Biennial Report
1979/80

Institute for Genetics and for Toxicology

Kernforschungszentrum Karlsruhe

Kernforschungszentrum Karlsruhe

Institut für Genetik und für Toxikologie

KfK 3200

Biennial Report 1979/80

Institute for Genetics and for Toxicology

(in association with the Institute of Genetics,
University of Karlsruhe, and the chair of Radiotoxicology,
University of Heidelberg)

Editor: G. Hotz

Kernforschungszentrum Karlsruhe GmbH, Karlsruhe

Abstract

The research activities of the Genetics and the Toxicology Divisions of the Institute for Genetics and for Toxicology of Fissile Materials during the period January 1979 to December 1980 are described. In addition to scientific reports on the various research topics the Report gives an overview of the external scientific and teaching activities of the staff members during the review period. The main emphasis of the toxicology program has been on studies of the radiotoxicology of the actinides and other heavy metals, especially in relation to chelation therapy and to the development of biochemical and physical methods for investigation of their metabolic behaviour. In the field of radiation genetics most of the interest has been focussed on the mechanisms of gene repair, gene regulation and the molecular biology of tumor viruses.

Zusammenfassung

Es wird über die Tätigkeiten des Teilinstituts Genetik und des Teilinstituts Toxikologie innerhalb des Instituts für Genetik und für Toxikologie von Spaltstoffen von Januar 1979 bis Dezember 1980 berichtet. Neben kurzen wissenschaftlichen Berichten über wichtige Forschungsergebnisse gibt der Report einen Überblick über sämtliche wissenschaftlichen und akademischen Aktivitäten der Mitarbeiter während des Berichtzeitraums. Schwerpunkte des Forschungsprogramms bilden Arbeiten über die Strahlentoxikologie von Actiniden und anderen Schwermetallen sowie ihre Therapie, die Entwicklung biochemischer und physikalischer Methoden und ihre Anwendung auf diesem Gebiet. Im Bereich der strahlengenetischen Untersuchungen interessieren vor allem Fragen zu den Mechanismen der Genreparatur, Genregulation und zur molekularen Biologie von Tumorviren.

This report is the first of a series which will be issued
biennially by this institute to inform our friends and colleagues,
where ever they may be, about what is going on in the IGT. This
report covers the scientific activity during the years 1979 and
1980.

It should be stated that all experiments on recombinant DNA done
in the IGT are carried out under biological safety regulations as
defined by the Federal Ministry of Research and Technology of the
Federal Republic of Germany, regulations which are comparable
to those defined by the NIH Recombinant DNA Research Guidelines.
Similarly all studies with living animals are performed in
accordance with ethical and husbandry standards recommended
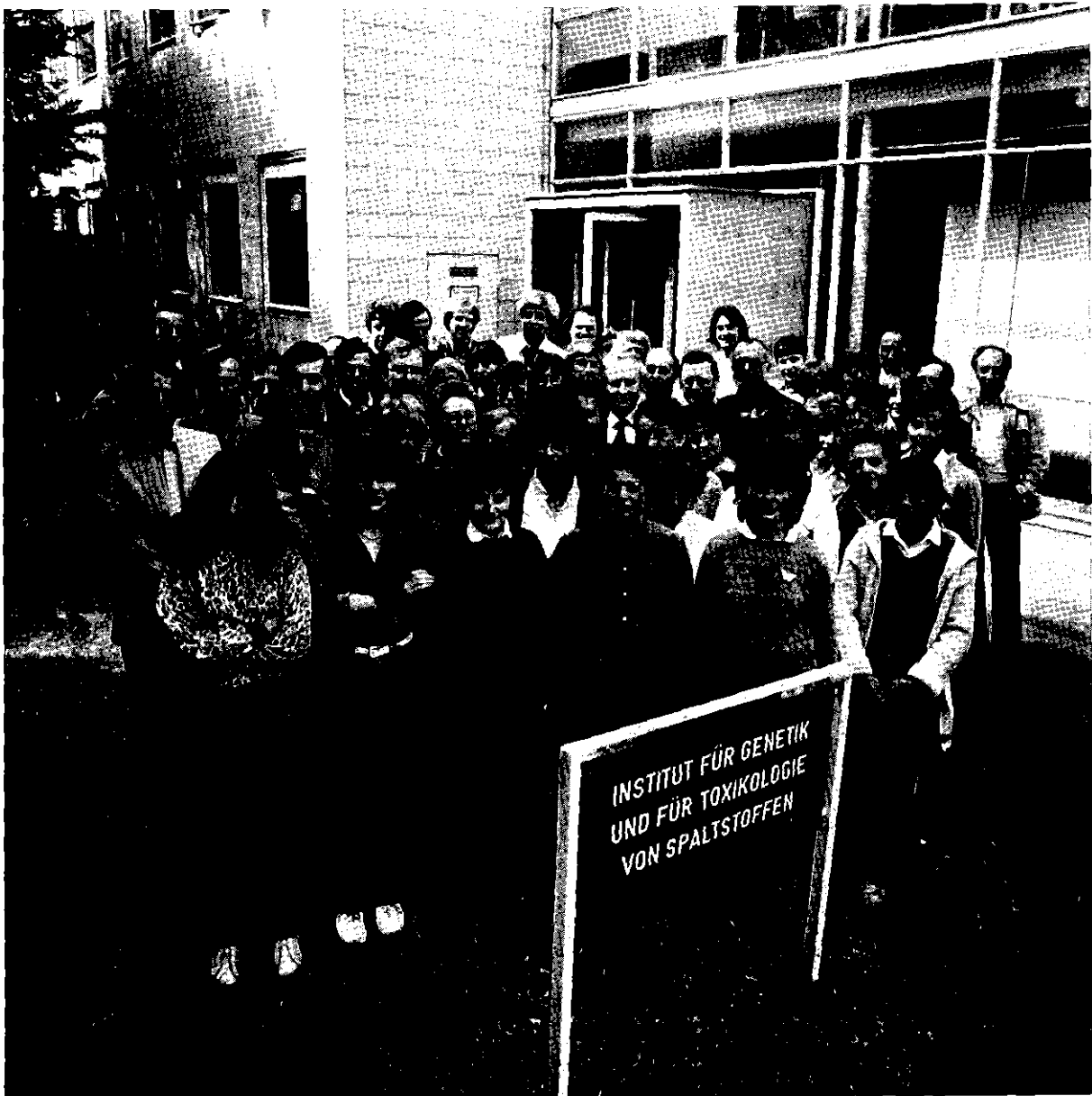both nationally and internationally.

REPORT 1979/80

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INSTITUT FÜR GENETIK
UND FÜR TOXIKOLOGIE
VON SPALTSTOFFEN

1. Introduction

The Institute was first established in 1958 as the Institut für Strahlenbiologie (IStB) of the Kernforschungszentrum Karlsruhe. In those early days the aim was to start again in a field of bio-physical research which had been studied prior to World War II in the Genetics Department of the Kaiser Wilhelm Institut in Berlin under the direction of such well known scientists as M.Delbrück, N.W.Timofeeff-Ressovsky and K.G.Zimmer. K.G.Zimmer started building the new Institute in Karlsruhe in 1957 and,together with the late Professor Alexander Catsch, directed the Institute for the next twenty years. Under the direction of Professors Zimmer and Catsch the Institute's scientists made many contributions to the analysis of radiation damage in phage DNA and to the problems of accelerating the removal of radionuclides from the body. Thus the tradition of carrying out both fundamental and applied research was firmly established.

Following the untimely death of Alexander Catsch in 1976 and with the retirement of K.G.Zimmer in sight, the Institute re-organised into two new divisions, genetics and radiotoxicology. The Institute was renamed as the Institut für Genetik und für Toxikologie von Spaltstoffen on 1 July 1977. The Institute has close university affiliations and the two present directors also occupy respectively the Chairs of Genetics in the University of Karlsruhe (Peter Herrlich) and Radiotoxicology in the University of Heidelberg (David M.Taylor). These strong university affiliations are reflected in a steady stream of diploma and doctoral students and by the active programme of seminars, lectures, practical courses, workshops and journal clubs. Close links are maintained with other institutions having similar interest throughout the world and several collaborative studies are being carried out. During the last two years, 41 scientists and 40 non-scientific staff worked in the Institute. The staff members coming from Germany, the United States of America, Belgium, Great Britain, Israel and Italy.

The research programmes of the Institute are assessed annually by
the Board of Management of Kernforschungszentrum Karlsruhe, assisted
by an independent Scientific Advisory Board, and the work is funded
almost entirely from the budget of Kernforschungszentrum but at
the present time 3.5 scientific posts are financed by the University
of Karlsruhe and other bodies.

Research in Genetics

The genetic research is devoted to basic studies of the mechanisms
of gene regulation and gene repair. Current interests are concerned
with the links in the chain of events which starts with exposure
of a cell to agents such as radiation, tumour promoters or hormones
and ends with the development of a tumour (Table 1). Clearly within
such a complex sequence of events, the areas chosen for experimental
work must be selective and the current studies are concerned with
DNA repair and gene activation.

Table 1

Agents	Early events	Secondary events	Endpoints
Radiation,	such as	such as chromosome rearrange-	such as
Mutagens,	gene activations	ments,	tumor
Tumor promoters,	and gene repair	activation of tumor viruses,	generation
Inhibitors of		chromosome aberrations,	
DNA synthesis		cell cycle changes	
Hormones			

We chose to examine the repair of DNA double strand breaks because
these seem particularly important for cell death and for the
induction of secondary events such as cell cycle changes and
chromosome aberrations. Both bacterial and mammalian cells can
rejoin DNA double strand breaks as judged from size measurements
by neutral filter elution. By means of kinetic and genetic studies

at least two typs of rejoining have been detected. The discovery
of human genetic deficiencies resulting in a reduced ability
to rejoin breaks opens up an approach to the mechanism of repair
in human cells. All the agents listed in the scheme lead to another
early response: the activation of genes.The pattern of new protein
production is cell-type specific but not agent-specific. All
treatments have elicted the same uniform response in one and the
same cell. Efforts are now being directed towards the mechanism
of gene activation and the functions of the induced gene products.
Circumstantial evidence to be discussed in the research reports,
suggests a relationship between the induced protein (s) and sub-
sequent pleiotropic effects such as tumorvirus induction and
chromosomal rearrangements.

The damage to DNA produced by irradiation or treatment of cells
with tumor promoters affects the progression through the cell
cycle. This endpoint may be mediated by signals other than those
responsible for the induction of genes. But this is not yet clear.
Because only relatively low doses of radiation are required to cause
the changes in the cell cycle, the target must be large and certainly
resembles, in this respect, the gene-inducing signal. Cells are
arrested in very late S phase or G2 and may progress into mitosis
after a delay. Shortening of the delay by treatment with caffeine
causes increased cell death, indicating that the delay is important
for an essential process, probably repair.

Most effort and man power goes into the investigation of one
specific gene system: the mouse mammary tumor provirus. The
expression of this gene obeys to several regulatory signals. In
its "silent" form which is present in all mice, it is activated
by mutagens or ionizing irradiation. Upon change of location
in the genome (e.g. transfection of cloned provirus) the gene
becomes active and its expression is magnified by hormonal stimula-
tion. This field of research connects with several most interesting
topics such as gene structur, mechanism of hormone action and tumor
generation. These perspectives are described in the respective
research reports.

Research in Toxicology

The aim of the toxicology research programme is to contribute
to our understanding of the risks to health which may result from
the uptake into the human body of very small amounts of
plutonium-239 and other components of the nuclear fuel cycle.
Because the available evidence suggests that incidence of tumours,
or other deleterious effects, to be expected after uptake of
very small doses of these materials will be extremely low,
conventional toxicological methods are inappropriate and new
approaches are required. Our research programme is founded on the
belief that a primary requirement for such investigations is
a clear understanding of the mechanisms controlling the metabo-
lism of the radioelements and the induction of their harmful
effects. Within this framework we have selected three areas for
investigation: the biochemical mechanisms controlling the
deposition of actinide elements, and non-radioactive metals,
in liver and other tissues: the microdistribution of radiation
dose from alpha particle emitting nuclides in bone: the development
of methods for accelerating the normally slow excretion of
plutonium and related elements from the body.

The biochemical studies have been concerned, so far, with the
sub-cellular distribution of plutonium in the liver of various
species of animal. These studies have demonstrated that lysosomes
are important deposition sites for plutonium, and some other
metals, in liver and probably most other tissues. Current studies
are now directed towards the determination of the chemical form
of plutonium in the lysosomes and the mechanisms of its transfer
from the blood, through the cell membrane to the lysosomes,
processes in which iron binding proteins may play a key role.

For the studies of microdosimetry in bone automated, computer
controlled scanning microdensitometric equipment has been developed
which permits the determination of the distribution of radioactivity

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on bone surfaces to been made from autoradiographs of bone sections. Further the same equipment is used to study the growth and remodelling of bone using calcein as an indicator. The techniques are now being used to build up a picture of the radiation dose to the bone surfaces and of the changes in dose distribution produced by bone growth and re-modelling and by normal or accelerated metabolic loss of plutonium-239 and other actinides.

The third area of study concerned with the development of therapeutic methods for the accelerated removal of plutonium and thorium from the body. For both plutonium and thorium DTPA remains the most effective agent for chelation therapy and current studies are concerned with the elucidation of the most effective treatment modalities, using DTPA alone or in combination with other agents.

More detailed discussions of all the aspects of this work will be found in the individual research reports. Some studies of the radiotoxicity of plutonium-239 in the tree shrew, Tupaia belangeri, are in progress and further radiotoxicological studies will be begun as improved animal facilities become available.

In both the genetics and toxicology programmes fundamental and applied studies are closely integrated. The combination of genetics and toxicology in one institution is quite unique and offers the chance to develop the programmes outlined which in fact cover interconnecting aspects of a joint problem. The combination has been designed to yield data which ultimately will assist in the evaluation of the effects of low doses of radiation in man.

<div align="right">P. Herrlich   D. M. Taylor</div>

Karlsruhe, April 1981
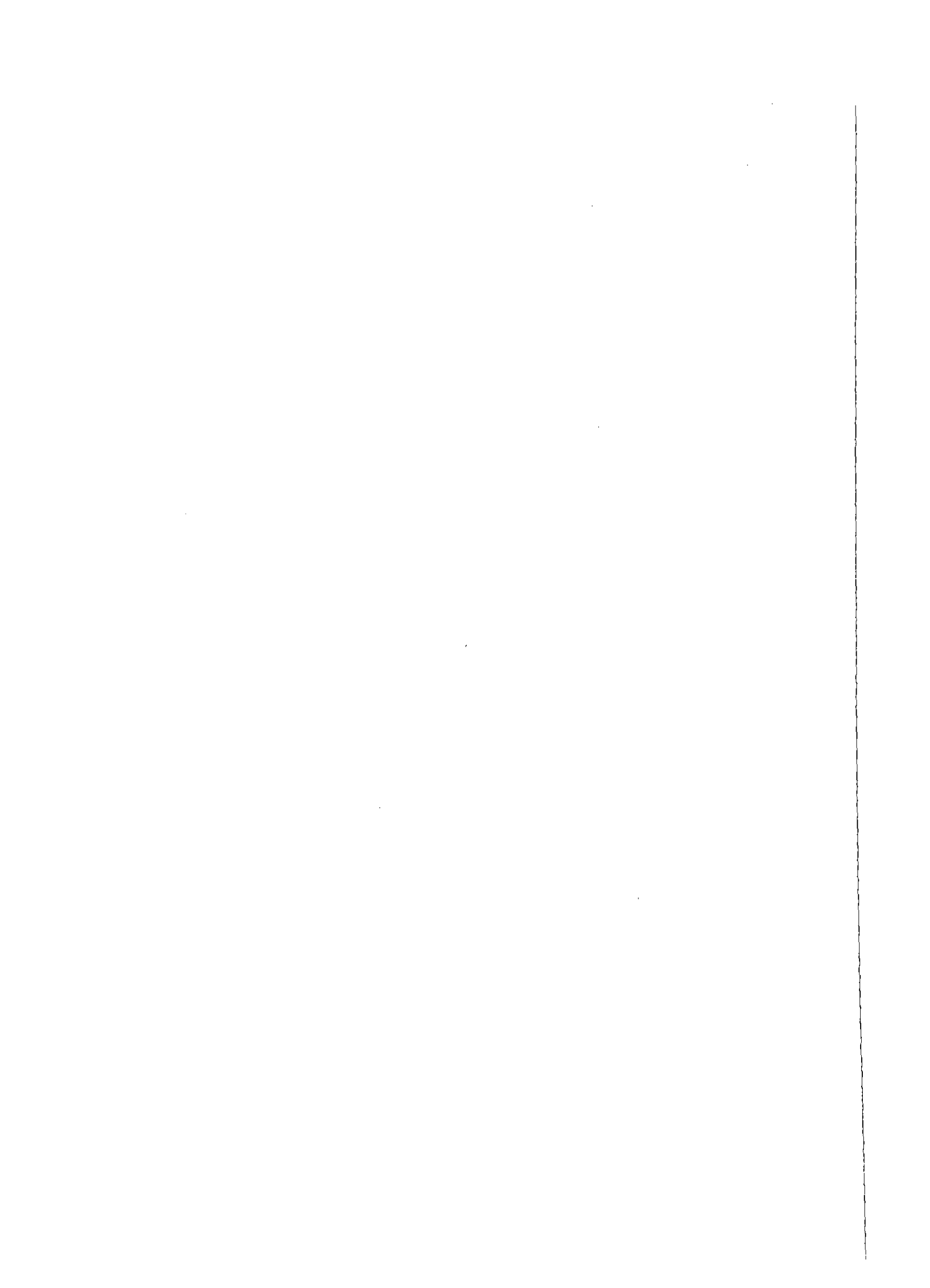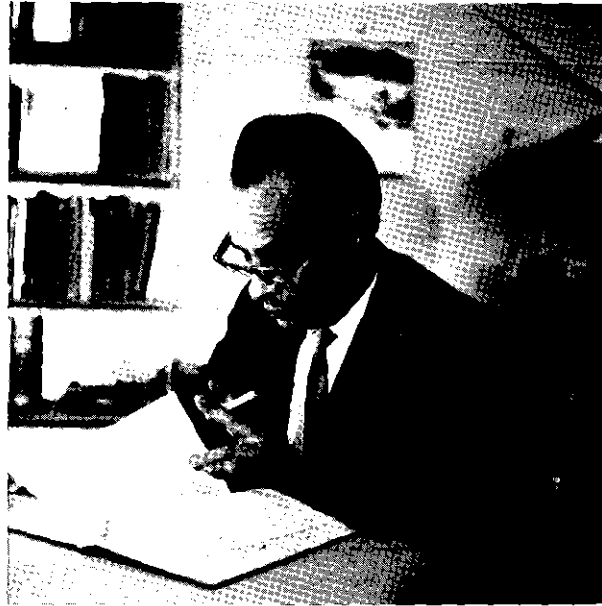
In memoriam ALEXANDER CATSCH

On February 16, 1976 Alexander Catsch, one of the two founder-
directors of the Institut für Strahlenbiologie and Professor
of Radiobiology in the University of Karlsruhe, died shortly
before his 63rd birthday. With his untimely death the Institute
lost a gifted scientist and teacher, a friend and stimulating
colleague. Educated as a physician Alexander Catsch began his
scientific career at the Kaiser-Wilhelm Institut in Berlin in
the field of experimental genetics, working with N.W. Timofeeff-
Ressovsky. In those early days his colleagues included other
young scientists including Max Delbrück and K.G. Zimmer, who was
later to become his fellow director in Karlsruhe. Although he
started his scientific work in the field of genetics, as early
as 1944 Catsch had become interested in the distribution and
toxicity of incorporated radionuclides, the field which he was
later to pursue with conspicuous success during his eighteen
years in Karlsruhe.

