expressed in (individual x $\mu g/m^3$).

From this indicator, the number of health effects was derived by using various "multistage" models for the extrapolation of exposure effect relationships at very low exposures. Results in terms of avoided health effects as well as derived man-Sievert values are shown in the following table, which indicates the corresponding values for a 1 300 MWe nuclear power reactor, for the sake of comparison.

	Avoided health effects 1981 Millions francs/effects	Value of man-Sv 1981 Millions francs/man-Sv
VC plant	1 950 - 23 500	40 - 486
Old PVC plant	3 - 40	0,06 - 0,8
New PVC plant	330 - 4 000	7 - 83
Nuclear reactor	530	11

As may be seen, values of avoided health effect can be very large in this chemical sector. This is to be interpreted as the consequence of the use of Best Available Technologies instead of ALARA technologies.

Progress Report
1982

Contractor:

Imperial College of Science
and Technology
Exhibition Road
GB-London SW7 2BX

Contract no.: BIO-F-423-81-F

sub-contract : SC-001-UK

Head(s) of research team(s):

Dr. A.J.H. Goddard
Mechanical Engineering Dep.
Imp. Coll. of Sc. and Techn.
Exhibition Road
GB-London SW7 2BX

General subject of the contract:

Assessment of collective doses from planned and unplanned releases to the atmosphere.

List of projects:

1. Assessment of collective doses from planned and unplanned releases to the atmosphere.

BIO-F-423-81-F Sub-Contract SC-001-UK

Assessment of Collective Doses from Planned and Unplanned Releases to the Atmosphere

Dr A.J.H. Goddard, Dr H.M. ApSimon, Dr J. Wrigley, Dr S. Crompton

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The task of the final year has been to bring together all the results produced in the 5 year study and produce a final contract report. This report is now complete, and hence only a brief summary of the contents is included for this progress report.

The contract had two main purposes related to dispersal of atmospheric releases of radioactive nuclides out to longer distances of several hundred kilometres or more; the first was the evaluation of collective doses resulting from routine releases from nuclear installations; the second involved statistical distributions of levels of contamination at remote receptor points (for example across a frontier in a neighbouring country) for short accidental releases. The MESOS model was developed and incorporated into computer programs to simulate the dilution and dispersal of such releases over the required distances, and was used in conjunction with two extensive data bases of meteorological data over Western Europe to study a large number of release periods of a variety of nuclides from 5 different source locations within the Community.

The final report contains a detailed description of the MESOS model and the meteorological data bases used with it and the estimation of doses resulting from contamination of air and ground. There is also a critical review of the model, commenting on the uncertainties and difficulties of predicting contamination patterns for specific situations, and describing a comparison between model prediction and observations for a validation study based on the Windscale release of 1957. The second part of the report contains a summary of the results. Complete results are given for collective dose distributions across Community countries which would result from unit releases from the 5 individual sources considered in the study; potential doses from several foodstuffs are included as well as doses from

external irradiation and inhalation; isopleths of contamination of air, and ground by dry and wet deposition are also given. Illustrative statistical results for the short accidental releases are presented; the project generated a very large amount of numerical information on this aspect of the study and further work is in progress to organise and present this data in the form of a simple model applicable to a wide range of sources and release durations. Considerable supplementary information and analysis of the results is also included in the report to help explain particular aspects - for example trajectory analysis and studies of release episodes leading to relatively high contamination at a remote receptor point. There are also 3 appendices covering material not specifically included in the contract requirements: for example comparisons with other models, and the development of a simple model for evaluating collective dose from routine continuous releases.

Progress Report
1982

Contractor:

Laboratoire de Statistiques et
d'Etudes, Economiques et
Sociales, LSEES
Rue d'Ulm 26
F-75005 Paris

Contract no.: BIO-F-423-81-F

sub-contract : SC-005-F

Head(s) of research team(s):

Dr.J.P. Pagès
LSEES
B.P. n° 48
F-92260 Fontenay-aux-Roses

General subject of the contract:

The variability of individual doses as a factor in the assessment of the costs of the man-Sievert.

List of projects:

1. The variability of individual doses as a factor in the assessment of the costs of the man-Sievert.

Title of project nr : BIO-F-423-81-F SC.005-F
"The variability of individual doses as a factor in the assessment of the Cost of the man-Sievert".

Head of project and scientific staff :

Dr J.P. PAGES
DR J. LOMBARD

Many people in charge with radiological protection aspects think that an explicit consideration of individual doses would increase the validity of the optimisation process which presently takes only collective dose into account.