Catsch's important work in the field of the uptake, distribution
and, above all, the accelerated removal or 'decorporation' of
radioactive materials in the mammalian body was published in a
host of scientific papers and in two monographs. This work brought
wide international recognition and provided much of the foundations
for our knowledge about the treatment of human contamination by
radioactive metals. In his scientific work and in his relations
with his colleagues Catsch was far sighted and open minded and
possessed a seemingly inexhaustable ability to provide inspiration,
guidance and new ideas to all with whom he came into contact,
powers which were still at their peak when he passed from our
midst.

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K.G. ZIMMER: 70th Birthday

On July 12 K.G. Zimmer is celebrating his 70th birthday. In the
mid-thirties the young physicist became interested in biological
problems. This was a period, only a few years after Muller's discovery
that ultraviolet and ionizing radiations were mutagenic, when radiation
genetics offered a broad field to be worked on by physicists and
chemists. In 1934 he started experimental work in the Kaiser-Wilhelm-
Institut in Berlin-Buch under Timoféeff-Ressovsky with the classical
tool of genetics: Drosophila. Already in 1935 a fundamental paper was
published by three men who today can be called the ancestors of
modern radiation genetics: M. Delbrück, N.W. Timoféeff-Ressovsky
and K.G. Zimmer. The paper, entitled "Über die Natur der Genmutation
und der Genstruktur", stated that genes are physicochemical units,
and that a mutation is a change in this structure. As Max Delbrück
in his Nobel Lecture pointed out very well: "...in the midthirties....
it was anything but clear that these units were molecules analyzable
in terms of structural chemistry". In about 130 papers Zimmer
published his successful experimental and theoretical studies on
general problems in radioprotection, radiotherapy and radiodiagnosis
as well as on neutron dosimetry, the analysis of radiobiological
dose-effect curves in terms of the "hit theory" and statistical
ultramicrometry with ionizing radiation. His biophysical monograph
"Das Trefferprinzip in der Biologie" published together with
Timoféeff-Ressovsky showed up both the possibilities and the limits
of the analytical "Treffertheorie", as well a fruitful stimulation
was given to quantitative radiobiology. K.G. Zimmer was one of the
first to apply the new method of electron-spin-resonance in radio-
biology. This technique made possible the quantitative and qualitative
analysis of radiation-induced radicals in biomolecules especially

in DNA. In 1957 Zimmer started as a director of the "Institut für Strahlenbiologie" in Karlsruhe and professor of radiation biology at the University of Heidelberg, to analyse, together with his coworkers, radiation damage in phage DNA by means of modern physical and biochemical methods. His monograph "Studies on quantitative radiobiology" exists in German, English and Russian versions and K.G. Zimmer's name is closely connected with the development of modern molecular radiobiology. He retired in 1978 but retains an active connection with his old institute. - For his 70th Birthday and the future we offer him our congratulations and very best wishes.

4. Staff of the Institute

Directors:

> Prof. David M. Taylor (Toxicology)
> (Administration of the institute during the
> period 1980/81)
>
> Prof. Peter Herrlich (Genetics)
> (Administration of the institute during the
> period 1978/79).

Research scientists:

> Prof. Helmut Appel
> Dr. Heidi Martin-Bertram (until May 1979)
> Dr. Thérèse Coquerelle
> Prof. Hermann Dertinger
> Dr. Bernd Groner
> Dr. Horst Haffner
> Prof. Ulrich Hagen (until Jan. 1979)
> Prof. Gerhart Hotz
> Dr. Nancy Hynes
> Dr. Nicholas Kennedy
> Dr. Martin Lehmann
> Dr. Christine Lücke
> Dr. Udo Mallick
> Dr. Felicitas Planas
> Dr. Erich Polig
> Dr. Helmut Ponta
> Dr. Hans-Jobst Rahmsdorf
> Dr. Ursula Rahmsdorf
> Dr. Gisela Schäfer (until August 1979)
> Dr. Helmut Schlag (until April 1980)
> Dr. Arnulf Seidel
> Dr. Werner Sontag

Dr. Nikolaus Spoerel (until Aug. 1979)
Dr. Wolf-Gerolf Thies
Prof. Vladimir Volf
Dr. Karl-Friedrich Weibezahn

Graduate Students

Sylvia Adelski
Christel Gärtner (until May 1979)
Roland Heidinger
Sigrid Herrmann (until April 1980)
Ludwig Hieber
Gudrun Hinz
Gudrun Knedlitschek
Thomas Krüger
Eva Peter
Joachim Raudies
Ulrike Sütterlin
Gerhard Then
Ruth Winter (until March 1980)

Visiting Scientists

Dr. Lucia Fabiani
Dr. Hisao Osawa (until Dec. 1980)

Research Technicians

Margarete Bächle
Tania Cresswell
Ute Guhl (until Dec. 1979)
Marion Hoidn (until March 1979)
Inge Knoch (until July 1980)
Evelyn Krüger
Frank Litfin (until May 1980)
Kornelia Loch (until Sept. 1980)
Rosemarie Mauser
Helga Olinger
Monika Pech
Irmgard Persohn

Gertrud Regula
Petra Schlenker
Gisela Schütz
Angelika Seiter
Christel Sexauer (June 1979)
Ruth Walser

Administrative and General Staff

Ulrike Baltzer
Else Baum
Barbara Braunstein
Brigitte Eggmann
Karoline Friedl
Erna Funke (until May 1980)
Bernd Gloss(until Sept. 1980)
Wilhelm Heger
Gisela Heil
Christel Heinold
Bettina Herzog
Maria Hofheinz
Gisela Kammerer
Peter Kindermann
Theo Mohr
Jutta Müller (until Sept. 1980)
Emma Ölbach (died Oct. 1980)
Mathilde Petereit (until April 1980)
Katharina Roth
Gisela Siegel
Irmgard Trautwein
Martha Zimmermann

5. Advisory Board

The following members belonged to the
Scientific Advisory Board of the IGT
during the years 1979/80:

Ministerialrat Dr. K. Bauer
Bundesministerium für
Forschung und Technologie, Bonn-Bad Godesberg

Prof. Dr. Lars Ehrenberg
Stockholms Universitet

Prof. Dr. L.E. Feinendegen
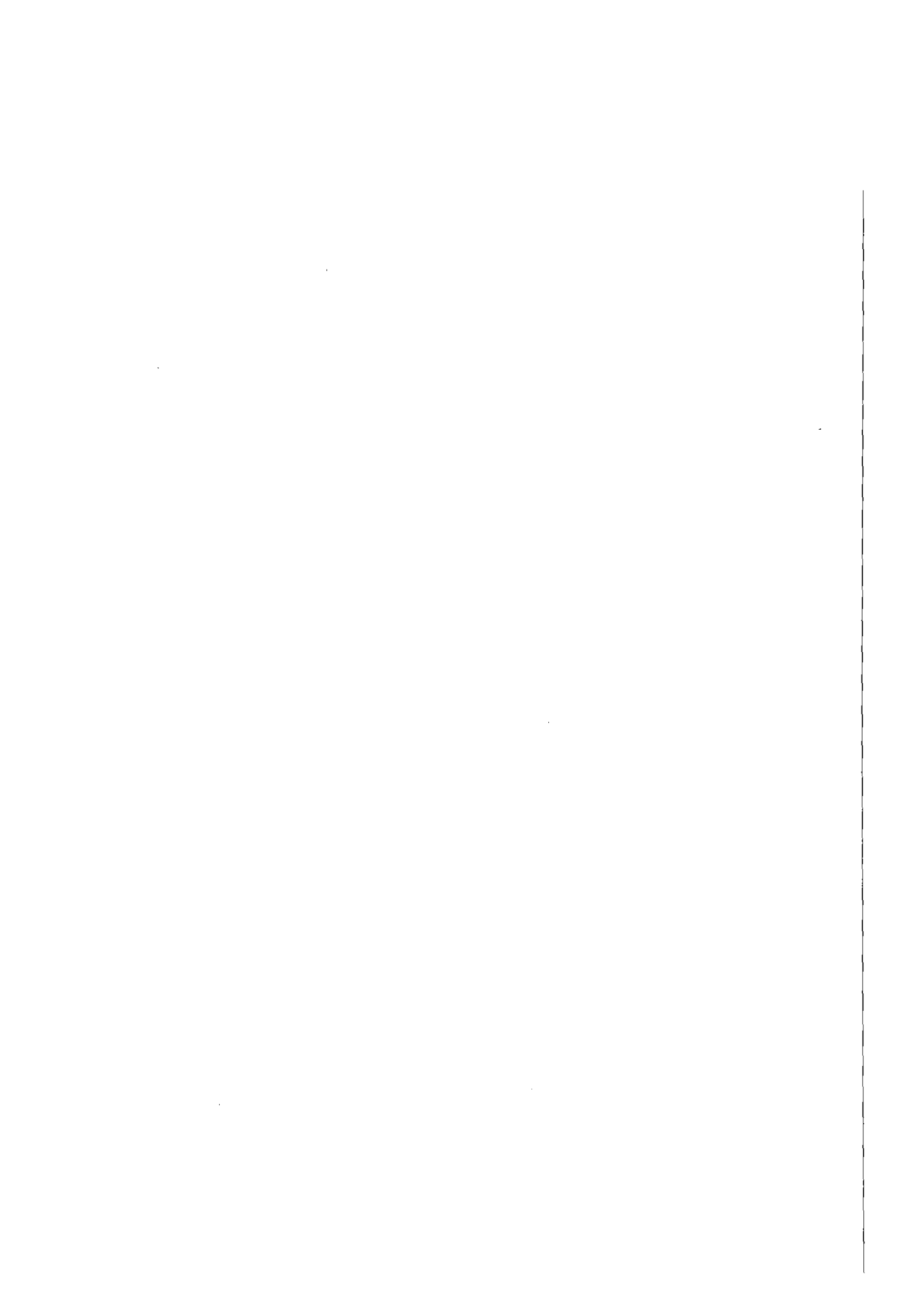Institut für Medizin
der KFA Jülich

Prof. Dr. H. Hoffmann-Berling
Max-Planck-Institut für
Medizinische Forschung, Heidelberg

Prof. Dr. G. Schatz
Biozentrum Basel

Prof. Dr. H.G. Wittmann
Max-Planck-Institut für
Molekulare Genetik, Berlin

6. Year-round Research (Short scientific reports)

a) Radiationtoxicology of Actinides

THE SUBCELLULAR DISTRIBUTION OF ACTINIDES

D.M. Taylor, A. Seidel, R. Gruner, U. Sütterlin, R. Winter

The association of monomeric and colloidal plutonium, americium and curium with lysosomal structures in rat liver, and of the monomeric metals with the mitochondrial-lysosomal fraction of dog liver, was recognized more than ten years ago (1). However, it was not clear whether this lysosomal association of the actinides was a universal phenomenon or if it was specific for the rat, a species in which the major part of the liver burden of plutonium, americium and curium is removed with a half time of a few days.

In order to investigate the role of the lysosomes in the fixation of actinides in the liver of animals which retain the metals with long as well as short half times a comparative study of the subcellular distribution of plutonium has been carried out in the livers of rats, mice, Syrian and Chinese hamsters. In rats and mice, following injection of monomeric plutonium, more than 70 per cent of the liver burden is removed with a half time of between 3 and 16 days, the remainder being removed with a half time of at least 120 days. In contrast to the bi-exponential pattern of removal of plutonium from rat and mouse liver, hamsters show only a mono exponential clearance with a half time of the order of 100 days in the Syrian hamster and at least an order of magnitude greater in the Chinese hamster.

In the study of the subcellular distribution of actinides in liver or other tissues an essential requirement is an experimental method which permits an unequivocal separation of lysosomes from mitochondria and other organelles. In our studies this has been achieved using sucrose density gradient centrifugation following administration of the detergent Triton WR-1339, and more recently by the use of Metrizamide gradients.

The profiles obtained from sucrose density gradient centrifugation of the post-nuclear supernatent from homogenates of rat, mouse, Syrian and Chinese hamster liver, with and without pretreatment (4 days previously) with Triton WR-1339, are shown in figure 1.
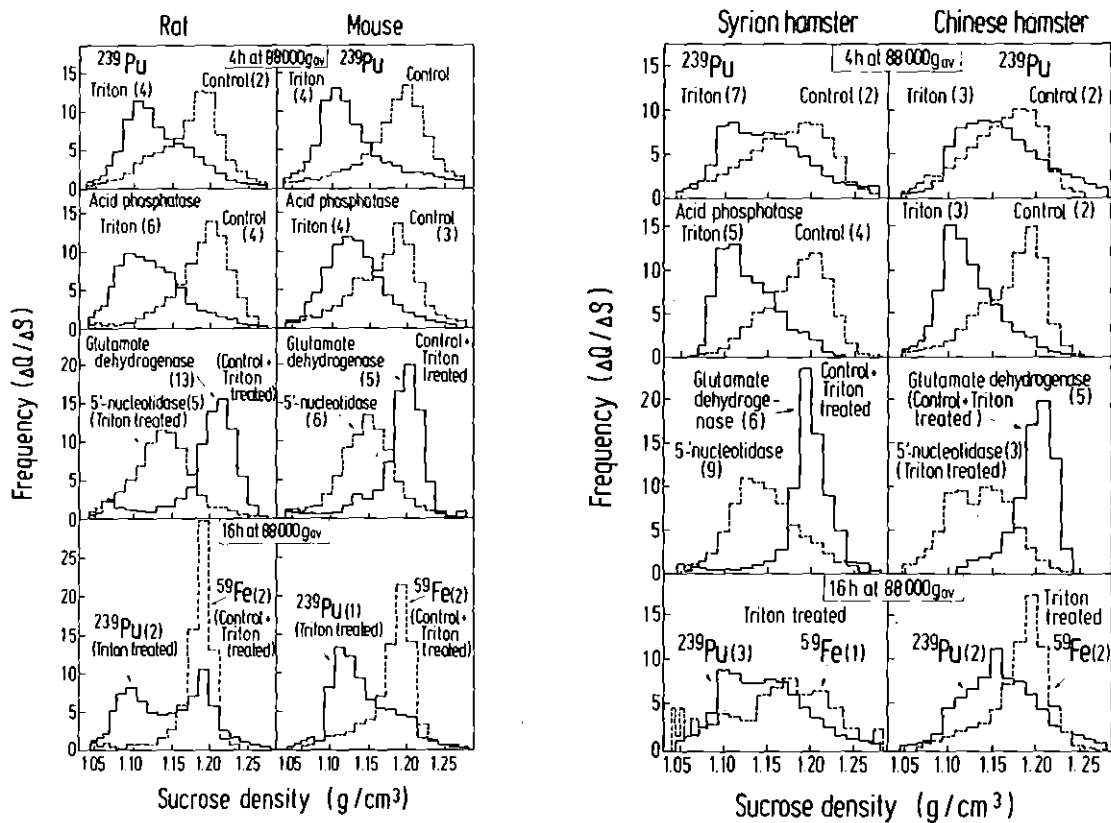
Fig. 1 The distribution of radioactivity and marker enzymes
after sucrose gradient centrifugation of the post-nuclear
supernatants from the livers of control rats, mice,
Syrian hamsters and Chinese hamsters and from animals
injected with Triton WR-1339 4 days previously. All
animals were killed 10 days after ^{239}Pu citrate injec-
tion. Δq is the fractional amount of the constituent
present in the fraction and Δp is the density increment
from fraction to fraction. Figures in parentheses are
number of experiments performed.

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These isopycnic centrifugation profiles measured 10 days after plutonium injection show certain results which are common to all four species but also some important differences. The shift of the lysosomal marker enzyme, acid phosphatase, activity towards a lower density after Triton WR-1339 injection is very similar and in all four species the profile of the mitochondrial marker, glutamate dehydrogenase, is unaffected by Triton WR-1339. However, whereas the plutonium profiles in the rat and mouse closely parallel the acid phosphatase shift, in the two hamster species the profiles are broader and the proportion of the plutonium which is shifted unequivocably is considerably smaller than in the rats and mice. The data obtained following concomitant injection of ^{239}Pu and ^{59}Fe indicate a clear difference between the deposition of iron and plutonium in the liver cell in all four species.

These results provide clear evidence that in the rat and mouse, and probably in Syrian hamsters, plutonium is associated with lysosomal structures, and that neither mitochondria nor the plasma membrane play any major role in binding plutonium. However, in the Chinese hamster the situation is equivocal.

Recent studies using Metrizamide gradient centrifugation do show clear evidence for an association of plutonium with lysosomes in the Chinese hamster liver, at least on the tenth day after injection, but the results of the more detailed studies over a larger time period after plutonium administration, which are currently under way, are needed in order to clarify the binding site for plutonium in the Chinese hamster liver.

The role of lysosomes in the binding of plutonium in other tissues is not yet clear neither is the chemical form in which the element is bound within the lysosomes, or the transport mechanisms from the blood, through the cell membrane into the lysosomes. Preliminary studies of the relationship between the plutonium deposition in the soft tissues of rats and the activity of lysosomal enzymes in the tissues suggest that for both plutonium and thorium there is a strong positive-correlation between actinide uptake and lysosomal enzyme activity, a similar relationship has been demonstrated for gallium in rats (Hammersley and Taylor 1979). Thus the association of hydrolysable heavy metals with lysosomes may be a common

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detoxification process in most tissues. However, further studies which are currently in progress are needed to establish this and to elucidate the transport and binding mechanisms for the different actinides and other metals.

References:

(1) Taylor, D.M., Health Phys. 22, 575 (1972).

(2) Hammersley, P.A.G., Taylor, D.M., Europ. J. Nucl. Med. 4, 261 (1979).

DECORPORATION OF PLUTONIUM AND THORIUM IN HAMSTER AND RAT

V. Volf, E. Peter, M. Lehmann

In contrast to plutonium for which there exists a large amount of experience, obtained in our own as well as in other laboratories (1), there are few data available for thorium. During pilot studies it soon became evident that in spite of the chemical similarities between plutonium and thorium the latter can not be always considered as a suitable biological model for plutonium. The upper part of Table 1 shows that a much smaller fraction of administered carrier-free ^{234}Th is taken up by the liver of control rats in comparison with ^{239}Pu. Furthermore, while ^{239}Pu can be relatively easily removed from the rat liver by Ca-DTPA, the fractional removal of ^{234}Th is even less than that from bone. These findings indicate that different binding mechanisms might operate for ^{239}Pu and ^{234}Th in rat tissue.