The integration of the individual doses in the optimisation process can be explained by many considerations based either on a biological or decisional grounds.

We have then made a review of the various ways which can be envisaged in order to integrate individual doses in the optimisation process :

- Calcul of health effects by using relevant non linear dose-effect relationships.
- Use of a more sophisticated optimisation procedure.
- Modification of the cost-benefit analysis.
- Implementation of more severe dose limits.

From the analysis it appears that :

1) The calculation of health effects is the best method when biological grounds explain the use for integrating individual doses in the optimisation process.

2) The modification of the cost-benefit procedure is the best manner of integrating individual doses within the framework of the various decisional grounds.

The practical modification of the cost-benefit procedure requires some decision : which formula to adopt ? $Y = \sum \alpha i \; ni \; di$ ou $\alpha S + \beta \sum nj \; f(Hj)$? How should the value of the man-Sievert vary according to the per caput dose ? The application of the modified cost benefit procedure in two different practical cases shows its feasability and, accessorily, its complexity. This application shows that the modification is of little interest when potential doses are very small (doses to the public surrounding of power plant for example). But on the other hand, results vary significantly when doses are relatively high, and heterogeneously distributed (case of uranium mining for example).

Progress Report
1982

Contractor:

Laboratoire de Statistiques et
d'Etudes Economiques et
Sociales, LSEES
Rue d'Ulm 26
F-75005 Paris

Contract no.: BIO-F-423-81-F

sub-contract : SC-006-F

Head(s) of research team(s):

Dr. J.P. Pagès
LSEES
B.P. n°48
F-92260 Fontenay-aux-Roses

General subject of the contract:

Comparative analysis of diffusion and transfer models for releases in marine waters.

List of projects:

1. Comparative analysis of diffusion and transfer models for releases in marine waters.

BIO-F-423-81-F Subcontract SC-006-F

COMPARATIVE ANALYSIS OF DIFFUSION AND TRANSFER MODELS FOR RELEASES IN MARINE WATERS

Dr J.P. PAGES
Dr. J. BRENOT, Dr M.J. MEJON

Two classes of models use differential equations. The first class is based on compartmental analysis ; the model is presented in the joint report by NRPB and CEA "Methodology for evaluating the radiological consequences of radioactive effluents released in normal operations" CEC 1979. We focus on the second class : ocean hydrodynamics 'theory to model sea waters' movements and diffusion equation for effluents' activity. For North European waters, and specialy North Sea, models proposed by J.C.J. Nihoul and Y. Ronday both of Liege University were studied ; in the simplification process of hydrodynamic equations, we pay attention to the assumptions done in each step : oceanographic knowledge, use of in situ measures, mathematical analogies, computational convenience and fitting attempts. For the Channel, models in use at Electricité de France have also been reviewed.

To our knowledge, one can find hydrodynamic models for each marine region - for example Channel, North Sea, ... - but none for the whole North European waters. A such synthesis seems interesting and useful for a better understanding of actual sea radioactivity and radiological impacts of future releases.

Progress Report
1982

Contractor:

International Commission on
Radiological Protection
Clifton Avenue
Sutton
GB-Surrey SM2 5PU

Contract no.: BIO-F-425-81-UK

Head(s) of research team(s):

Dr. F. D. Sowby
ICRP
Clifton Avenue
Sutton
GB-Surrey SM2 5PU

General subject of the contract:

Development of fundamental data for radiation protection.

List of projects:

1. Examination of the scientific problems related to radiation protection with a view to improve the planning and application of the results of research to this problem.

Title of project:	Examination of the scientific problems related to radiation protection with a view to improve the planning and application of the results of research to this problem.
Contract No:	BIO-F-425-81-UK
Head of project:	Dr. F. D. Sowby, ICRP, Clifton Avenue, Sutton, Surrey SM2 5PU, England.

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2. Committees of the ICRP, and the Commission itself, met during 1982 to review current work and to plan future work.

Topics being studied include:

- Index of harm,
- Exposure of the public from radon,
- Non-stochastic effects of radiation,
- Developmental effects of irradiation of the embryo and foetus,
- Cancer induction following exposure in utero,
- Quantification of radiation detriment,
- Continuing review of epidemiological studies on the effects of low doses,
- ALIs for members of the public,
- Metabolism of plutonium and related elements,
- Dose to patients from radiopharmaceuticals,
- Conversion factors for use in the protection of workers exposed to external radiation,
- Protection of the patient in nuclear medicine,
- Protection of the patient in radiotherapy,
- Application of basic radiation protection principles to radioactive waste disposal,
- Protection of the public in the event of a radiation accident,
- Assessment of internal contamination from occupational exposure,
- Monitoring principles for the radiation protection of the public,

- Principles for limiting exposure of the public to natural sources of radiation.

3. The following ICRP reports were issued in 1982:

Index to ICRP Publication 30 and Supplements to Part 3:

Limits for Intakes of Radionuclides by Workers.

ICRP Publication 33:

Protection against ionizing radiation from external sources used in medicine.

ICRP Publication 34:

Protection of the Patient in Diagnostic Radiology. (Revision of ICRP 16)

ICRP Publication 35:

General Principles of Monitoring for Radiation Protection of Workers (Revision of ICRP 12)

ICRP Publication 36:

Radiation Protection in the Teaching of Science (Revision of ICRP 13).

IV

KOORDINIERUNGSTÄTIGKEIT

COORDINATION

ACTIVITES DE COORDINATION

IV. COORDINATION

Study group meetings, seminars, symposia and conferences have proved to be a most effective means of coordination because they are naturally adapted to scientific work and easily accepted by scientists. These meetings, focussing on the evaluation of particular subject areas of the Radiation Protection Programme, are attended by research workers involved in the contract programme, as well as scientists from non-participating laboratories or organizations and scientific staff members of the Commission.

On the following pages the various meetings held in 1982 are listed:

A. Meetings of Study Groups, where scientists involved in the contract programme, independent experts and staff members of the Commission discuss specific subject areas of the programme.

B. Meetings organized or co-organized by the Commission on special subject areas of interest for radiation protection and where contacts among scientists from a wider range of disciplines and countries might be established.

C. Meetings of Experts, whose activities have the effects both of coordinating and stimulating efforts towards practical measures of radiation protection, in accordance with Chapter III of the Euratom Treaty.

A

MEETINGS OF STUDY GROUPS IN 1982

Study Group "Radiation Risks resulting from Nuclear Activities"

Neuherberg, 1-2 February 1982
13 participants from 3 countries and the Commission.

Principal subjects :
- Discussion of the state-of-the-art of ongoing research.
- Planning of future activities.
- Identification and discussion of the priorities of the 1985-89 Radiation Protection Research Programme.

Study Group "Radiation protection dosimetry"

Luxembourg, 8-9 February 1982
29 participants from 9 countries and the Commission

Principal subject:
- Intercomparison programme, radon measurements in mines.
- Discussion on future action in the field of radiation protection measurements.

Study Group "Radiation Epidemiology"

Brussels, 12 February 1982
10 participants from 3 countries and the Commission.

Principal subjects :
- Discussion of the state-of-the-art of ongoing research.
- Planning of future activities.
- Identification and discussion of the priorities of the 1985-89 Radiation Protection Research Programme.

Study Group "Dosimetry intercomparison programme"

Luxembourg, 24-25 March 1982
12 participants from 5 countries and the Commission

Principal subject:
- Analysis of the photon intercomparison programme.

Study Group "Priorities in Radiation Protection - Behaviour and Control of Radionuclides in the Marine Environment"

Brussels, 29 March 1982
12 participants from 8 countries and the Commission.

Principal subject :
Definition of the relevant research areas (research priorities and scientific documentation).

Study Group "Priorities in Radiation Protection - Behaviour and Control of Radionuclides in the Terrestrial Environment"

Brussels, 31 March 1982
13 participants from 9 countries and the Commission.

Principal subject :
Definition of the relevant research areas (research priorities and scientific documentation).

Study Group "Priorities in Radiation Protection - Evaluation of Radiation Risks"

Brussels, 28 April 1982
11 participants from 5 countries and the Commission.

Principal subject :
- Future research areas in the sector "Evaluation of Radiation Risks".

Study Group "European Dosimetry Group" (EURADOS)

Brussels, 7 June 1982
16 participants from 5 countries and the Commission.

Principal subjects:
Preparation of the activities of Eurados on:
- dissimination of information,
- initiation and planning of collaboration between scientists and laboratories,
- collection and evaluation of dosimetric data,
- joint development and application of dosimetric methods, instruments and computer programs,
- organisation of specialist meetings, workshops and training courses,
- publication of monographs on particular fields of dosimetry.