The lower part of Table 1 summarizes both our previous results with mixed ligand treatment of plutonium (2) and recent data obtained with thorium. These studies were initiated following the world wide publicity on a method removing completely plutonium from bone (3). Although the original animal data were later withdrawn by the senior author, the validity of the underlying working hypothesis for mixed ligand therapy, which was based on in vitro binding experiments between mixed ligands with thorium has been re-stated recently (4). Therefore, we tested some of the recommended mixtures with thorium in vivo. However, neither the combination of DTPA with sodium salicylate (aspirin) nor with pyrocatechol (catechol) or pyrocatechol disulphonate (Tiron) was more effective than DTPA alone. Although the experiments with ^{234}Th and ^{239}Pu were performed under somewhat different conditions (earlier treatment with higher chelate doses and a double molar excess of secondary ligands in the case of ^{234}Th), it is evident that there is no reason to assume any enhanced effect due to any of these chelate mixtures. The effectiveness of the hydroxamic acid derivative desferrioxamine (DFOA) and of its combination with Ca-DTPA for the removal of plutonium has been characterized in detail previously (1). Studies with thorium have shown that there

Table 1. Effect of prompt single treatment with Ca-DTPA or its combinations with other ligands on the retention of ^{234}Th and ^{238}Pu in female rats*

Group	Experiments with thorium			Experiments with plutonium		
	Chelate dose (µmol/kg)	% of injected ^{234}Th dose		Chelate dose (µmol/kg)	% of injected ^{238}Pu dose	
		Skeleton	Liver		Skeleton	Liver
Controls	0	90.1 ± 2.4	4.5 ± 0.2	0	61.8 ± 0.7	16.0 ± 0.4
Ca-DTPA	50	33.6 ± 2.1	2.9 ± 0.1	15	33.6 ± 1.6	9.2 ± 0.8
	100	18.6 ± 0.8	2.3 ± 0.2	30	23.9 ± 1.6	5.6 ± 0.3
Ca-DTPA+DFOA	50 + 50	24.6 ± 1.2	5.9 ± 0.2	15 + 15	17.1 ± 2.0	4.0 ± 0.8
Ca-DTPA+salicylate	50 +100	40.0 ± 2.3	2.6 ± 0.02	15 + 15	24.8 ± 3.7	7.6 ± 0.8
Ca-DTPA+catechol	50 +100	35.6 ± 2.4	2.5 ± 0.04	15 + 15	37.3 ± 0.8	8.4 ± 0.4
Ca-DTPA+Tiron	50 +100	46.9 ± 2.2	3.0 ± 0.04	15 + 15	28.5 ± 3.3	9.9 ± 1.1

*Chelates were injected i.p. at 1.5 min post i.v. injection of carrier-free ^{234}Th-nitrate and s.c. at 1.5 hr post i.v. injection of ^{238}Pu-citrate. Rats were sacrificed 7 d later. Arithmetic means ± S.E. of at least five animals. Skeleton: 1 femur times 20.

Table 2. Effect of delayed repeated treatment with Ca-DTPA, Ca-Puchel or their combination on the retention of ^{239}Pu in rats and hamster*

Group	Experiments on rats			Experiments on hamsters		
	Chelate dose (µmol/kg)	% of injected ^{239}Pu dose		Chelate dose (µmol/kg)	% of injected ^{239}Pu dose	
		Skeleton	Liver		Skeleton	Liver
Controls	0	62.8 ± 4.5	10.1 ± 1.2	0	36.6 ± 0.8	42.4 ± 2.7
Ca-DTPA	100	48.3 ± 3.6	3.7 ± 0.8	200	26.6 ± 1.9	3.3 ± 0.5
Ca-Puchel	100	56.6 ± 5.2	8.0 ± 1.4	200	24.0 ± 1.6	6.5 ± 0.3
Ca-DTPA + Ca-Puchel	50 + 50	51.1 ± 2.3	3.6 ± 0.5	100+100	21.2 ± 1.1	3.9 ± 0.4

* Five daily s.c. injections of the chelates beginning 4 d after ^{239}Pu-citrate (i.v. in female rats, s.c. in male hamsters). Animals were sacrificed 11 d post ^{239}Pu injection. Arithmetic means ± S.E. of five animals. Skeleton: 1 femur times 20 (rats) or times 21.5 (hamsters).

is a definite, partly additive effect of DTPA plus DFOA on ^{234}Th only in the skeleton, but that the retention in the liver is even higher than that in control animals. Thus, in addition to the poor effectiveness of a large dose of DFOA which has been reported previously (5), it appears that also the administration of DFOA with DTPA does not result in substantially enhanced effectiveness. It seems therefore unlikely that mixed complex formation with thorium occurs. From the practical point of view no advantage may be expected by combining DTPA with DFOA for the decorporation of thorium.

A lipophilic derivative of DTPA with the code name Puchel has been reported to be extremely effective for the removal of plutonium from hamster lung and liver (6, 7). We tested this substance recently with ^{234}Th in rats (8). The results indicate (Fig. 1) that Puchel, alone or in combination with DTPA is less effective than an equimolar amount of DTPA alone. Moreover, Puchel actually increases the retention of ^{234}Th by the rat liver, and this becomes even more pronounced after repeated administration. Our experiments with Puchel and ^{239}Pu (Tab. 2) suggest that in rats Puchel does not cause any elevation of the ^{239}Pu content in the liver, in contrast to the observation with ^{234}Th. However, also with ^{239}Pu Puchel alone is less effective than DTPA and so is the combination of Puchel and DTPA.

Our data after injection of ^{239}Pu and Puchel in Chinese hamsters parallel those obtained from rats. Again they indicate that even with ^{239}Pu in the liver, Puchel alone or in combination with DTPA is no more effective than DTPA alone. Thus we could not confirm the results obtained at Harwell. However, these authors administered only single doses of Puchel to Syrian hamsters and in our preliminary experiment with this substance we also observed significantly greater removal of ^{239}Pu from hamster liver than when DTPA was administered as single injection 4 days after ^{239}Pu. Thus it seems that the favourable effect of the first Puchel administration is more than outweighed by the lower effectiveness of succeeding treatments.

In conclusion, after a thorough investigation Puchel seems to have no advantage over DTPA and does not enhance the effect of DTPA on injected plutonium or thorium in hamsters and rats. Since it is

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LIVER

☐ DTPA
▨ PUCHEL
▩ DTPA + PUCHEL

Th-234 RETENTION (% OF CONTROL)

200
180
160
140
120
100
80
60
40
20
0

SKELETON

100
80
60
40
20
0

EARLY TREATMENT   LATE TREATMENT   REPEATED TREATMENT

PROTEIN CONCENTRATION ΔE_{280}

RADIOACTIVITY [cpm/3ml]

^{59}Fe   (a)

^{234}Th   (c)

FRACTION NUMBER

PROTEIN CONCENTRATION ΔE_{280}

RADIOACTIVITY [cpm/2ml]

NaCl - GRADIENT [M]

^{59}Fe   (b)

^{234}Th   (d)

FRACTION NUMBER
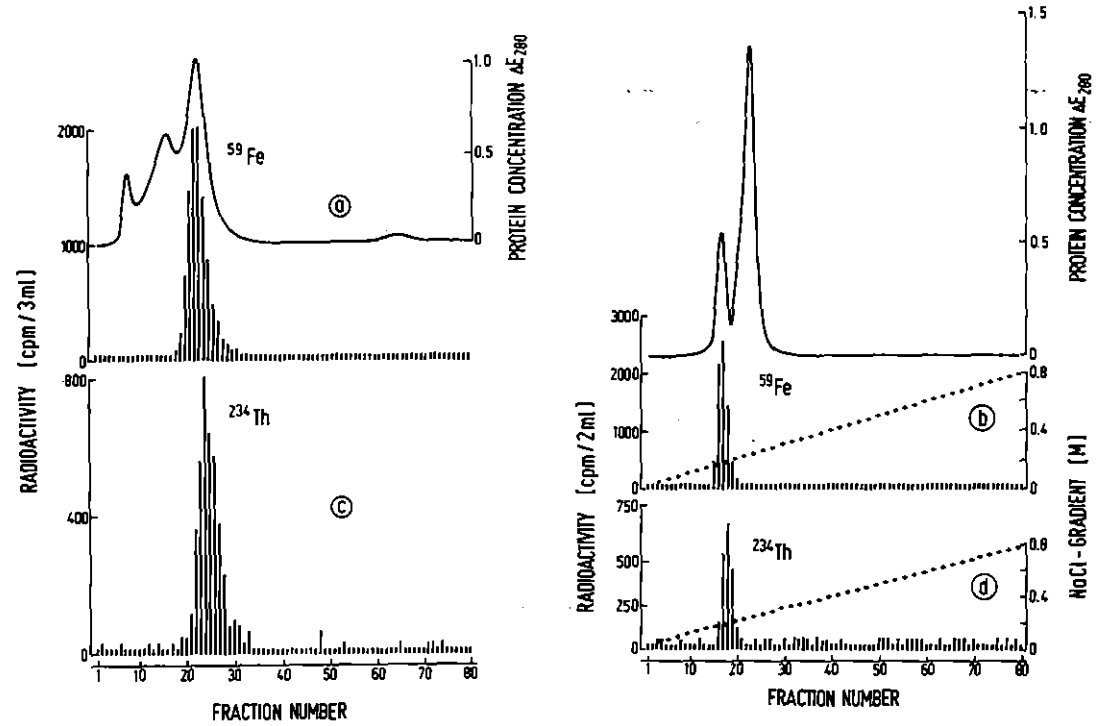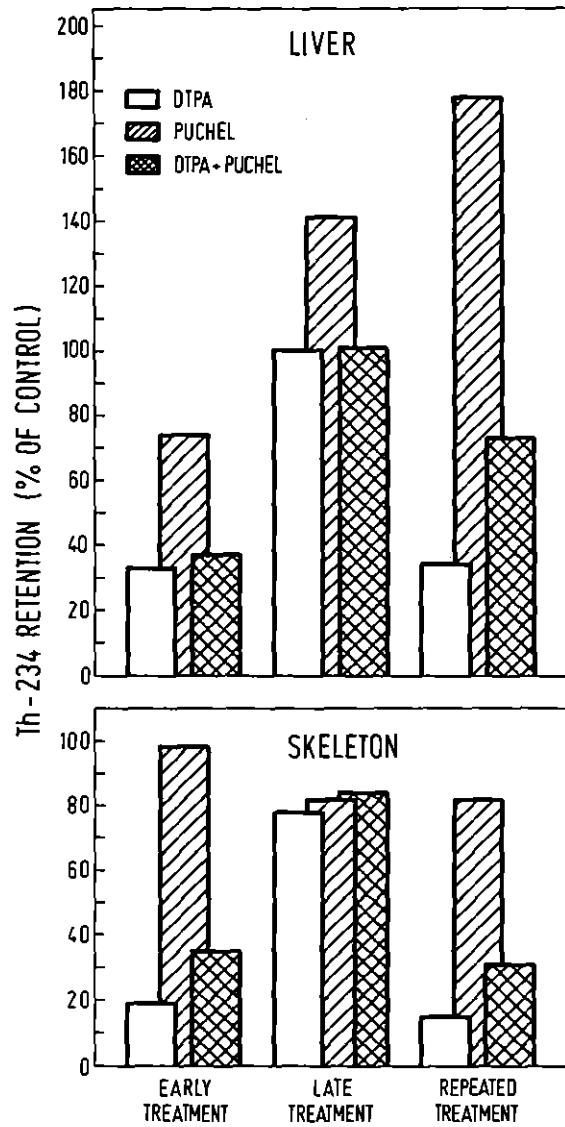
Fig. 1   The influence of single and multiple treatments with Puchel and or DTPA on the retention of ^{234}Th in the rat.

Fig. 2   The distribution of ^{234}Th and ^{59}Fe among rat serum proteins after chromatograhy on Sephacryl S-200 (a+c) and among the transferrin/albumin fraction after further chromatography on DEAE-Cellulose.

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certainly not less toxic than Ca-DTPA, there is at present no reason to recommend it for treatment of these radionuclides circulating in blood and/or deposited in internal organs.

On the other hand, experience gained with Puchel is of considerable interest from the point of view of the mechanism of chelate action as well as of binding mechanisms for radionuclides in the liver. The different response to Puchel of thorium and plutonium in rats (reported here) as well as the different excretion patterns of plutonium after Puchel and DTPA (reported from Harwell) indicate the need for comprehensive studies to determine the ability of various chelating agents to remove different, but chemically related radionuclides and of the usefulness for detecting difference in the fractions bound by the liver.

In spite of the dissimilarities shown above in the distribution patterns of plutonium and thorium and of their reaction to chelate treatment, their behaviour in blood appears to be similar. The distribution of ^{234}Th was investigated in rat serum (9) by gel-filtration and ion-exchange chromatography and the iron- and plutonium-binding protein transferrin was identified as the thorium-binding protein (Fig. 2). Furthermore, it was shown that after saturation of transferrin in vivo by iron loading with an iron-sorbitol-citrate complex (Jectofer$^{(R)}$), ^{234}Th is bound mainly to albumin and perhaps other serum constituents, but not to transferrin. Thus, iron apparently competes with thorium for the common binding sites, as previously observed in other laboratories with plutonium. Although plutonium and thorium are both bound to transferrin in vivo, there is no reason to suppose that they are necessarily bound to the transferrin binding sites in an identical manner. Further, known difference in the sizes of the Pu(IV) and Th(IV) ions suggest that the stability of the thorium complex may be lower than those of plutonium and iron. Experiments on the influence of iron on the overall behavior of ^{234}Th in the body under various conditions are in progress.

References:

(1) Volf, V., Treatment of Incorporated Transuranium Elements, IAEA, Vienna, Technical Reports Series No. 184 (1978).

(2) Volf, V., Health Phys. $\underline{39}$, 364 (1980).

(3) Schubert, J., Derr, S.K., Nature $\underline{275}$, 311 (1978).

(4) Schubert, J., Trends Pharmacol. Sci. $\underline{2}$, 50 (1981).

(5) Taylor, D.M., Health Phys. $\underline{13}$, 135 (1967).

(6) Bulman, R.A., Stather, J.W., Strong, J.C., Rodwell, P., Griffin, R.J., Hodgson, A., NRPB/R&D3 Report, Harwell, 129 (1979).

(7) Stradling, G.N., Stather, J.W., Ham, S.E., Summer, S.A., Health Phys., in press (1981).

(8) Peter, E., Volf, V., Health Phys., in press (1981).

(9) Peter, E., Lehmann, M., submitted for publication (1981).

THEORETICAL CONSIDERATIONS ON BONE REMODELLING AND OSTEOSARCOMA INDUCTION

E. Polig

A theory of bone remodelling

Remodelling may be defined as the process by which old bone is removed by resorption through osteoclasts, replaced by osteoid, laid down by osteoblasts, and calcified to form new bone material. In the adult skeleton the amount of bone resorbed approximately balances the amount formed and thus no net change in skeletal mass or structural integrity occurs. A basic principle stated by Frost (1) says that remodelling occurs in discrete packages of bone called bone metabolic units (BMU). Fig. 1 represents in a highly schematic and simplified form a segment of bone surface made of BMUs. In order to describe the remodelling of cancellous bone by means of a mathematical model the basic assumptions adopted by Kimmel and Jee (2) in their Monte Carlo calculation of 239-Pu deposition have been used. BMUs are assumed to have a lifetime t_L (fig. 1), determined by some stochastic process of ageing, and to be replaced during a time t_{RA} by the resorption and apposition of new bone. The stochastic law of ageing is not known at present, but if one sets the probability $p(dt)$ of a BMU being remodelled within an interval dt after it has existed for a time t

$$p(dt) = \lambda t^\beta dt \qquad (1)$$

then the nature of the remodelling process can be specified by choosing an appropriate value of β. Eq. 1 comprises the limiting case for both probabilistic ($\beta = 0$) and linear remodelling ($\beta \to \infty$) as defined by Frost (3). Eq. 1 and the associated value of β can be used to give these somewhat vaguely defined terms a precise mathematical meaning.

From the above equation the differential equation

$$\frac{dp}{dt} = \lambda(1-p(t))t^\beta \qquad (2)$$

may be derived which yields as solution the cummulative distribution function for the lifetime of BMUs.
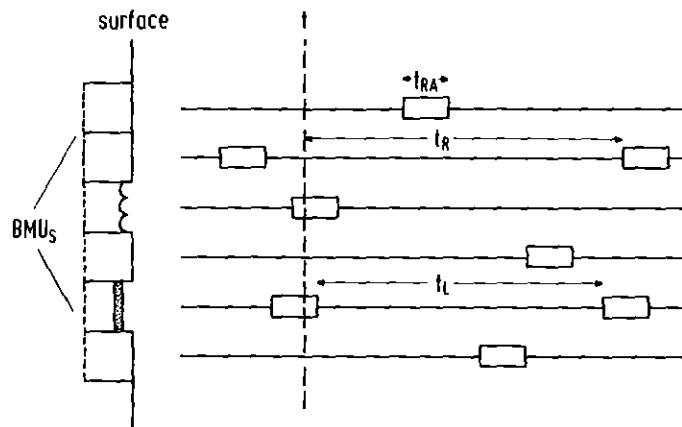
Fig. 1   Time scale of remodelling of BMUs showing lifetime t_L,
residual lifetime t_R and the time for resorption and
apposition t_{RA}. The scheme on the left represents the
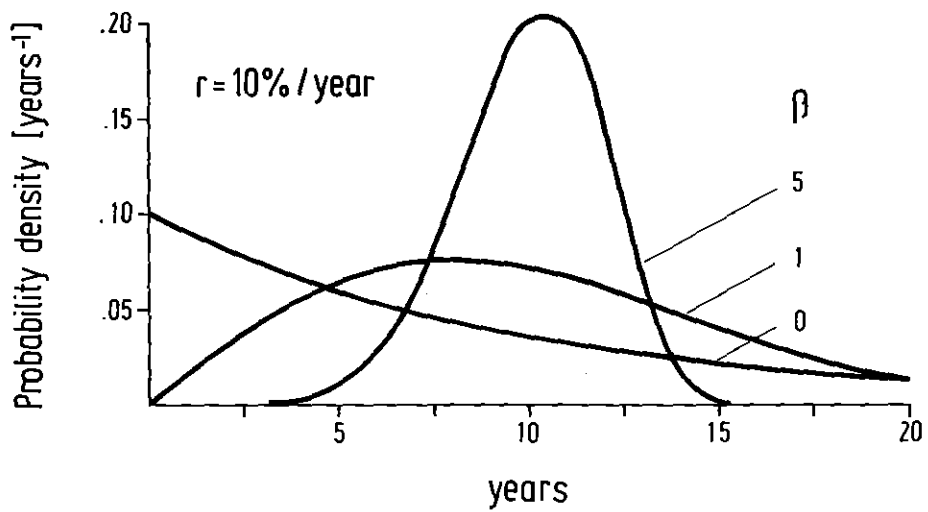associated status of BMUs.

Fig. 2   Probability density $\phi(t)$ for the lifetime t_L of BMUs
for different values of β, characterizing the age
dependence of remodelling.

$$p(t) = 1 - \exp\left(- \frac{\lambda}{\beta+1} t^{\beta+1}\right) \qquad (3)$$

Differentiation and normalization of eq. 3 yields the more illustrative probability density of lifetimes

$$\phi(t) = (\beta+1)\left(r\Gamma\left(\frac{\beta+2}{\beta+1}\right)\right)^{\beta+1} t^{\beta+1} \exp\left(-\left(r\Gamma\left(\frac{\beta+2}{\beta+1}\right)t\right)^{\beta+1}\right) \qquad (4)$$

which specifies the fraction of BMUs $\phi(t)dt$ that are being resorbed within a time interval dt after they have existed for a time t ($\Gamma(\cdot)$ is the gamma function). In the derivation of eq. 4 use was made of the relationship

$$\mu = \frac{1}{r} = \int_0^\infty t\phi(t)dt \qquad (5)$$

which relates the mean lifetime μ to the bone turnover rate r. It is also assumed that the time interval t_{RA} (Fig. 1) for resorption and formation is small compared to $\mu \cdot \phi(t)$ is identical to the exponential distribution for probabilistic remodelling (Fig. 2). In this case remodelling is not age dependent. For increasing β, $\phi(t)$ as illustrated in fig. 2 for a turnover rate of 10 %/year becomes more peaked around the mean lifetime μ.