Study Group "Radon and radon daughter products, measurement in mines"

Luxembourg, 7-8 June 1982
17 participants from 5 countries and the Commission

Principal subject:
- Discussion on measurement of exposure to radon and its daughter products in mines.

Study Group "Dosimetry"

Brussels, 8 June 1982
16 participants from 5 countries and the Commission.

Principal subject:
Discussion of future research projects in dosimetry and exchange of information on the following subjects:
- Quantities and units
- Reference radiations
- Health physics dosimetry and instrumentation
- Microdosimetric studies and physical data
- RBE
- Intercomparisons.

Study Group "Collection and Evaluation of Neutron Dosimetry Data" (CENDOS Committee)

Bologna, 23-24 June 1982
5 participants from 4 countries and the Commission.

Principal subject:
Discussion of the status of projects and planning of future activities.

Study Group "Calibration of dosimeters used in radiation protection"

Luxembourg, 8-9 July 1982
12 participants from 6 countries and the Commission

Principal subject:
- Calibration of measuring instruments for use in Radiation Protection.

Study Group on "Accident Consequence Assessment"

Luxembourg, 22 July 1982
9 participants from 2 countries and the Commission.

Principal subjects :
- Discussion of the state-of-the-art of ongoing research at NRPB (UK) and KFK (FRG).
- Definition of short term research needs to be included in a joint research programme.

Study Group "Irradiation and Thyroid disease"

Brussels, 3-4 September 1982
18 participants from 9 countries and the Commission.

Principal subject:
Evaluation of the risk from thyroid irradiation.
Possible future epidemiological studies.

Study Group "Reduction of Radiation Exposure from Medical Diagnostic Procedures"

Fontenay-aux-Roses, 23-24 September 1982
18 participants from 6 countries and the Commission.

Principal subject:
Data collection, comparison and harmonization of data evaluation for the establishment of dose reducing measures in medical diagnosis; work orientation for 1983.

Study Group "European Radiation Dosimetry Group" (EURADOS)

Jülich, 1 October 1982
15 participants from 5 countries and the Commission.

Principal subject:
Formation of working committees on:
- application of microdosimetric priniciples in radiation protection,
- beta and low energy photon dosimetry,
- application of thermomulinescence to routine personal dosimetry,
- computer codes.

Study Group "Non-stochastic effects in Lung"

London, 29 October 1982
16 participants from 4 countries and the Commission.

Principal subject :
Effects of external and internal (Pu) radiation on lung fibrosis and macrophages.

Study Group "Radiation protection in nuclear power plants"

Luxembourg, 8-9 November 1982
20 participants from 8 countries and the Commission

Principal subject:
- Exchange of experience between nuclear power station operators.
- Job-related dose recording.

Study Group "Effects of Radiation on the hemopoeitic and immune system"

Brussels, 2 December 1982
14 participants from 5 countries.

Principal subject:
Methods of cell separation to be used for bone marrow transplantation after irradiation.

B

MEETINGS ORGANIZED OR CO-ORGANIZED

BY THE COMMISSION OF THE EUROPEAN COMMUNITIES IN 1982

Workshop on the Metabolism of Technetium

Co-organized with the Laboratoire de Biochimie - Université de Nantes

La Baule, 25-26 February 1982
11 participants from 4 countries and the Commission.

Principal subjects :
- Cytotoxicity of Technetium.
- Behaviour of Technetium in terrestrial and marine ecosystems.
- Ongoing research at the different laboratories and co-ordination of the work.

European Seminar on Neutron Carcinogenesis

Coorganized with the TNO Rijswijk - The Netherlands
Rijswijk, 30 March- 1 April 1982
60 participants from 10 countries and the Commission.

Principal subjects:
- Review of existing data on neutron carcinogenesis in animals
- Review of human data
- Discussion of theoretical considerations
- Discussion of risk assessment

Conference on Radioprotectors and Anticarcinogens

Coorganized with the National Bureau of Standards USA, National Cancer Institute USA, Federal Emergency Management Agency USA, US. Radiation Research Society, National Council on Radiation Protection
Gaithersburg Md, USA 21-24 June 1982
100 participants

Principal subjects:
- Radical kinetics and mechanisms after irradiation
- Radioprotectors and protection in vitro and in vivo
- Carcinogenesis and anticarcinogenesis
- Individual resistance factors

Workshop on Natural Irradiation and Intercomparison of passive and active Radon Measurement Devices

Co-organized with the National Radiological Protection Board

Chilton, 20-22 September 1982
42 participants from 11 countries and the Commission.