An important parameter related to the problems of the deposition of radionuclides onto bone surfaces is the residual lifetime t_R (Fig. 1) of the BMUs. This is the interval from a particular time defined by some event such as the injection and deposition of the nuclide in the skeleton, until resorption. In other words it is the residence time of a surface deposit on a particular BMU. The derivation of the probability density for the residual lifetimes $\rho(t)$ rests on the stochastic theory of renewal processes. As a result one obtains the expressions

$$\rho(t) = r \exp\left(-\left(rt\Gamma\left(\frac{\beta+2}{\beta+1}\right)\right)^{\beta+1}\right) \qquad (6)$$

and for the mean residual lifetime τ.

$$\tau = \frac{(\beta+1) \; \Gamma \; (2/(\beta+1))}{r\Gamma \; (1/(\beta+1))} \qquad (7)$$

For probabilistic remodelling ($\beta=0$) eq. 7 yields $\tau=1/r=\mu$, i.e. the mean residual lifetime is equal to the mean lifetime. This means the residual lifetime of an individual BMU is independent of its past. For $\beta>0$ the mean residual lifetime is longer (!) than the mean lifetime. This apparent paradox is due to length biased sampling. For increasing β, $\rho(t)$ approximates to a step function (Fig. 3) which is to be expected for linear remodelling. In this limiting case each BMU has a definete lifetime between two remodelling cycles and the residual lifetimes are uniformly distributed between 0 and μ. It should be noted from fig. 3 that the stronger the age dependence of remodelling for a constant turnover rate, the more shorter residual lifetimes are favoured, as it becomes more and more improbable that a BMU will survive for prolonged times. In conclusion it should be noted that the theory outlined above does not take into account the variations with age of parameters such as turnover rates and t_{RA}. This might be an undue simplification in the case of the immature skeleton.

Induction of osteosarcomas

The malignant transformation of osteogenic cells has been studied using the results of the theory of remodelling above. As the mechanism of osteosarcoma induction is not known at present, a specific hypothesis was tested which stated that the cells at risk are located close to bone surfaces and that the nucleus is the sensitive region containing two sensitive targets either of which must be hit by at least one α-particle to induce transformation (induction). It is further postulated that only the initial surface deposition of the nuclide induces malignancies, and then only until its resorption, and that osteogenic cells are resting on quiescent BMUs until a remodelling cycle starts. Upon receiving the signal for re-building a resorbed BMU a tumour may then start to grow immediately from a single transformed cell (promotion). The assumption of a two target initiation seems to have a sound basis from current experimental data (4). For the sake of simplicity the nucleus was approximated by a sphere of 3.16 μm radius (r_n) with the sensitive targets characterized by a transformation cross section σ and located at random within the sphere (Fig. 4). For parallel beam incidence the probability of one target p_h being hit
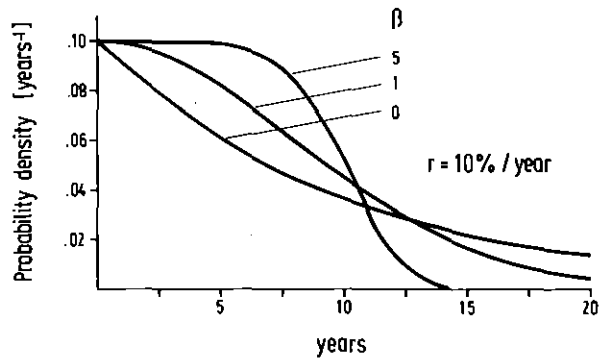
Fig. 3 Probability density ρ(t) for the residual lifetime t_R of BMUs for different values of β, characterizing the age dependence of remodelling.
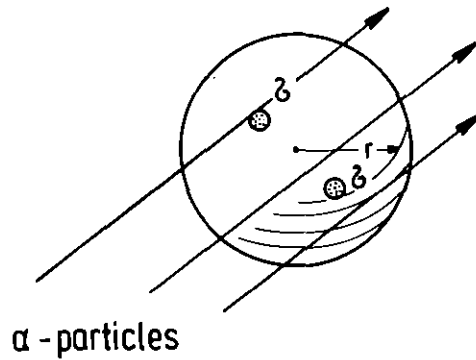
α -particles

Fig. 4 Schematic representation of two targets with transformation cross section σ located at random inside a spherical nucleus that is crossed by α-particles.
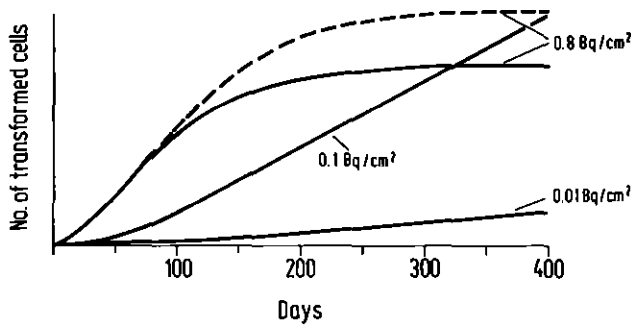
Fig. 5 Number of transformed cells (relative scale) as dependent on time after contamination of the surfaces (239-Pu) for different values of specific surface activity (β=0). The dashed line is for β=5 (age dependent remodelling). Remodelling rate = 100 %/year, p_k = 0.8.

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by a particle crossing the sensitive volume is then

$$p_h = \frac{3\sigma}{2\pi r_n^2} \qquad (8)$$

The parallel beam approximation is also acceptable in the case of a nucleus touching a radioactive surface, as for instance with 239-Pu alphas where more than 53 % of the particles hitting the nucleus originate from a distance of more than 10 μm away. For small σ the probability of hitting both targets if ν particles are crossing the nucleus can be shown to be $\approx \nu^2 p_h^2$. This then leads to the overall induction probability

$$p_i = p_h^2 \sum_{\nu=1}^{\infty} \nu^2 p_\nu p_s^\nu \qquad (9)$$

where p_ν is the probability of ν hits to the nucleus and p_s the probability that a cell receiving a nuclear hit survives. Values of p_s are ranging from 0.14 to 0.95 (5). Flat cells may survive even after traversal by more than 14 particles (7). p_ν is a function of the residual lifetime of the BMUs. Employing a result derived earlier and the probability distribution of residual lifetimes $\rho(t)$ one gets

$$p_\nu = \frac{(aG)^\nu}{\nu!} \int_0^t t'^\nu \exp(-aGt')\rho(t')dt' \qquad (10)$$

where 'G' is a geometric factor determined by the size of the nucleus and its position relative to the surface and 'a' is the specific surface activity in disintegrations/unit area. Insertion of eq. 10 into eq. 9 and some arithmetic manipulation finally yields the expression

$$p_i = p_h^2 (\frac{1}{p_k^{-1}}) p_1(a,t,r,\beta,G') + 2\ p_h^2 (\frac{1}{p_k^{-1}})^2 p_2(a,t,r,\beta,G') \qquad (11)$$

for the probability of tumour induction.
As is indicated in the list of arguments for $p_{1,2}$ eq. 10 has to be evaluated using geometry factor $G' = G\ p_k$ where $p_k = 1 - p_s$ is the probability of killing. In other words this means that for the kinetics of tumour induction, cell killing can be accounted for by using a corrected value G' instead of the true geometric factor. The first and the second term in eq. 11 may be interpreted as

representing the production of malignant lesions from one hit and more than one hit to the nucleus, respectively.

In this respect the result is analoguous to the intratrack and intertrack contributions in the Kellerer and Rossi Theory of dual radiation action. The kinetics of transformation for 239-Pu alpha particles resulting from eq. 11 is shown in fig. 5 for a turnover rate of 100 %/year. This value is typical for adult rats. With increasing specific surface burden transformed cells appear earlier. At later times, however, for a high specific activity the effect may be less than for a lower surface burden due to cell killing. The curve for 0.8 Bq/cm^2 in fig. 5 predicts a shorter latency period than was found experimentally (7). A calculation for a = 0.03 Bq/cm^2 and r = 100 %/year, both values typical for some dog experiments, yields a median apperance time of about 600 days. Age dependence of remodelling tends to increase the number of lesions over that for probabilistic remodelling.

The variation of the transformation probability with the turnover rate depends on the level of specific surface activity (Fig. 6). For high surface burdens and probabilistic remodelling the yield of lesions increases with turnover rate; for low surface burdens it is approximately constant above 100 %/year, for intermediate values there is an increase for small and a decrease for large values of r. This effect of displaying a maximum is even more pronounced for age dependent remodelling (Fig. 6). It should be noted at this point that different bones in the skeleton may have widely differing turnover rates. At 0.03 Bq/cm^2 and for a time of 3000 days, which is typical for the time span over which osteosarcomas appear in dogs, the model predicts that skeletal sites with the lowest turnover rates should display the highest tumour incidence. This is definitely at variance with experimental observations (2). Assuming a mean skeletal turnover rate of 2 %/year in man, 50 %/year in dogs and 400 %/year in rats and mice eq. 11 yields the scale of risks depicted in fig 7. The curves refer to the quantity $p=p_i/p_h^2$. For decreasing turnover rates the specific surface burdens for maximum effect shift to lower values, man being the most sensitive species in that respect. Fig. 7 however represents a relative scaling, neglecting the overall sensitivity of the endosteum, characterized by the product number cells at risk X p_h^2. On the basis of Mole's hypothesis of equal sensitivity of the endosteum and using a formula relating 'a' to the specific
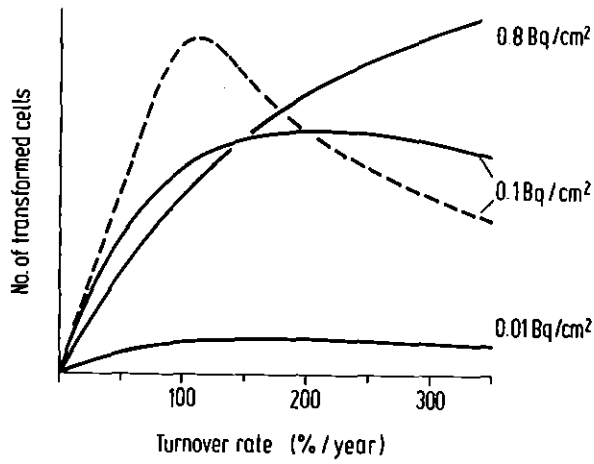
Fig. 6 Number of transformed cells (relative scale) as a function
of the turnover rate for 239-Pu α-particles and different
specific surface activities (β=0). Dashed line: β=5.
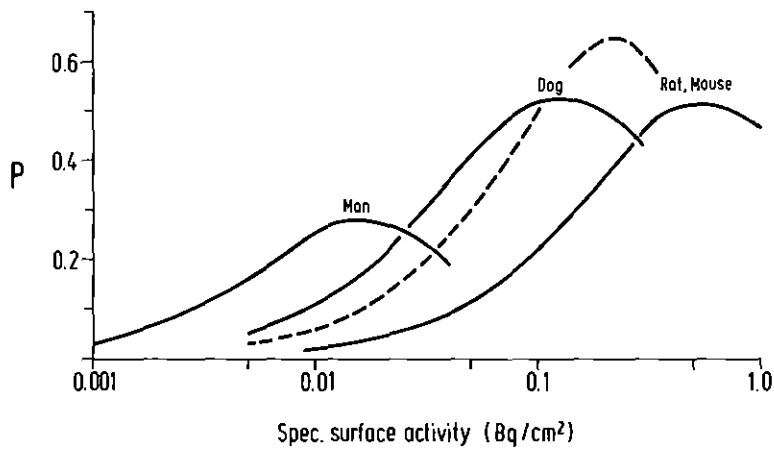
Fig. 7 The quantity $p = p_i/p_h^2$ (see text) as a function of the
specific surface activity. p is the number of transformed
cells per unit endosteal sensitivity. Solid curves: β=0
(probabilistic remodelling), broken curve: β=5 (age
dependent remodelling).
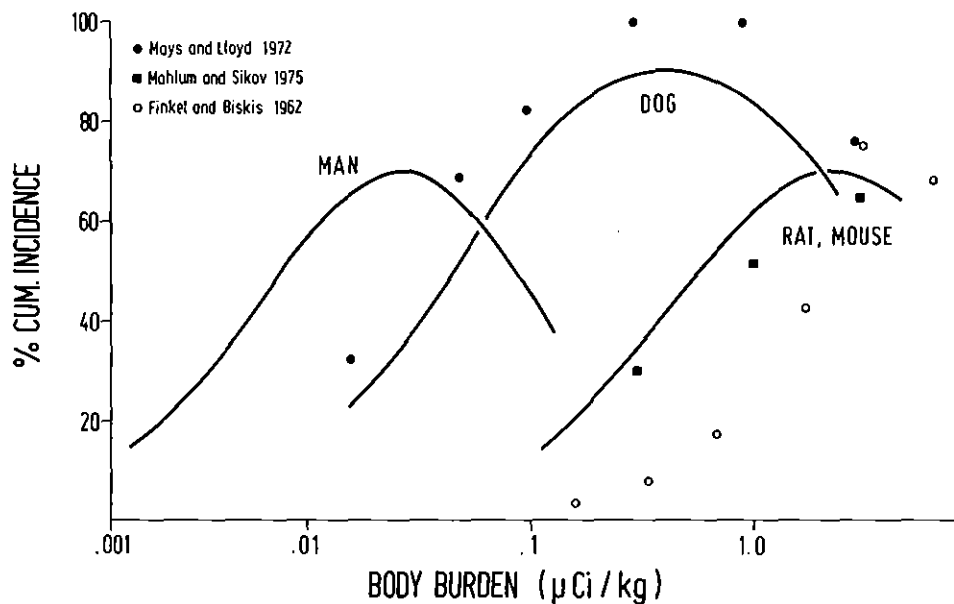
Fig. 8 Cummulative tumour incidence (per individual) as dependent
on body burden for four species. The curve for man assumes
an endosteal sensitivity equal to that of the dog.
Experimental points: ● dog, ■ rat, o mouse.

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dose of 239-Pu injected one might venture into calculating an
absolute risk as done in fig. 8. A mean surface/volume ratio of
500 cm^{-1} and 200 cm^{-1} was assumed for rat, mouse and dog, man,
respectively. The calculations take into account the occurence
of multiple tumours and roughly fit the experimental results.

Summary and conclusion

The postulate that osteogenic tumours from surface seeking α-
emitters are caused by the initial surface deposits irradiating
resting osteogenic cells only until the first remodelling cycle
at a site occurs, leads to tumours appearance times shorter than
observed experimentally, even if the time for tumour growth is
taken into account. Contrary to experimental results it predicts
a high incidence for low turnover sites, under conditions similar
to those in some dog experiments. However, the cummulative
incidences as a function of the specific body burden are in
approximate agreement with the experimental data. From these
discrepancies it may be suspected that factors other than those
stated in the original hypothesis play a decisive role in the
induction of osteosarcomas. These could be for instance that
diffuse deposits may at later stages contribute to cell trans-
formation and or that the promotion of induced cells requires
more than are remodelling cycle.

References:

(1) Frost, H.M., In Bone Biodynamics. H.M. Frost (Ed.). Little,
    Brown and Co., Boston, 1964.

(2) Kimmel, D.B., Jee, W.S.S., Rep. COO-119-254, p. 55 (1979).

(3) Frost, H.M., Mathematical Elements of Lamellar Bone Remodelling.
    Charles C. Thomas, Publisher, Springfield, 1964.

(4) Marshall, J.H., Groer, P.G., Radiat. Res. 71, 149 (1977).

(5) Elkind, M.M., Whitmore, G.F., In The Radiobiology of Cultured
    Mammalian Cells. Gordon and Breach, New York, 1967.
(6) Lloyd, E.T. et al., Int. J. Radiat. Biol. 35, 23 (1979).
(7) Bensted, J.P.M. et al., Brit. J. Radiol. 38, 920 (1965).

BONE TURNOVER-RATE IN THE MIDSHAFT OF THE FEMUR - A COMPARATIVE STUDY OF MALE AND FEMALE RATS

W. Sontag

Male and female rats differ widely in bodyweight, shape and size. After incorporation of plutonium-239 male rats have a higher osteosarcoma incidence than female rats at the same age and the same dose per unit of bodyweight (1,2). One of the parameters which may influence the tumour incidence is the bone turnover-rate. In order to study this possible influence an automatic microspectro-photometric scanning method following vital staining of growing bone was developed (3) and used to examine rats of both sexes over a period of between 60 days and 820 days. In this comparative study, only the differences in the fundamental parameters bone volume, surface/volume-ratio, appositional rate, formation rate, and resorption rate, in the femur midshaft, considered as an example of cortical bone, are discussed.

Male and female rats of the Heiligenberg strain in groups of the same age but different bodyweight receive one or several injections of calcein. At different times after the first injection two animals were killed, the midshaft of the femur was fixed, embedded in methyl-methacrylate and cut into about 80 micrometer thick sections on a Leitz sawing-microtome. The sections were analysed with a Leitz microphotometer controlled by a minicomputer (PDP 8/E) and the volumes of the total bone, the bone marrow, the newly forming bone, and the bone surface were calculated. Additionally the shape and diameters of the midshaft were measured by hand between birth and 820 days. From the data for about 180 animals a mathematical model of the midshaft of the femur was developed which reproduced the experimental data within an error of 10 per cent. The data calculated with this model are presented here.

The femur midshaft is per difinition the region between the distal end and the proximal end of the femur where no trabeculae exist but only cortical bone. The length of the midshaft is 30 % of the length of the whole femur. Fig. 1 shows the basis of the midshaft model: The shape of the cross section through the midshaft is
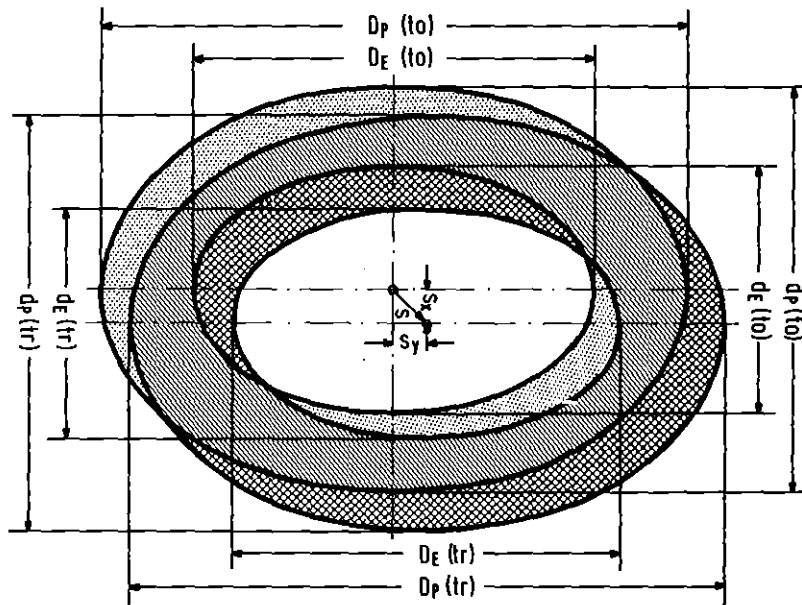
Fig. 1 Basis of the midshaft model: The periosteale and
endosteale surfaces would described by two ellipses,
defined by four diameters D_p, D_E, d_p and d_E and a
shift function S. It is shown the development of the
midshaft between age t_o and t_r, the hatched area is
the old bone, the crossed area is the formed bone and
the pointed area is the resorped bone.

elliptical, thus the time dependence can be described by four functions - two for the periosteal and two for the endosteal diameters. Simultaneously the whole midshaft moves in one transverse direction and lengthens the movements can be described by a shift-function and a longitudinal growth-function. These six functions completely describe the behaviour of the midshaft with time.

Fig. 2 shows the movement of the midshaft in space and time. It is presented as a section through the major axis of the ellipses, whereby the zero point is the midpoint of the ellipses at birth. In this presentation both the shift of the whole midshaft, which has a maximum at birth and decreases with increasing age, and the changes in the endosteal and periosteal diameters with age can be seen. The shift is similar in male and female rats but at the same age the diameter is greater in the male rats. This can be seen better in the next figure (Fig. 3) which shows the total bone volumes and the surface/volume-ratios. Whereas within the experimental error the surface/volume-ratio is equal for both sexes the volume of the male rat is always greater than that of the female rat. 900 days after birth the female rats have only 60 % of the volume of the male rats. This is in agreement with the bodyweight which in the female rats at the same age is only 55 % of that of the male rats. The surface/volume-ratio is very high (18.) in the newborn, but decreases rapidly and reaches a constant value of 4.48 at the age 300 days.

The bone surface can be classified into three types:

Group 1:   The growing surface - where new bone is forming
Group 2:   The resting surface - with no change in the bone
Group 3:   The resorbing surface - from where old bone is removed.

The appositional rate (4) describes the growth of the surface in micrometer per day. From fig. 1 we know that the surface does not growth homogeneously, therefore the appositional rate shown in fig. 4 describes the mean growth rate of the periosteal and endosteal surfaces. The appositional rates are maximal at birth (about 60 μm/d) and decrease rapidly with increasing age until at the end of the life of the lifespan the are only about .1 μm/d. In middle-age (280 days) there is a disturbance in the curves, which comes from
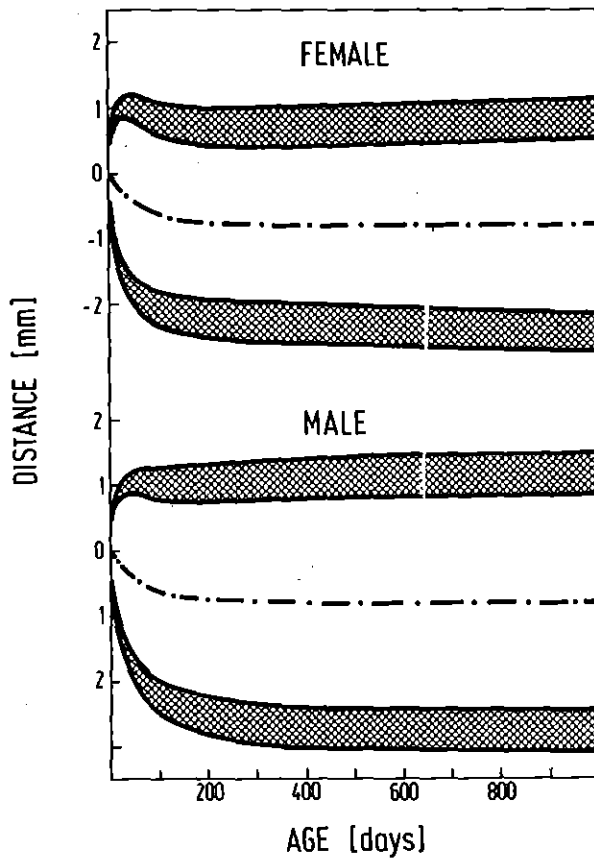
Fig. 2 Schematic description of the movement of the femur-
midshaft in space and time. The picture show a section
through the major axis of the ellipses (see Fig. 1) as
a function of age for both sexes. The point 0 is the
midpoint of the ellipses at birth and the crossed area
is the cortical bone. This presentation shows the
combined movement caused from the shift of the centre
(— · —) and the increase of the inner and outer diameter.
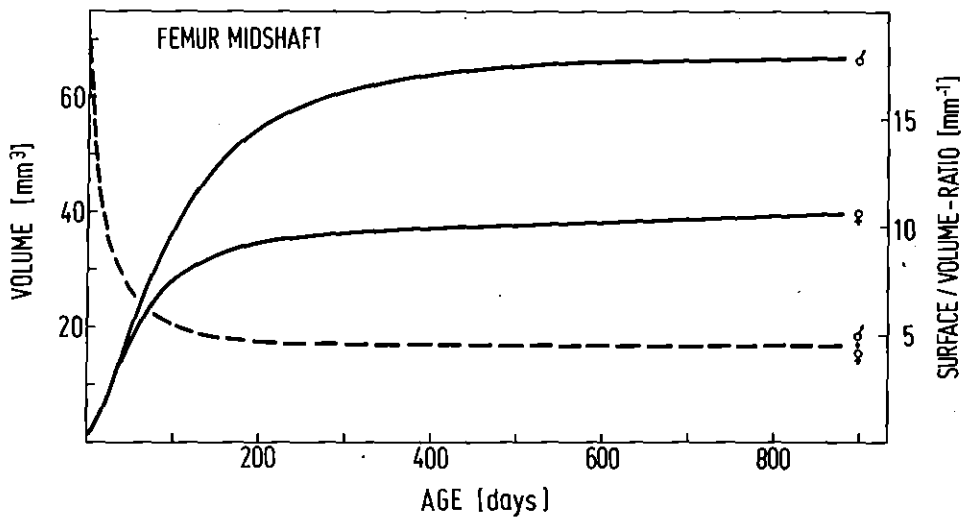
Fig. 3 Bone volume (———) and the surface/volume-ratio (— —)
as a function of age.

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the state of transition between the shift of the midshaft and the growing diameter. The ratio of the appositional growth rate and the appositional resorption rate in both sexes is about 1.5 in the younger animals falling after age 200 days to a constant value of 1. after 600 days.

The newly-forming bone volumes are a product of the appositional rates and the area of the growing bone surface. Over the whole measurement period the growing surface is about 50 % and the resorbing surface is also 50 % of the total area, thus the midshaft is a system with no resting surfaces. The distribution of the growing and resorbing areas on the endosteal and periosteal surfaces is a function of age. In younger animals both surfaces have equal proportions of growing and resorbing areas, but by age 200 days the proportion of growing surface decreases on the endosteal and increases on the periosteal surfaces. whereas the resorbing surface shows the opposite behaviour. After 400 days in female rats, 280 days in male rats, the whole endosteal surface becomes a resorbing area and the whole periosteal surface a growing area.

Fig. 6 shows the formation- and resorption-rates expressed in the conventional way as per cent per year. The curves show the same time course as the appositional rates in fig. 4. The greatest turnover rates occur at birth (50.000) but thereafter rapidly decrease with increasing age to a value of 5 %/y at 900 days, thus between birth and the end of the life the bone turnover rates in the midshaft of the rat femur change by a factor of 10.000. At the same age the resorption rate is always lower than the formation rate, except in old animals, but this difference is not significant; so that we can assume, that after 550 days the formation rates and the resorption rates are equal.

Comparing the data for the two sexes we find that the ratio between male and female rats in the daily appositional rate is about 3.6 at birth, then falls to a constant value of about 0.9 between 50 and 150 days but rises again to a maximum of about 2.7 at 290 days before decreasing again with increasing age to about 1.0. The corresponding ratio for the daily resorption rate is unity at birth, rises between about 200 and 600 days, reaching a maximum of 2.5 at 290 days, and then returns to approximately unity. A similar pattern is seen with the ratios of the yearly formation
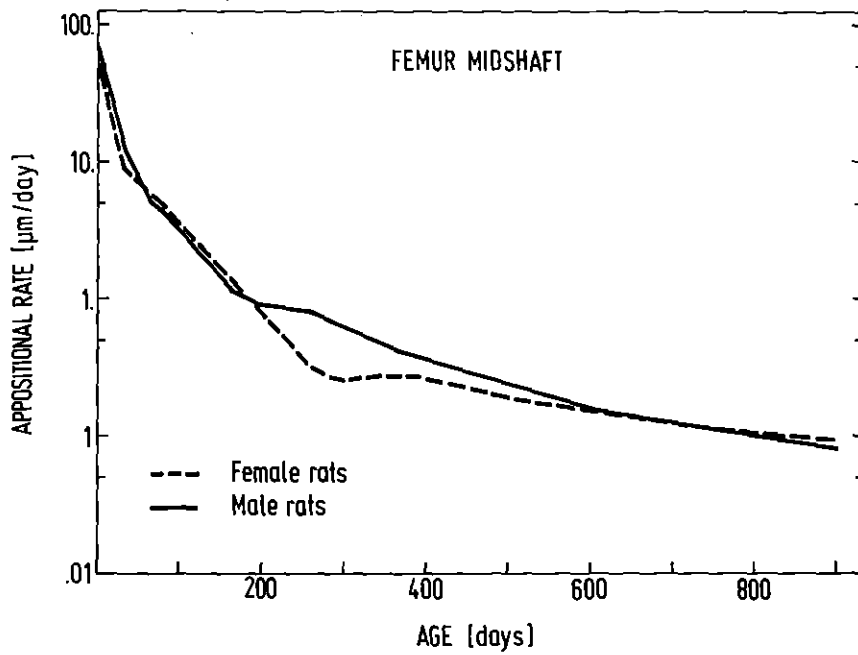
Fig. 4   Appositional rate as a function of age.
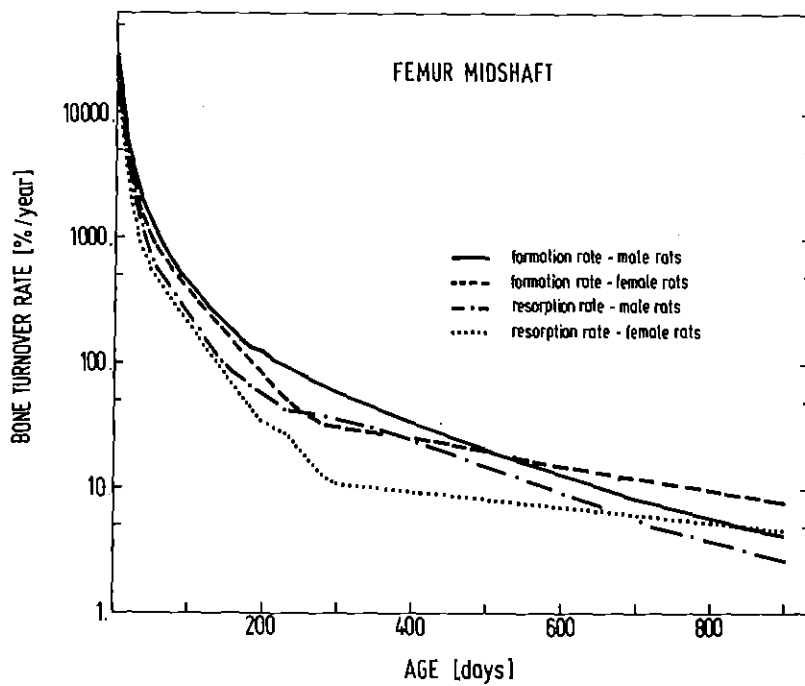
Fig. 5   Formation rate and resorption rate as a function of age.

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and resorption rates. Thus the differences between male and female rats in bone formation and resorption rates are greatest between 250 and 400 days of age, with a maximum value of around 2.5 at 290 days. This is understandable if we look at fig. 2, in the young animals both volume curves do not differ considerably, whereas in the old animals the volume curve of the female rats rises more steeply than the curve of the male rats; in the meantime between 100 days and 400 days we have the greatest disperse of the two curves.

In summary all the parameters measured (Fig. 4 and 5) are very sensitive in relation to the age of the animals having a maximum at birth and decreasing with increasing age over the whole life-span. The formation rate is always greater than the resorption rate, except in the old animals where they are about equal. The yearly formation and resorption rates are always greater in male than in female rats, only in the old animals is the turnover rate higher in the female rats.

The Heiligenberg strain of rat is unique to this laboratory, therefore we have made some experiments with female Sprague-Dawley rats, which are larger and heavier than the Heiligenberg rats, and found that the yearly formation and resorption rates are equal, but the appositional rate is about 10 % greater in the Sprague-Dawley rats. This result is in agreement with those of Stenström et al. (5) in female Sprague-Dawley rats, Raman (6) in male Wistar rats and Aries (7) in male albino rats all of whom reported appositional rates which were about 5 % to 12 % greater than those found in the Heiligenberg strain.

References:

(1) Mahlum, D.D., Sikov, M.R., Influence of age on the development of bone tumors in rats injected with ^{239}Pu-citrate. BNWL-1950 PT1, 79-81 (1975).

(2) Sinyakov, E.G., Radiobiol. 15, 937 (1975).

(3) Sontag, W., Calcif. Tissue Int. 32, 63 (1980).

(4) Frost, H.M., Bone remodelling dynamics. Charles C. Thomas, Springfield, Ill. (1963).

(5) Stenström, A., Hanson, L.I., Thorngren, K.G., Calcif. Tissue Res. <u>23</u>, 161 (1977).

(6) Raman, A., Acta Orthop. Scand. <u>40</u>, 193 (1969).

(7) Aries, L.J., Surgery, Gynec. Obstet. <u>72</u>, 679 (1941).
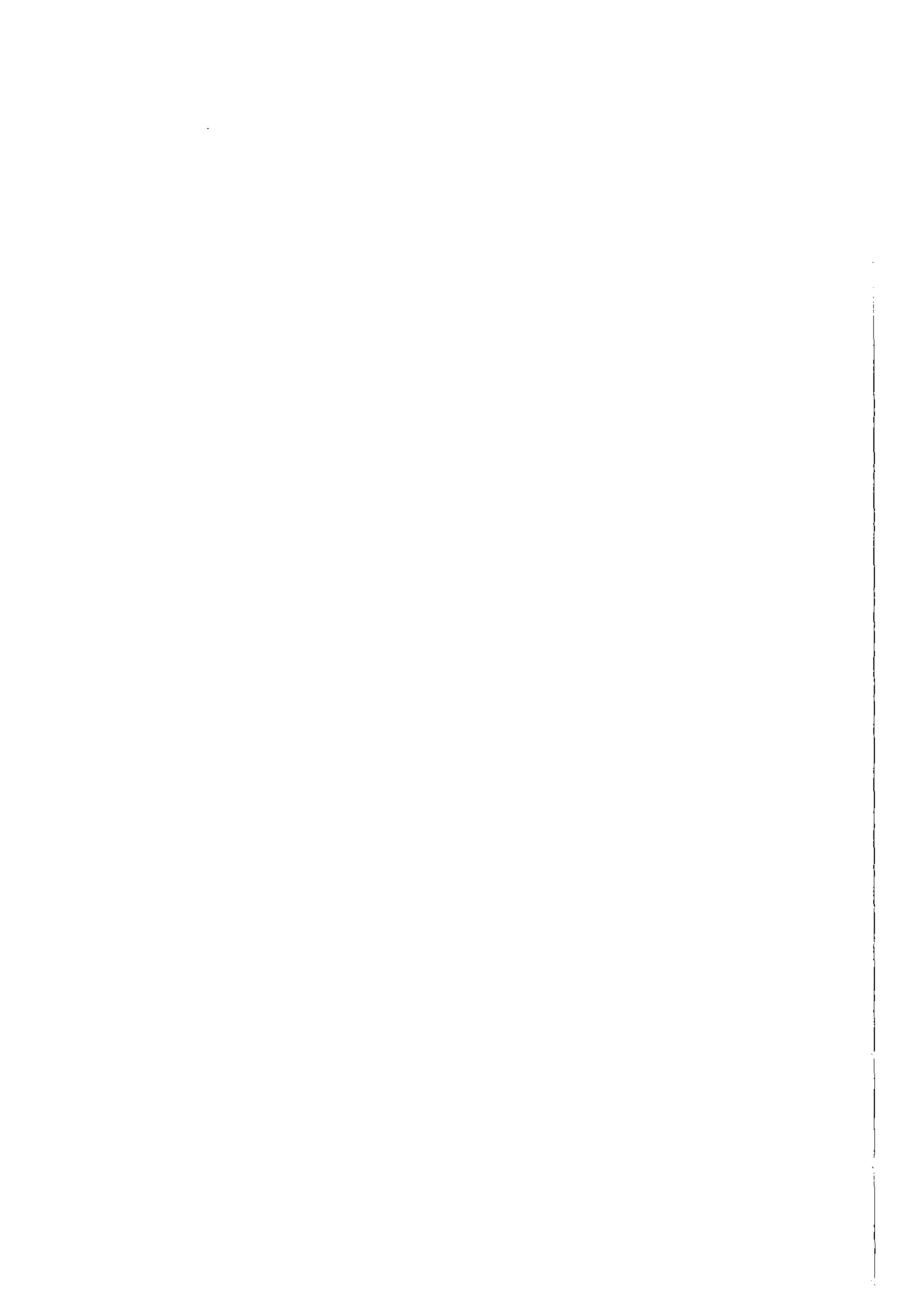
6. Year-round Research (Short scientific reports)

b) Biochemistry of Heavy Metals

DECORPORATION OF MERCURY

F. Planas-Bohne

During the last years investigations have shown that in comparison
to many other chelating agents 2,3-dimercaptopropane-1-sulfonate
(DMPS) shows the highest efficiency for the removal of Hg^{II} from
the mammalian body (1). In contrast to 2,3-dimercaptopropanol
(BAL) which is now used for the treatment of mercury intoxication,
DMPS has some favourable qualities such as low toxicity (2), and
a high absorption from the gastrointestinal tract (3), which
permits the peroral administration of DMPS.

In addition to intoxication with inorganic mercury (esp. Hg^{II}) the
toxicity of organo mercury compounds, especially methylmercury
(CH_3Hg-) was recognised some years ago.

The toxic symptoms of inorganic and organic mercury poisoning are
distinctly different. In the mammalian body organic mercury
compounds such as CH_3Hg do not undergo extensive metabolic degrada-
tion with the result that the biodistribution and tissue binding
patterns are different from those of inorganic mercury. However,
in liver and more especially in the kidneys part of the organic
complex is transformed and within a few hours after the intake
of CH_3Hg both inorganic and organic mercury are found in these
tissues (Fig. 1).

Most of the chelating agents which remove inorganic mercury from
mammalian tissues are ineffective against methylmercury. However,
it was shown, that DMPS could mobilize mercury from methylmercury,
especially from liver and kidneys (4,5). An even better efficacy
was found with 2,3-dimercaptosuccinic acid (DMSA) particularly
in those organs where DMPS is not so effective. However, in the
case of a delayed start of the treatment DMPS removes · much
more mercury from the kidneys than DMSA so that the body burden is
reduced about equally by the two substances (Tab. 1). These
observations led to the supposition that they have a different
mechanism of action. The higher efficiency of DMPS against methyl-
mercury is seen mainly in those organs in which decomposition of
CH_3Hg- to Hg^{II} occurs indicating the elimination of Hg^{II} and further demethyla-

Table 1. The body burden of CH_3HgCl- in rats which received treatment with chelating agents once (experiment I) or 4 times per week for 4 weeks (experiment II). Values are given as % of control animals. Means of 6 animals.

| Experiment | time after CH_3Hg | | DMPS | DMSA | DMPS + DMSA* |
	start of therapy	dissection			
I (total dose: 2 mmol/kg)	1 hr	1 day	57.4	43.5	-
	1 day	2 days	60.1	50.5	-
	4 days	5 "	67.8	57.5	-
	10 "	11 "	71.6	66.3	-
II (total dose: 1.6 mmol/kg)	1 day	25 days	42.2	40.1	-
	7 days	32 "	46.2	45.7	21.4
	14 "	39 "	55.2	51.6	28.8

* total dose 1.6 mmol/kg each

tion of CH_3Hg- by disturbation of the equilibrium between the two fractions. In contrast DMSA mainly (or solely) binds organic mercury. Seperate determinations of inorganic and organic mercury in the liver and kidneys of rats after incorporation of methyl-mercury corroborated these assumptions. These results caused us to investigate the effect of a combined treatment of DMPS plus DMSA. Table 1 shows that such combined treatment can substancially reduce the body burden. The effect is higher than that which on the basis of previous work would have been expected by doubling the dose of either one of the two chelating agents (5). The data in Table 1 also indicate that long term treatment is effective even when started after a delay of 2 weeks. Since, because of the delayed onset of adverse effects methylmercury poisoning is often not recognised until some time after incorporation this is of particular importance.

All animal experiments like these raise the question how can these results be extrapolated to man. As a direct comparison is not possible, model systems like cell cultures are often used. Methylmercury shows a high affinity for erythrocytes, and the possibility of using this system for testing the ability of various compounds to remove mercury from these cells has been tested. An erythrocyte-buffer-system was used as a model to compare the effectiveness of DMPS and DMSA in removing CH_3Hg from human and rat erythrocytes. As in the in vivo studies in rats DMSA was more effective than DMPS in releasing mercury from both rat and human erythrocytes (Fig. 2).