Principal subjects :
- Nation-wide surveys on indoor exposure to radon and daughter products and to gamma rays.
- Radon exhalation of building materials.
- Attachment of radon-daughters onto aerosol particles.
- Results of the intercomparison of passive radon measurement devices.
- Intercomparison of active radon measurement devices in the NRPB-steel room.

8th Symposium on Microdosimetry

Jülich, 27 September - 1 October 1982
165 participants from 19 countries, international organizations and the Commission.

Principal subjects:
- radiation interaction and energy deposition,
- radiation quality,
- biomolecular and radio-chemical effects in relation to energy-transfer and energy-deposition processes,
- interpretation of biological effects,
- analysis and interpretation of dose-effect relationships,
- microdosimetric detectors for radiation protection problems.

Workshop on the Behaviour of Americium and Curium in the Environment

Co-organized with the International Laboratory of Marine Radioactivity IAEA, Monaco

Monaco, 12-13 October 1982
12 participants from 6 countries and the Commission

Principal subjects :
- Environmental behaviour of Am and Cm.
- Discussion of results recently obtained.
- Definition of research areas which deserve priority.

Fourth Information Seminar on the radiation protection dosimeter intercomparison programme (Photon intercomparison 1980/82

Bilthoven (Netherlands) 25-27 October 1982
60 participants from 10 countries and the Commission

Principal subject:
- The European radiation protection dosimeter intercomparison programme.

European Seminar on Risks from Tritium Exposure

Coorganized with the CEN/SCK, Mol, Belgium
Mol 22-24 November 1982
50 participants from 11 countries and the Commission

Principal subjects:
- Sources of Tritium
- Behaviour of Tritium in the environment
- The problem of organic tritium
- Tritium toxicology

Meeting on information and training on radiation protection for Trade Union representatives from the Member States of the European Communities

Luxembourg, 13-14 December 1982
26 participants from 10 countries and the Commission

Principal subject:
- Information on and training in Radiation Protection for Trade Union representatives of the European Trade Union Confederation. The subjects dealt with were the progress achieved in applying the Euratom Basic Safety Standards of 15 July 1980, the evolution of the doses received by nuclear workers, the compensation for industrial diseases caused by ionizing radiation, the practical organization of radiation protection in nuclear power stations and the health hazards of the industrial use of Laser rays.

C

MEETINGS OF EXPERTS IN 1982

Group of experts mentioned in art. 31 of the Euratom Treaty

Brussels, 29-30.4.1982
25 participants
Subject : Euratom Basic Safety Standards

Group of experts mentioned in art. 31 of the Euratom Treaty

Luxembourg, 24-25.11.1982
25 participants
Subject : Euratom Basic Safety Standards

V

AUSWAHL EINIGER AUF VERANLASSUNG DER KOMMISSION ERSCHIENENER VERÖFFENTLICHUNGEN

SELECTION OF PUBLICATIONS ISSUED ON THE INITIATIVE OF THE COMMISSION

CHOIX DE PUBLICATIONS EDITEES A L'INITIATIVE DE LA COMMISSION

V. PUBLICATIONS 1982

The scientific research results of the Commission's Radiation Protection Programme are presented in articles published in scientific journals. References to these are given in the corresponding Progress Reports. In certain cases the Commission initiated surveys of detailed results of specific activities in the field of radiation protection and published them as monographs, proceedings and radiological protection data as announced on the following pages:

A. Monographs and Proceedings
B. Radiological Protection Data
C. Other publications.

A

MONOGRAPHS AND PROCEEDINGS

8th Symposium on Microdosimetry

Proceedings of the 8th Symposium organized in collaboration with the Institut für Medizin of the Kernforschungsanalage Jülich GmbH, Jülich, Federal Republic of Germany, Jülich, 27 September - 1 October 1982

Edited by J. BOOZ, H.G. EBERT

The proceedings contain all scientific contributions to the symposium: papers, poster papers, reports and comments on poster sessions and discussions. The different contributions have been regrouped into three thematically oriented chapters:

I. Microdosimetric description of radiation interactions with matter;
II. Measurements and interpretation of radiation action and
III. Employment of microdosimetric principles in radiation dosimetry and protection.