Dimercaptosuccinic acid as well as dimercaptopropanesulfonate are chelating agents which combine high decorporating efficiency in case of mercury intoxication with low toxicity (2,6) and good absorption from the gastrointestinal tract (3). Therefore, they show considerable promising potential for the treatment. of Hg-poisoning in man.

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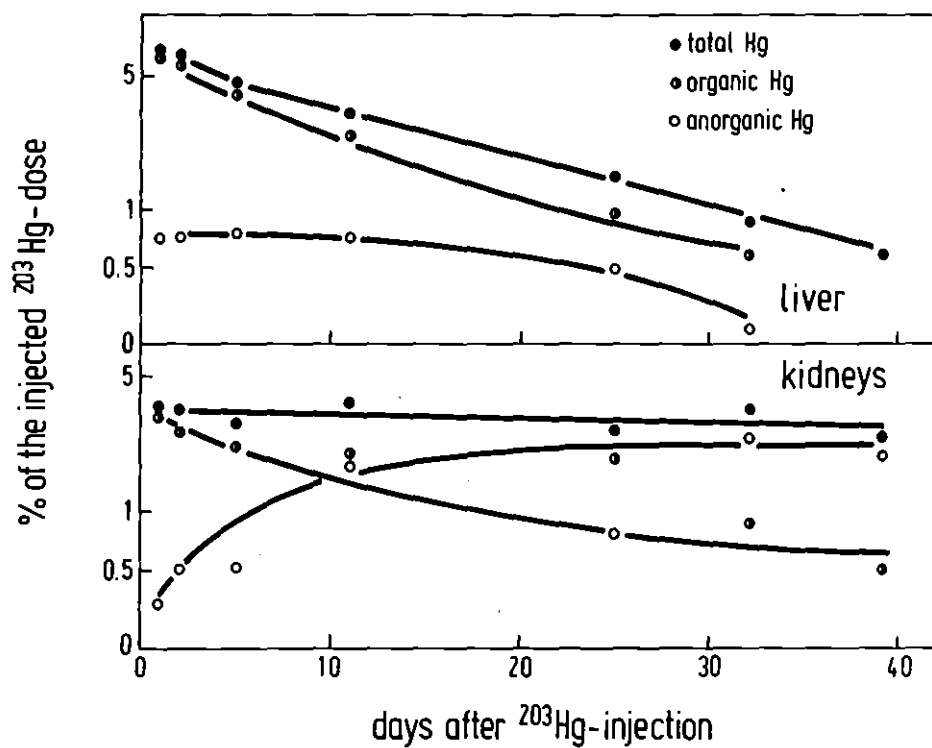
Fig. 1 Total mercury (closed symbols) organic- (half closed
symbols) and inorganic- (open symbols) mercury in liver
and kidneys of rats at different times after a single
intraperitoneal dose of CH_3HgCl (^{203}Hg). Means of
6 animals.
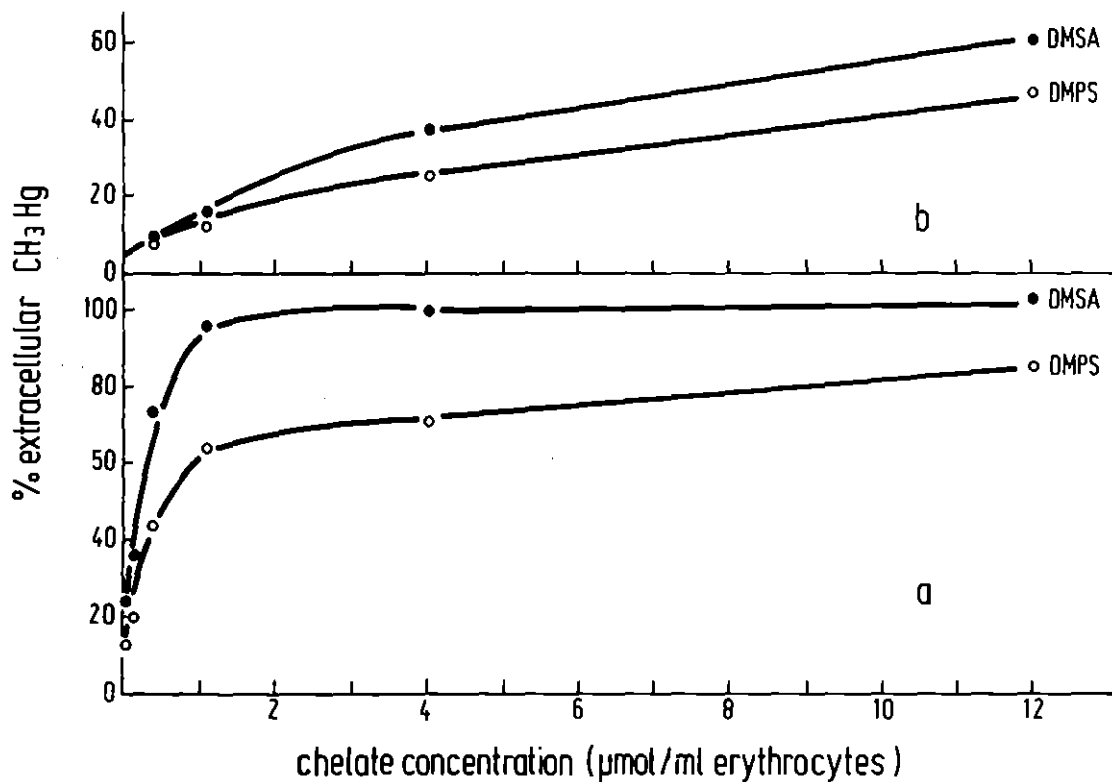
Fig. 2 Mobilization of methylmercuric chloride from a) human,
b) rat erythrocytes. Chelating agents were added 30 min
after CH_3 ^{203}Hg Cl (40 µM). 10 min later erythrocytes
were seperated from the supernatant and the mercury
content of both fractions was measured.

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References:

(1) Gabard, B., Arch. Toxicol. 35, 15 (1976).

(2) Planas-Bohne, F., Gabard, B., Schäffer, E., Arzneim. Forsch. (drug-res.) 30, 1291 (1980).

(3) Gabard, B., Arch. Toxicol. 39, 289 (1978).

(4) Gabard, B., Acta pharmacol. et toxicol. 39, 250 (1976).

(5) Gabard, B., Toxicol. Appl. Pharmacol. 38, 415 (1976).

(6) Graziano, J.H., Cuccia, D., Friedheim, E., J. Pharmacol. Exper. Ther. 207, 1051 (1978).
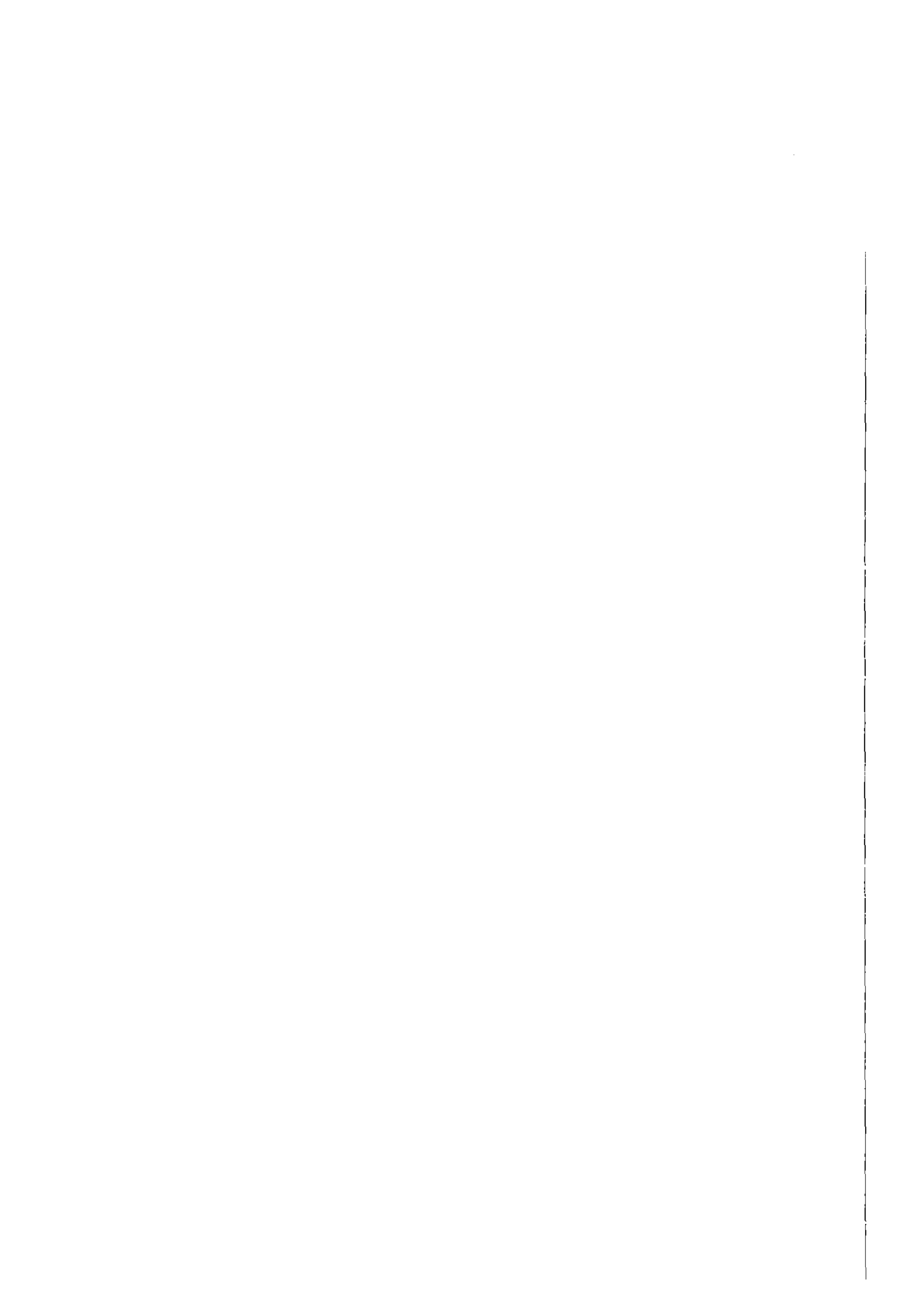
6. Year-round Research (Short scientific reports)

c) Development and Application of Physical Methods
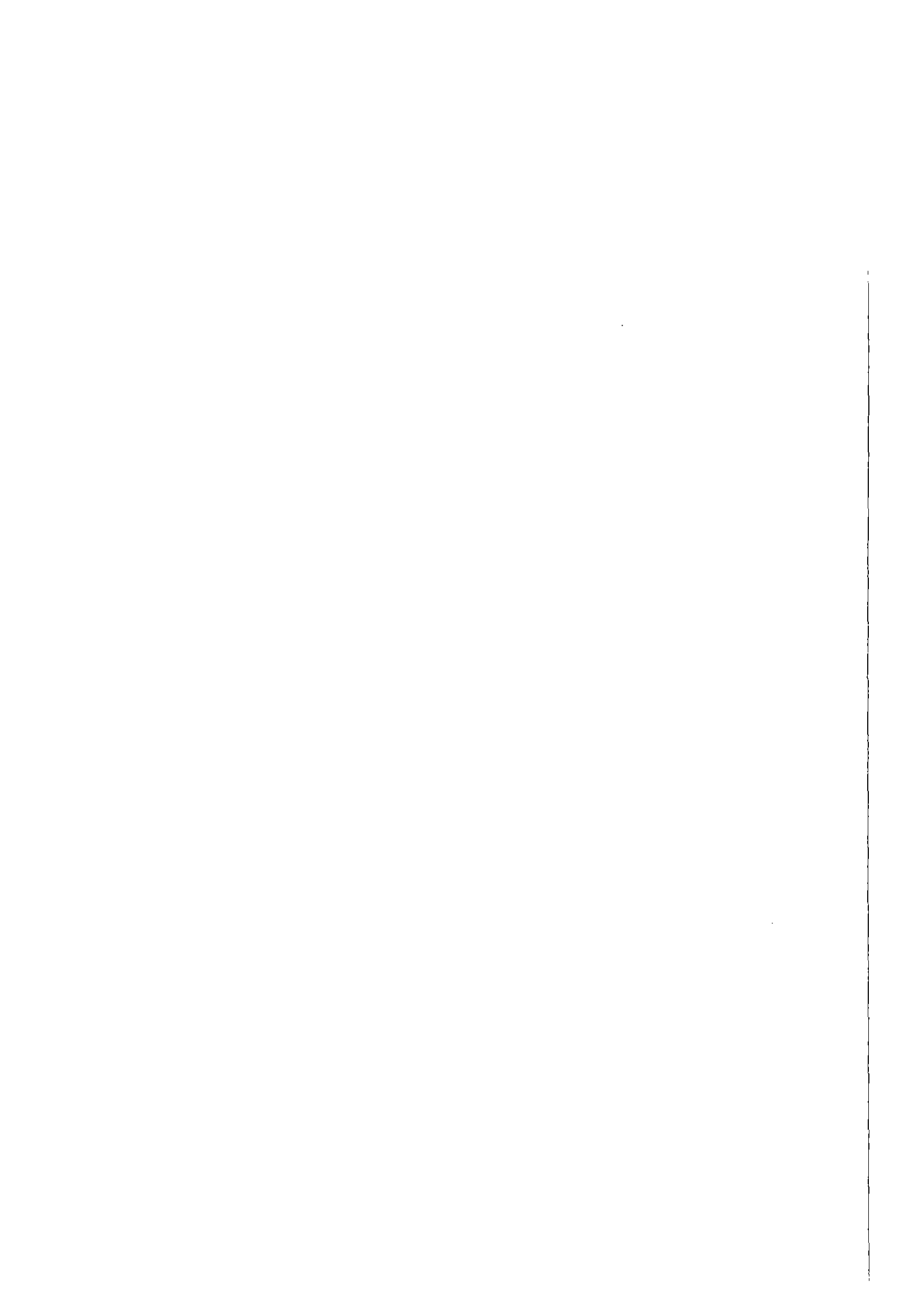
THE DEVELOPMENT AND APPLICATION OF PHYSICAL METHODS FOR THE

STUDY OF THE BINDING OF ACTINIDES AND HEAVY METALS IN TISSUES

H. Appel, H. Haffner, W.G. Thies

In recent years much attention has been paid to transport and deposition phenomena of metals in the body. This interest arose in particular in the study of the biological behaviour of plutonium (1). It has been extended since to other actinide elements and to metals in general.

These studies have shown that transferrin, the transport protein of iron metabolism, is the major metal binding serum protein in man (2). Also that ferritin, the iron storage protein, may incorporate transuranic elements in a similar way to iron (3).

It is well known that iron is bound by transferrin at two specific sites with well defined stoichiometry. In ferritin iron exists as polynuclear aggregates; over 2500 iron atoms may be bound by each ferritin molecule.

Although both proteins have been studied for more than four decades relatively little is known about the details of the metal binding. For transferrin not even the question whether the two sites are chemically equivalent or different or whether the sites are independent or positively cooperative has been definitely answered.

Moessbauer-measurements

Since April 1980 a program is being pursued to investigate the chemical character of the iron binding sites in transferrin and ferritin by Moessbauer methods and complementary hyperfine inter-action techniques. It was convincingly demonstrated earlier (4) that the observation of isomeric shifts and electric quadrupole splittings is a very sensitive and specific method for the investigation of chemical bonds in biomolecules.

As in previous experiments line doublets were observed for ^{57}Fe-transferrin and ferritin at T = 293 K (see Figs. 1 and 2). As yet, this result allows only the ambiguous interpretation: Either
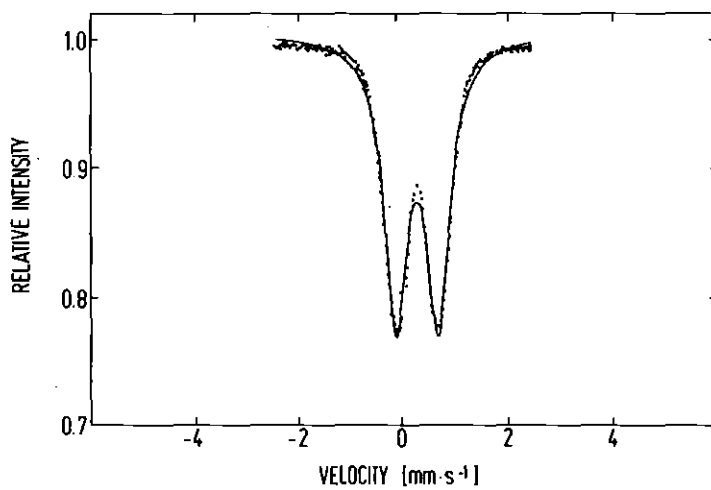
Fig. 1   Moessbauer spectrum of polycrystalline transferrin
at T = 293 K. The stoichiometrically bound iron was
enriched in ^{57}Fe to about 96 %.
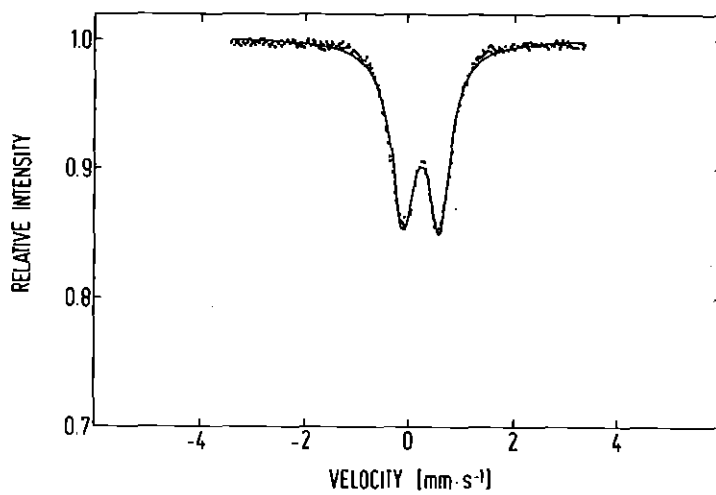
Fig. 2   Moessbauer spectrum of polycrystalline ferritin at
T = 293 K.

each of the two lines has to be attributed to a definite binding site with characteristic isomeric shift δ or the two sites are chemically equivalent and the doublet results from a quadrupole splitting ΔE.

Further measurements of the dependence of δ and ΔE on temperature and pH-value should permit the clarification of this ambiguity.

Studies using perturbed angular $\gamma\gamma$-correlations

Another experimental method which, in principle, provides equivalent information employs the so-called perturbed angular $\gamma\gamma$-correlations. Contrary to the Moessbauer method it is, however, not sensitive to isomeric shifts and unfortunately as a rule it is not applicable to the same isotopes.

A suitable nuclide for this method is ^{111}In which may be easily bound to transferrin (5) and probably also to ferritin. Again the recorded spectra look rather similar (see Figs. 3a and 3b).

This result is somewhat surprising. If quadrupole interaction occurs then the electric field gradient distribution has necessarily to be broad. This, however, would be markedly different in comparison to the Moessbauer results from the iron loaded samples. In the case of undisturbed sites R(t) should be approximately -0.13 and constant in time. The observed pattern may in part be due to time dependent electric fields that arise from the electron-capture process in ^{111}In preceding the $\gamma\gamma$-correlation (the so-called "after effects"). This rather involved phenomenon sheds some light on the availability of conduction electrons within the molecule and can be influenced by different sample preparation techniques. This dependence will be employed for further investigation of the process.

References:

(1) Taylor, D.M., Handbook of Experimental Pharmacology Volume XXXVI, 323 (1973).