Important subjects treated are:
- radiation interactions and energy deposition,
- radiation quality,
- biomolecular and radio-chemical effects in relation to energy-transfer and energy-deposition processes,
- interpretation of biological effects,
- analysis and interpretation of dose-effect relationships,
- microdosimetric detectors for radiation protection problems.

EUR Report 8395 DE-EN-FR, to be published by Office for Official Publications of the European Communities, Boîte Postale 1003, L-2985 Luxembourg.

For information: C.E.C., DG XII/F
Rue de la Loi 200
B-1049 Brussels

Radiation Protection - Assessment of plutonium internal contamination in man

A report prepared under contract for the Commission of the European Communities (G.F. Clemente - A. Delle Site) - Comitato Nazionale per l'Energia Nucleare, Centro di Studi Nucleari della Casaccia - Italy

The objective of this report is to provide a complete critical analysis of all methods currently applied to the assessment of plutonium internal contamination. Plutonium isotopes may enter the body either by inhalation, by ingestion or through wounds. Inhalation is generally considered the main source of plutonium internal contamination for workers.

A general introduction outlined the role of the direct "in vivo" methods (lung counting) and of indirect methods based mainly on excreta analysis to measure the plutonium internal burden.

A brief review of the metabolic behaviour of plutonium and americium is also given with particular reference to the retention and excretion functions which may be applied to evaluate the systemic and lung burdens on the basis of the excretion data. The analysis of the radiation protection standards to be applied in the control of plutonium internal exposure following the recommendations based on ICRP publications is reported. It is clear that the present report responds to the need for a comprehensive review of the methods available for measuring plutonium internal contamination in order to comply with the revised basic safety standards for the health protection of the general public and of workers approved by the Council of the European Communities on 15 July 1980. The direct and indirect methods are analyzed in detail to give the reader a clear idea of the pros and cons of each method. Particular attention has been paid to the calibration techniques (phantom and 'in vivo') used in plutonium lung counting and to the evaluation of chest-wall thickness and its effects. The significance of bioassay data and sampling procedures is critically reviewed together with the choice of the radiochemical procedures and counting techniques used to measure plutonium and americium in the excreta.

The meaning and interpretation of excretion data is also analyzed in the most important practical cases to explain how the available metabolic models are used to evaluate internal plutonium burden from excretion data.

The indirect methods particularly designed for a rapid assessment of acute plutonium contaminations are reported together with a brief analysis of the techniques used to measure plutonium in wounds.

The conclusions of the report are aimed mainly to facilitate :

(a) correct application of the direct and indirect methods in the assessment of plutonium internal exposure,

(b) good management of cases of accidental exposure to acute plutonium internal contamination,

(c) guidelines for intercomparison of the methods applied to the measurement of plutonium internal contamination.

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Late effects after therapeutic whole body irradiation
Proceedings of a Symposium organized in collaboration with the European Late Effect Project Groups.

Edited by T.M. FLIEDNER, W. GÖSSNER and G. PATRICK

The symposium took place on the occasion of the 10th Congress of the International Society for Expemimental Haematology in Munich. The 11 papers discuss late effects after conditioning for bone marrow transplantation, and in particular, conditioning by high dose whole-body irradiation.

After an introduction explaining the background and purpose of this symposium, the proceedings summarize the clinical experience to date, both in Seattle where such works was begun, and also effects in various European centres. In terms of development of art, this form of treatment is a recent innovation and the experience in the clinics is therefore still very limited. It then discusses the basis in pre-clinical animal models from which expectations of late effects may be derived. Studies on dogs have taken place in three EULEP laboratories and the experimental approaches are presented separately; the resulting pathology is, however, summarized in a combined paper. A description is given of comparable work with monkeys, which is being carried out in only one European centre.

Next, consideration is given to the question of how it might be possible to influence the development of late effects after high doses of whole-body radiation, from studies on mice with various chemo-protectors. The symposium concludes with a Round Table Discussion on the principal questions which have been raised.

Report EUR 8070 EN, 139 pages, 1982, to be ordered through :

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Neutron Carcinogenesis
Proceedings of an European seminar organized in collaboration with the TNO, Rijswijk, 30 March to 1 April 1982.

Edited by J. BROERSE and G.B. GERBER

The proceedings on neutron carcinogenesis review critically, in 30 pages, the information presently available as based on animal experiments and human observations.

The relative biological effectiveness (RBE) for induction of leukemia and solid tumours was mainly based on the observations in survivors from the atomic bomb explosions at Hiroshima and Nagasaki. Recently, the stimate of the doses received by the survivors have been revised, with possible implications for the efficiency of radiation carcinogenesis in man. Studies on animal models can be of substantial value in the assessment of the stochastic risks of neutron exposure. The initial studies were concentrated on the RBE of neutrons for mammary carcinogenesis in Sprague-Dawley rats. More groups have recently collected information on tumour induction in different animal species and various strains.

The proceedings include papers on induction of different tumours by neutrons including myeloid leukaemia; lung tumours and mammary tumours; in vitro transformation studies; mathematical analysis of tumour rates and incidences; revisions in the dosimetry of the atomic bomb survivors and the implication for the RBE of fast neutrons. For comparison reviews on non-carcinogenic effects of neutron irradiation are included. The seminar was closed with a Round Table Discussion on the mechanisms of action, the range of values and the needs for further studies of neutron carcinogenesis. The discussions on the different subjects as summarized by discussion moderators are included as separate contributions in the proceedings.

Report EUR 8084 EN, 455 pages, 1982; to be ordered through
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Genetic effects of ionizing radiations in multicellular eukaryotes and the assessment of genetic radiation hazards in man

A monograph prepared, in the framework of the Association Euratom - Commissariat de l'Energie Atomique (Fontenay-aux-Roses, France), by: Professor K. Sankaranarayanan, Department of Radiation Genetics and Chemical Mutagenesis, Sylvius Laboratories, State University of Leiden, The Netherlands.

The aim of this monograph is to review the progress in studies on the genetic effects of radiation in multi-cellular eukaryotes and its bearing and relevance on the evaluation of genetic risks in man. Within this general framework, an attempt has been made to (i) present a broad overview of the lines of inquiry that have dominated this field and the nature and kinds of data that have accumulated as a result; (ii) delineate those aspects of current research that hold promise and (iii) identify and outline problem areas that may need more attention in the years to come, so that questions regarding genetic risk assessments and genetic health protection can be answered with greater confidence.

K. Sankaranarayanan, 1982. Genetic effects of ionizing radiations in multicellular eukaryotes and the assessment of genetic radiation hazards in man (385 pages, 67 tables, 1500 references).

To be ordered through Elsevier Biomedical Press, Amsterdam

Biochemistry of genetics and sensitivity and repair of DNA

Abstract of 36 papers presented at the meeting of contractants working in the sector on genetic effects which was organized on 5-6 October, 1981 at the University Jusdus Liebig, Giessen, FRG.

The book of abstracts summarize the research activities of the contractants during the year 1981. The field covered includes the assessment of variations in sensitivity and repair capacity in human cells, the identification of the molecular effects of ionizing radiations and the analysis of the biochemical pathways of repair in microorganisms, animal cells and human cells. Illustrations are presented of the utility of recombinant DNA techniques for the study of sensitivity and repair.

Published by Elsevier Biomedical Press in:
Mutation Research 96: 119-151, 1982

Cytogenetic response in vivo to ionizing radiations in somatic and germ cells of mammals including man

Contributions presented at the meeting of contractants working in the sector on genetic effects which was organized on 27 and 28 October 1981 in Brussels.

The contributions, published in extenso in a special issue of Mutation Research, cover the following areas of research:
- Origin, nature and frequency of chromosomal observations in somatic and germinal cells;
- Ultrastructural characterization of the meiotic prophase in man;
- Description of effects in exposed humans and irradiated animals;
- Extrapolation of data (from somatic to germinal cells and from animals to man)

Published by Elsevier Biomedical Press in:
Mutation Research 95: 1-77, 1982

Methods of evaluation of the consequences of irradiation of the population (General Report for the period 1976-1980)

Edited by "Commissariat à l'Energie Atomique" and "Commission des Communautés Européennes" - Contrat d'Association EURATOM/CEA No 099-76-1 PSAF

The present state of scientific knowledge does not make it possible to set a threshold for the effects of ionizing radiation on the health of individuals or of populations. The pessimistic assumption whereby any exposure is liable to have a harmful effect therefore remains the basis for the evaluation of standards. The resulting recommendations formulated by the ICRP Publication 26 consist in a system of dose limits, which constitute the new foundations of radiation protection, the aim being to ensure that :

- no source of exposure is unjustified, with due regard to the benefits occuring therefrom,
- all necessary exposure is kept as low as reasonably achievable, economic and social factors being taken into account,
- the dose equivalents received do not exceed certain specified limits.