(2) Stover, B.J., Bruenger, F.W., Stevens, F.W., Radiat. Res. 33, 490 (1968).
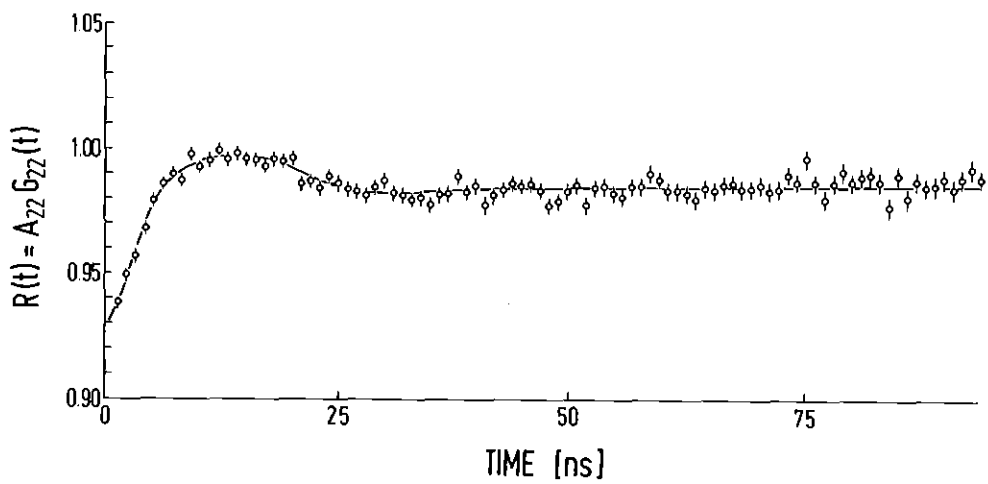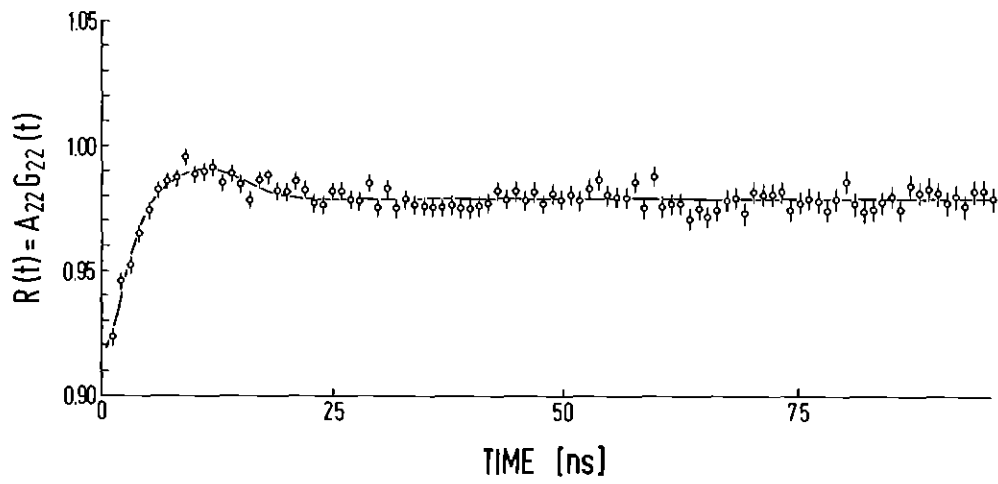
Fig. 3 Time differential perturbed angular correlation
measurements on

a) polycrystalline ^{111}In-transferrin

b) polycrystalline ^{111}In-ferritin

The perturbation factor R(t) is shown versus time.

(3) Taylor, D.M., Health Physics <u>22</u>, 575 (1972).

(4) Andl, A., Appel, H., Büche, G., Haffner, H., Wittek, P., Demonstration of a hydrogen bond in tobacco mosaic virus using ^{129}I, Hyperfine Interactions, to be published.

(5) Goodwin, D.A., Meares, C.F., Song, C.H., Radiology <u>105</u>, 699 (1972).
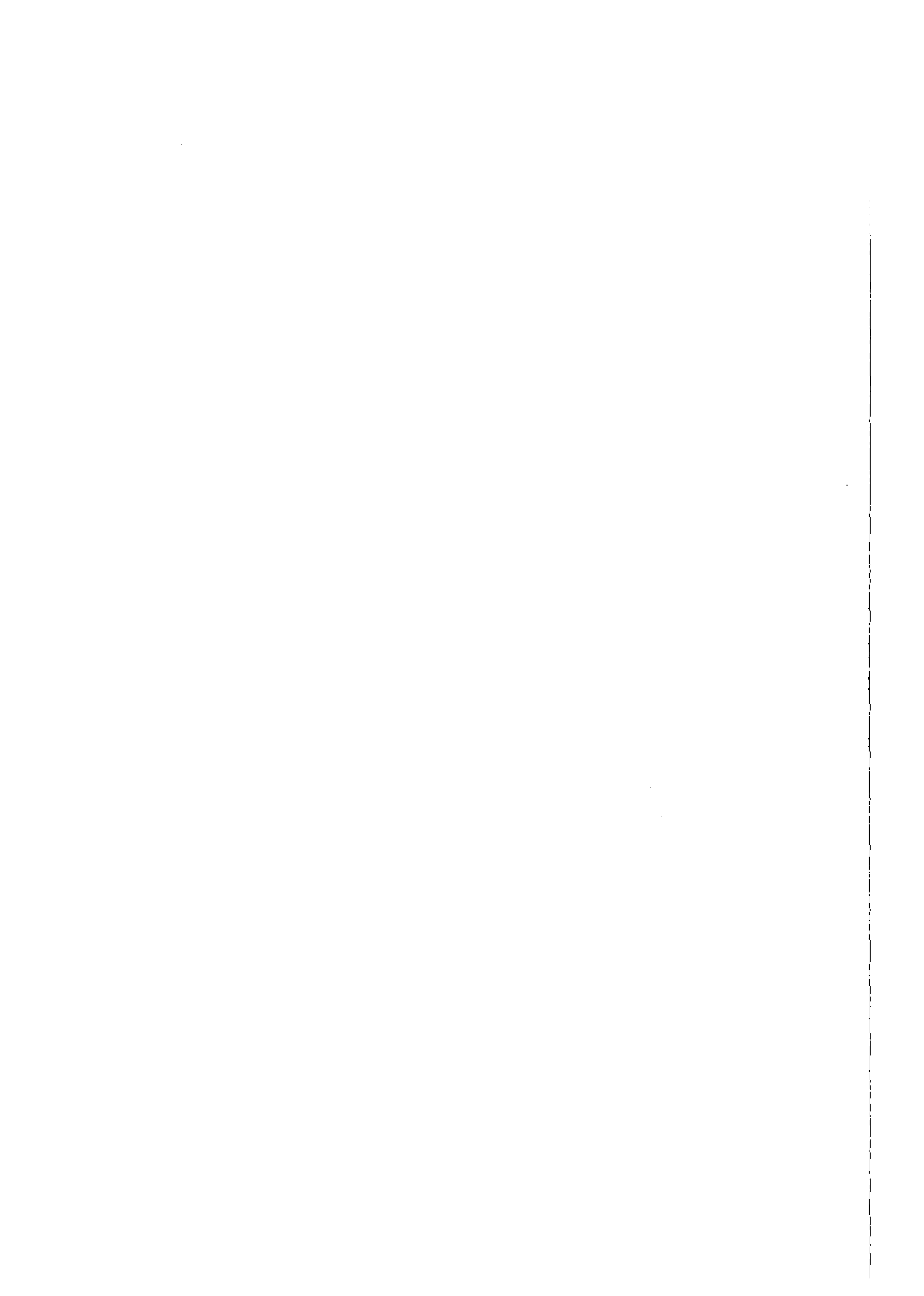
6. Year-round Research (Short scientific reports)

d) Genrepair

THE ROLE OF G2 PHASE WITH RESPECT TO GENOME INTEGRITY

C. LÜCKE-HUHLE, L. HIEBER

Exponentially growing mammalian cells duplicate their genome during the S-phase and divide at regular time intervals. DNA synthesis is preceded (G1) and followed (G2) by periods of no DNA synthesis. While G1 is a period of preparation for the subsequent S-phase by the synthesis of specific proteins, no clear function has yet been ascribed to G2 (1).

For the past 2 years our central theme has been to study cell cycle alterations after ionizing radiation in relation to the repair of DNA damage. Both, S and G2 are prolonged by X-irradiation. Since sparsely ionizing X-rays produce a number of different lesions such as strand breaks, base alterations and cross-links, it is reasonable to assume that some lesions will stop replication while others will cause a delay in G2. This hypothesis is strengthened by our results with densely ionizing radiation such as heavy ion beams (^{12}C, ^{20}Ne, ^{40}Ar) and 4 MeV alpha particles which produce mainly double strand breaks and arrest cells almost exclusively in G2.

The critical lesion for G2 delay

Alpha particles, as emitted by an Am-241 source, proved to be much more effective in causing a G2 block, measured by flow cytometry, than either X- or ^{60}Co-γ-irradiation. The number of cells arrested in G2 increased linearly with dose. The alpha particles, which had an LET (Linear Energy Transfer) of about 110 keV/μm showed a greater effectiveness by a factor of 8, than γ-rays. The G2 phase was defined operationally as the period of apparent 4n chromosomal content in a flow-cytogram. Mitotic indices were determined to prove that cells were arrested in the premitotic G2 phase and not at mitosis.
Doses as low as 0.03 Gy produced a measurable increase in the proportion of cells in G2, accompanied by a compatible decrease in the proportion of cells in G1 and S. At doses below 0.5 Gy cells leave G2 quantitatively after some period of arrest.
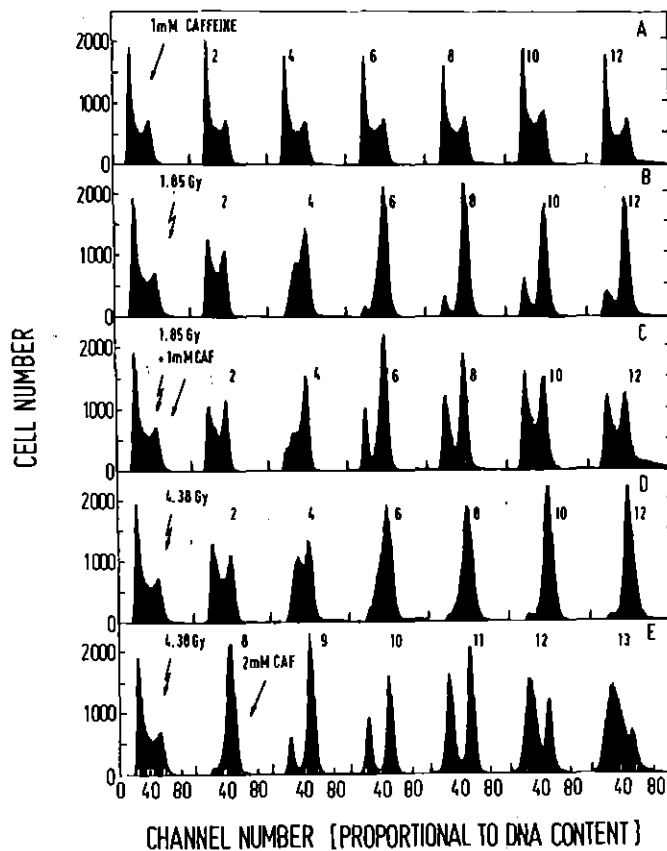
The effect of caffeine on DNA distribution, as measured by flow
cytofluorometry (the first peak represents cells in G1, the
second peak cells in G2) in Chinese hamster cells at specified
time intervals after drug administration and/or irradiation.

(A) 1 mM caffeine added to control cultures shows no effect.

(B) Irradiation with 1.85 Gy of MeV alpha particles produces
    a sharp increase in the proportion of cells in G2.

(C) 1 mM caffeine added before irradiation reduces the increase
    of cells in G2.

(D) Irradiation with 4.38 Gy of MeV alpha particles produces
    irreversible G2 arrest.

(E) 2 mM caffeine added 8 hcurs after irradiation releases G2
    cells from a formerly irreversible arrest.

At higher doses, however, an increasing fraction of the cells
remained arrested irreversible. This irreversible arrest in G2
prior to the first post-irradiation mitosis is typical of densely
ionizing radiation. After X-rays even non-surviving cells proceed
through several mitoses.

The effectiveness of doses of alpha particles as low as 0.03 Gy,
the importance of the radiation quality in causing G2 delay and
the irreversible arrest at doses higher than 0.5 Gy suggest,
that the characteristic DNA lesion found after exposure to densely
ionizing radiation - the DNA double strand break - might be the
critical lesion causing the G2 delay.

G2 delay - a DNA repair period

Since G2 delay is reversible at low doses we investigated the
hypothesis that the G2 delay is a period of repair. In order to
test this hypothesis we tried to interfere with repair by using
the purine derivative caffeine, which is known to interact with
nucleic acids or with the enzymes operating on nucleic acids.
Caffeine altered drastically the cellular response to densely
ionizing alpha radiation: Depending on concentration it reduced
(1 mM) or even completely abolished (2 mM) the arrest in G2.

In the presence of caffeine, even cells which would be blocked
irreversibly in G2 after 4.38 Gy of alpha particles proceeded
into mitosis and into the subsequent G1. Thus a cell blocked in
G2 must have contained all the macromolecules required for cell
division including the specific proteins, the spindle apparatus
and membrane components.

Although the mechanisms of caffeine action are far from understood
it seems that caffeine altered a process occuring on DNA during
G2 and permitted the cells to enter mitosis. Since caffeine at the
same time decreased survival if present during G2, we conclude
that whatever process is inhibited by caffeine it is surely
essential for cell survival. We favour the explanation that the
process involves repair of DNA double strand breaks during G2.

Genome aberrations in G2 arrested cells

Normally the chromosomes of eukaryotic cells are visible only
for a brief period during cell division. However, when interphase
cells are fused with mitotic cells by means of Sendai virus
"premature chromosome condensation" (PCC) is induced in the inter-
phase cells (2) permitting the examination of chromatin structure
by means of light microscopy during all cell cycle phases.

Preliminary results indicate that alpha irradiation induces "gaps"
within chromatids in G2 PCC's. We have not yet proved that these
gaps represent interruptions in both DNA strands. However, the
number of these chromatin aberrations rose with increasing dose
suggesting a role for these lesions in the irreversible arrest of
cells in G2.
Our working hypothesis is that part of the radiation-induced DNA
double strand breaks can be repaired during the G2 phase of the
cell cycle. If the repair capacity of a cell is exhausted un-
repaired double strand breaks will arrest cells in G2. Caffeine
treatment, which shortens G2 delay and decreases cell survival,
appears to overcome this regulatory mechanism in the cell, but
only at the price of cell death.

References:

(1) Pardee, A.B., Dubrow, R., Hamlin, J.L., Kletzien, R.F.,
    Ann. Rev. Biochem. 47, 715-750 (1978).

(2) Johnson, R.T., Rao, P.N., Nature 226, 717-722 (1970).

CELL-CELL INTERACTION INCREASES CELLULAR RADIORESISTANCE IN A THREE-DIMENSIONAL CULTURE SYSTEM

H.F. Dertinger, G.B. Hinz

DNA repair is a property of individual cells; this investigation probes the question whether the three-dimensional cell contact present in tissues modifies DNA repair as expressed by the radiosensitivity. We have compared different cell lines, which can grow either as monolayers or as multicell spheroids (1), for differences in radiosensitivity and other cellular properties. Some of these lines were indeed more radioresistant when growing as spheroids compared to singly growing or monolayer cells (Fig. 1). The radioresistance correlated well with the ability of the cells to form ionic coupling which was determined by microelectrode techniques and which is a measure of the presence of gap junctions. The L, HeLa and V79 cell lines did not transfer electrical signals to neighboring cells while the 3T3, B14 FAF 28 and BICR/M1R-K lines were strongly compled (Fig. 2). Only this latter group was more radioresistant in the spheroid form than as monolayers (cf. Fig. 1). Cells regardless of their location within the spheroid were radioresistant and the increased radioresistance disappeared after trypsination with a fairly long half life of approximately 4 hours. The contact-induced radioresistance involved reduced cell death as well as some cell cycle effects and also reduced the frequency of mutation and chromosome aberrations (2).

References:

(1) Dertinger, H., Hülser, D., Radiat. Environm. Biophys., in press

(2) Dertinger, H., Hinz, G., Hülser, D., Radiat. Environm. Biophys. 17, 316 (1980).

(3) Sutherland, R.M., Durand, R.E., Int. J. Radiat. Biol. 23, 235 (1973).
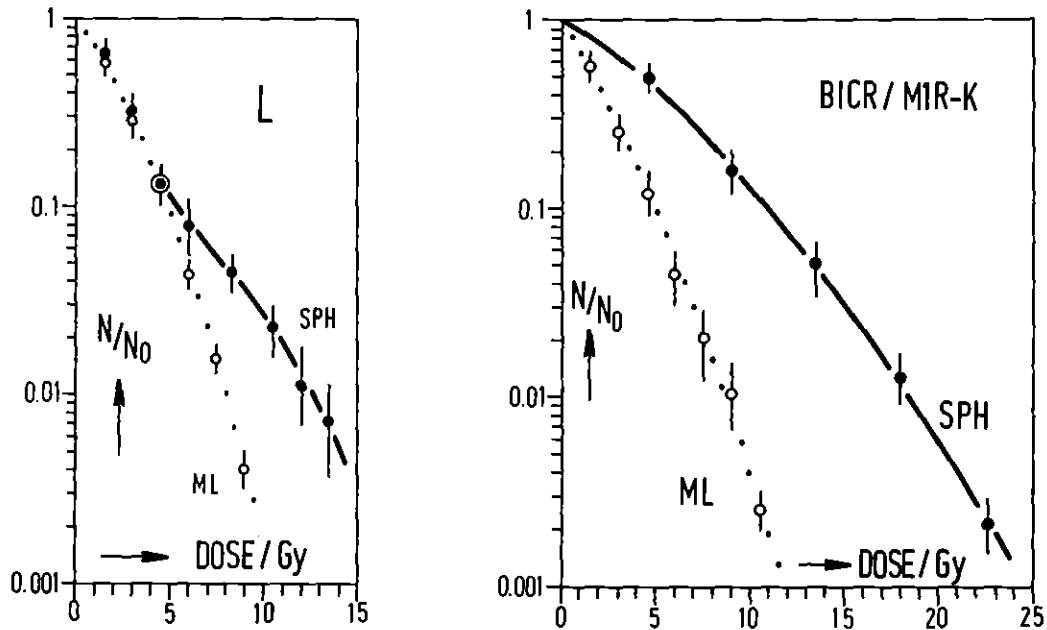
Fig. 1   Examples of survival curves (expressed as colony forming
         ability) for spheroid (SPH) and monolayer (ML) cells
         after γ-irradiation. Line BICR/M1R-K (coupled cell line)
         shows increased survival when cultured as spheroids,
         whereas the survival of L cells (not coupled) is independent
         of the culture method (The deviation in the survival below
         0.1 is due to a small fraction of radioresistant anoxic
         cells known to be located near the spheroid core (3)).
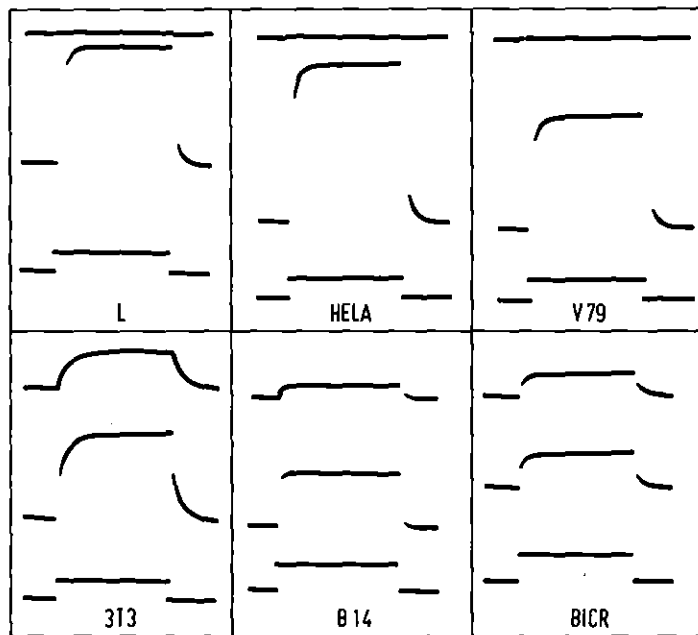
Fig. 2   Measurement of cell coupling by micro-electrode techniques.
         A current signal (lower oscilloscope trace) is injected
         into a cell and causes a voltage deflection which is
         monitored by a second electrode (middle trace). A third
         electrode records the voltage deflection in an adjacent
         cell (upper trace). Origin of the cells:HELA (Human),
         L and 3T3 (Mouse), V79 and B14 FAF 28 (Chinese hamster),
         BICR/M1R-K (Rat).