In order to attain this objective, it appeared necessary to have at our disposal, in the context of any project for an installation that is liable to cause exposure to ionizing radiation, means of evaluating the following :

1. The levels of exposure to the individuals and population groups concerned.
2. The resulting radiological detriment to man.
3. The economic and social impacts thereof.

In view of the foregoing, therefore, methods for making such evaluations had to be developed. This is the purpose of the Euratom/CEA Contract of Association, under which a research programme consisting of three projects was undertaken. These three projects are described below :

PROJECT 1 : Methods of evaluating individual and collective doses resulting from normal discharges and accidental releases

PROJECT 2 : Methods of evaluating radiological detriment

PROJECT 3 : Methods of evaluating the economic and social impacts of irradiation

EUR 8068 FR/EN, 1982, 450 pages
Office for Official Publications of the European Communities
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Proceedings of the Information Seminar on the problems of applying the Directive laying down the Euratom basic safety standards for the health protection of the general public and workers against the dangers of ionizing radiation - Papers presented at the seminar on 4 and 5 June 1981.

The Euratom Treaty has set the Commission the task to "establish uniform safety standards to protect the health of workers and of the general public and ensure that they are applied". In order to facilitate the adaptation of national radiation protection legislation to the provisions of the "Basic Safety Standards" Directive as they were adopted on 15 July 1980, an information seminar was held on 4 and 5 June 1981. This was primarily intended for representatives of the appropriate authorities responsible for introducing the principles defined by these revised basic safety standards into national law.

Luxembourg, 1982
EUR 8287 EN/FR
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B

RADIOLOGICAL PROTECTION DATA

Radiological protection - No 22
Results of environmental radioactivity measurements in the Member States of the European Community for Air - Deposition - Water - Milk.

This is the 20th report on ambient radioactivity published by the Health and Safety Directorate of the Commission of the European Communities. It was drawn up using the data collected by stations responsible for environmental radioactivity monitoring in Member States. The results are extracts from the data sent to the Commission under Article 36 of the Treaty of Rome establishing the European Atomic Community.

The results presented in this report deal with radioactivity of the air, deposition, surface water and milk during 1980 in the ten Member States of the European Community, viz. Belgium, Denmark, the Federal Republic of Germany, France, Greece, Ireland, Italy, Luxembourg, the Netherlands and the United Kingdom.

The results are presented under four main headings:

- artificial radioactivity in the air at ground level;
- artificial radioactivity in deposition;
- radioactivity of water;
- radioactivity of milk.

The report also contains the list of sampling stations and laboratories, together with a list of publications by Member States in this field.

This report places special emphasis on the measurement results for specific radionuclides, but it also contains data total beta activity so as to ensure continuity vis-à-vis previous and provide comparative values.

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OTHER PUBLICATIONS

Catalogue of contracts on the Commission's Radiation Protection Programme 1980-1984

This catalogue is being published in two volumes : Volume I containing information on the administrative features such as contractor, subject of the research projects, duration, budget, etc... and Volume II containing the scientific content of each project. Some 190 contracts covering more than 300 research projects are presented. The aim pursued through this publication is to convey a better transparency to the Commission's programme, and to serve as an aid for its management. It reflects the situation on 31 December 1981.

An updating reflecting the situation on 31 December 1982 will be published in the course of 1983.

For information :

C.E.C.
DG XII/F
Rue de la Loi 200
B-1049 Brussels

Radiation Protection Programme 1976-1980
Synthesis of Results

This report contains a synthesis of the results which have been obtained in the framework of the Radiation Protection Programme of the Commission during the period 1976-1980. Its objectives are to provide for the period considered, a short inventory of significant results and achievements which contribute to the evaluation of radiation hazards and to the protection of man against ionizing radiation, and to outline briefly some essential features (aims, budgetary means, management principles) of the programme.

Doc. Nr. XII/340/82, 1982, 175 pages

For information:

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Rue de la loi, 200
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EUR 8486 — Progress Report Programme — Radiation protection — 1982

EUR 8486 — Rapport d'Activité Programme — Radioprotection — 1982

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The role of this scientific research programme is:

(i) to study methods of reducing radiation exposure and minimizing the radiation hazards in all circumstances,

(ii) to study ways and means of acting rapidly and effectively in the event of an accident,

(iii) to acquire the research results necessary to update the basic principles of radiation protection, and

(iv) to supply sufficient information to contribute to a framework for making informed decisions on issues on which public opinion has become very sensitive.

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