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REJOINING OF DNA DOUBLE STRAND BREAKS INDUCED BY GAMMA-RAYS

IN BACTERIA AND MAMMALIAN CELLS

T. Coquerelle, K.F. Weibezahn, P. Herrlich

This project addresses the question whether bacterial and mammalian cells can rejoin DNA double strand breaks (DSB) induced by ionizing radiation. In the experiments presented here, we use the technique of neutral filter elution (1) based on the observation that double stranded DNA passes a membrane filter under non-denaturing conditions at a size dependent rate. We can detect DSB by this method in both bacteria and mammalian cells. The reconstitution of the original DNA filtration rate after incubation of the cells is taken as evidence of DSB rejoining. By screening the available mutants we are trying to characterize the enzymatic conditions required for this rejoining.

Neutral filter elution technique.

The rate at which DNA passes through a polycarbonate filter at non-denaturing pH, depends mainly on the size of the naked double stranded DNA fragments. Although our DNA isolates could still contain non-digestable protein complexed to DNA we favor the interpretation, that the rate of elution of our DNA fragments also depended on size. Upon fragmentation of DNA by gamma-irradiation, the elution rate through the filter indeed increased with the absorbed dose (Fig. 1). A dose of 10 Gy, equivalent to one DSB per 1.3×10^{10} dalton sufficed to increase the elution rate significantly (1 DSB in 4×10^4 kb).

Bacterial cells rejoin DSB.

When bacteria were incubated at growth temperature after irradiation, the DNA was reconstituted to near-normal size suggesting intracellular rejoining of the DNA fragments. If the percentage of rejoined DNA is plotted versus the time of incubation second order kinetics are observed with a very fast initial component and a second much slower one. From similar experiments with mutants it appeared that the rapid initial rejoining depended on the presence of an active ligase while the slow process of rejoining required rec functions (Fig. 2).
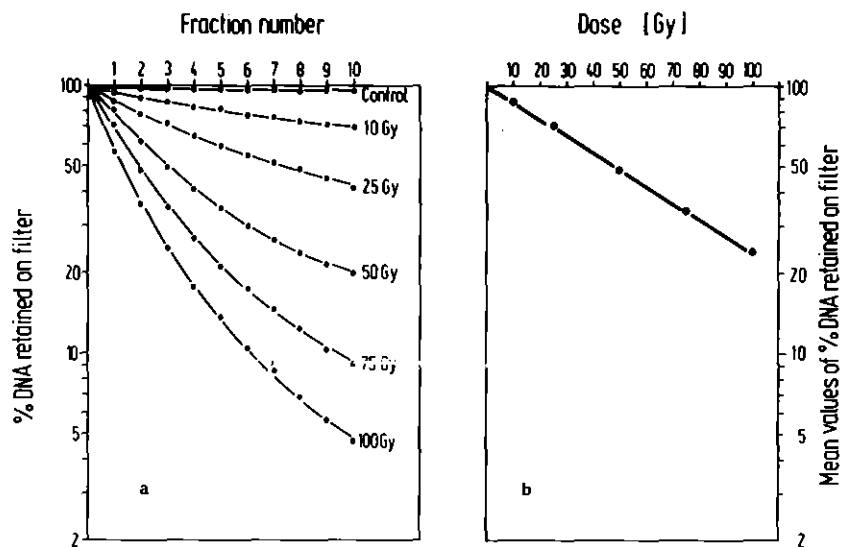
Fig. 1 Induction of DSB in DNA of V79 cells by gamma-rays.
(a) Percentage of DNA retained on the filter is plotted
as a function of the eluted fraction. Each fraction
represents elution of 90 min at a rate of 4 ml/h.
(b) Dose effect curve of DSB induction. The mean
values (MV) of the percentages of ten fractions of
the elution profile normalized to control are plotted
as a function of dose. Errors are within the size of
the symbols.
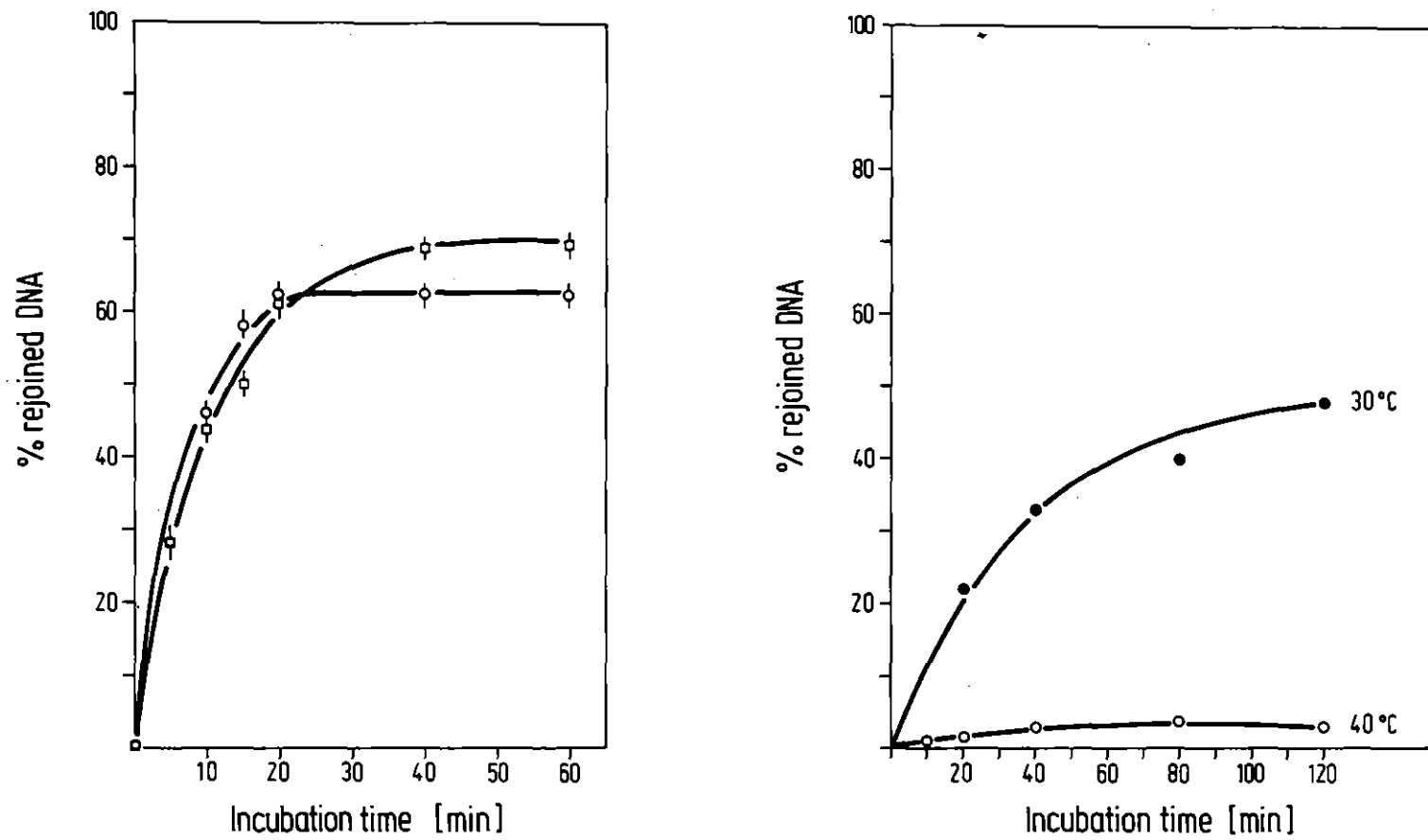
Fig. 2 a) Rejoining kinetics of DSB in bacterial DNA:
□ AB 1157 (wild type), o AB 2463 (rec A⁻).

b) Rejoining kinetics of DSB in ligase deficient
E.coli mutant KS 268 at permissive and nonpermissive
temperature.

Normal human and other mammalian fibroblasts rejoin DSB.

Following the same criteria, mammalian fibroblasts (human or V79
Chinese hamster cells) rejoined DSB. The kinetics were similar
to those of bacterial repair (Fig. 3). Within 8 min at 37 C, the
elution rate for V79 cells returned to half the control value
indicating rejoining of 50 % of the DSB. At 60 min, 90 % were
rejoined, but even after long periods, some of the DSB remained
unjoined. The rapid part of the rejoining kinetics has not been
detected previously. By analogy with the bacterial data, different
repair pathways could be postulated. The DSB may differ in their
ability to serve as substrates depending on their structure, and
some DSB may be non-repairable.

Human mutants with impaired DSB rejoining ability.

Ataxia telangiectasia (AT) and Fanconi's anemia (FA) are human
autosomal recessive diseases carrying a high risk of cancer.
Patients with AT suffer from neurologic disorders, ocular and
cutaneous telangiectasia and immune deficiencies, while FA is
characterized by progressive bone marrow insufficiency in conjunc-
tion with congenital anomalies and malfunctions. Cells from both
groups of patients are more sensitive to ionizing radiation and
to carcinogen treatment than normal cells, and the cells accumulate
chromosomal aberrations both spontaneously and after treatment
with mutagens. Double strand breaks are likely primary lesions
leading to chromosome aberrations. Therefore, we examined AT and
FA cells for their ability to rejoin DSB under our experimental
conditions. The data are presented in Fig. 4. In both groups of
patients, individuals with normal and with reduced ability to
rejoin DSB, were observed. This may suggest the existence of
different complementation groups in both AT and FA. In two cases
of FA and one of AT, the rejoining was impaired with both the rate
of initial rejoining and the extent of rejoining beeing reduced.
This throws some doubt on the interpretation of two independent
processes: fast and slow rejoining. Interestingly one of the FA
patients has earlier been reported to have reduced DNA ligase
activity. It is not surprising that the impairement was relative
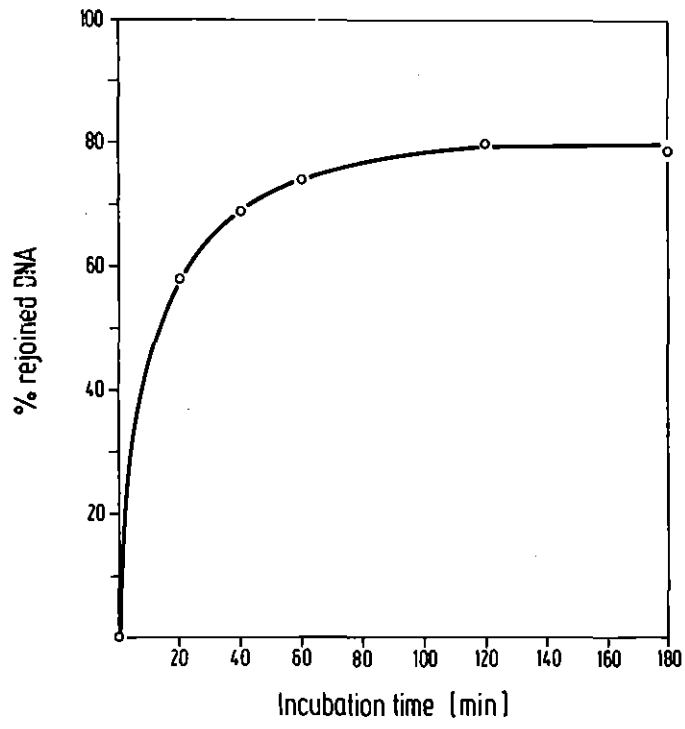
Fig. 3   Rejoining kinetics of DSB in V79 Chinese hamster cell
         DNA after a dose of 100 Gy γ-rays.
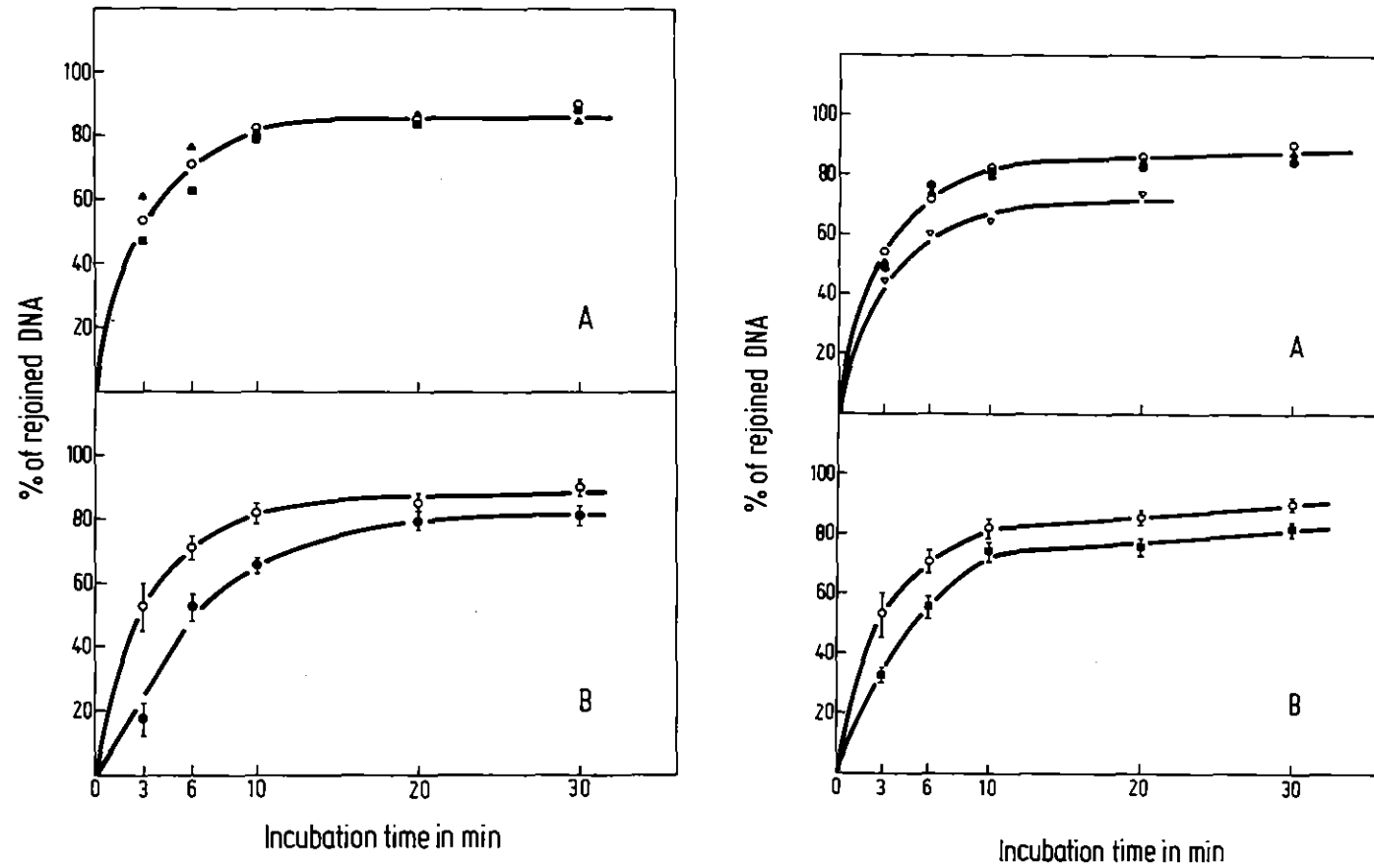
Fig. 4 a) Rate of DSB rejoining in normal and Ataxia telangiectasia
fibroblasts.
Panel A: o, normal fibroblasts Berlin; ▲, AT3BI;
■, AT5BI.
Panel B: o, normal fibroblasts; ●, AT2BE (CRL 1343).
Each point represents the mean value obtained from
3 experiments.

b) Rate of DSB rejoining in normal and Fanconi fibroblasts.
Panel A: o, normal fibroblasts Munich; ●, FA Buse;
▲, FA CRL 1196; ▽, FA 3557.
Panel B: o, normal fibroblasts Munich; ■, FA 1424.
Except for those of FA 3557, each point represents
the mean value obtained from 3 experiments.

and not complete, since in contrast to the bacterial mutants, the human mutants are viable and the functions for which these patients are deficient, are certainly required for essential physiologic steps.

References:

(1) Bradley, M.O., Kohn, K.W., Nucleic Acids Research 7, 793-804 (1979).

SYNERGISTIC INCREASE OF E.COLI MUTATION RATE BY COMBINED ACTION OF UV AND ALKYLATION

S. Herrmann, G. Hotz

In the context of our approaches to DNA repair we have examined the combined effect of mutagenic treatments in E.coli WP2, and found that ultraviolet light (254nm) and alkylation (ethylmethane-sulfonate = EMS) cause an overadditive increase in mutation rate (trp → trp$^+$). This is interesting with respect to the unravelling of the levels at which mutagens interact, and it may relate to the situation where man is exposed to a combination of mutagens.

In order to cover a wide range of mutagen dose-responses, two mutagenesis assays (1,2) of differing sensitivity (fluctuation test and liquid incubation test) were used and the data compiled. In the higher dose range of EMS following the UV irradiation, a strongly synergistic effect on mutation rate was observed in cells lacking the uvr A gene (Fig.). In wild type E.coli this synergism is far less pronounced. Apparently only in uvr A⁻ do major or minor products of each mutagenic treatment interfere with repair or mutagenesis of the other.

References:

(1) Green, M.H.L., Muriel, W.J., Bridges, B.A., Mutation Research 38, 33 (1976).

(2) Mitchell, I. de G., Mutation Research 54, 1 (1978).
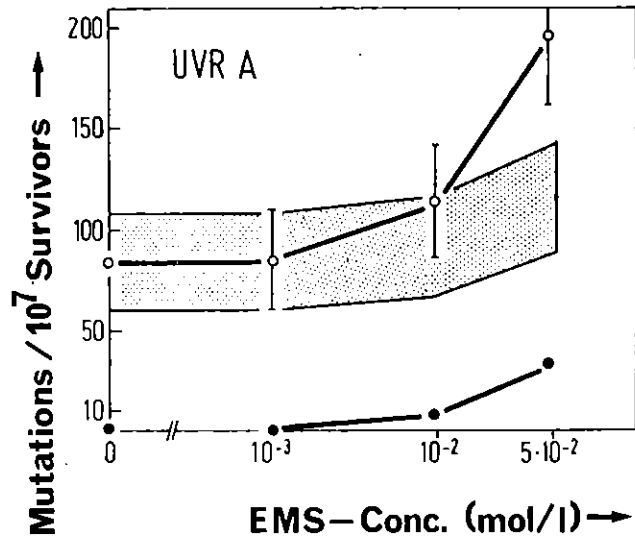
Mutation induction in E.coli WP2 uvr A after subsequent treatment
with UV (2.4 J/m^2) and EMS (2 hrs) measured in the liquid incuba-
tion test. ●—● EMS control. The marked area corresponds to the
additivity of the single mutation rates induced by UV and EMS,
respectively (p = 0.05).
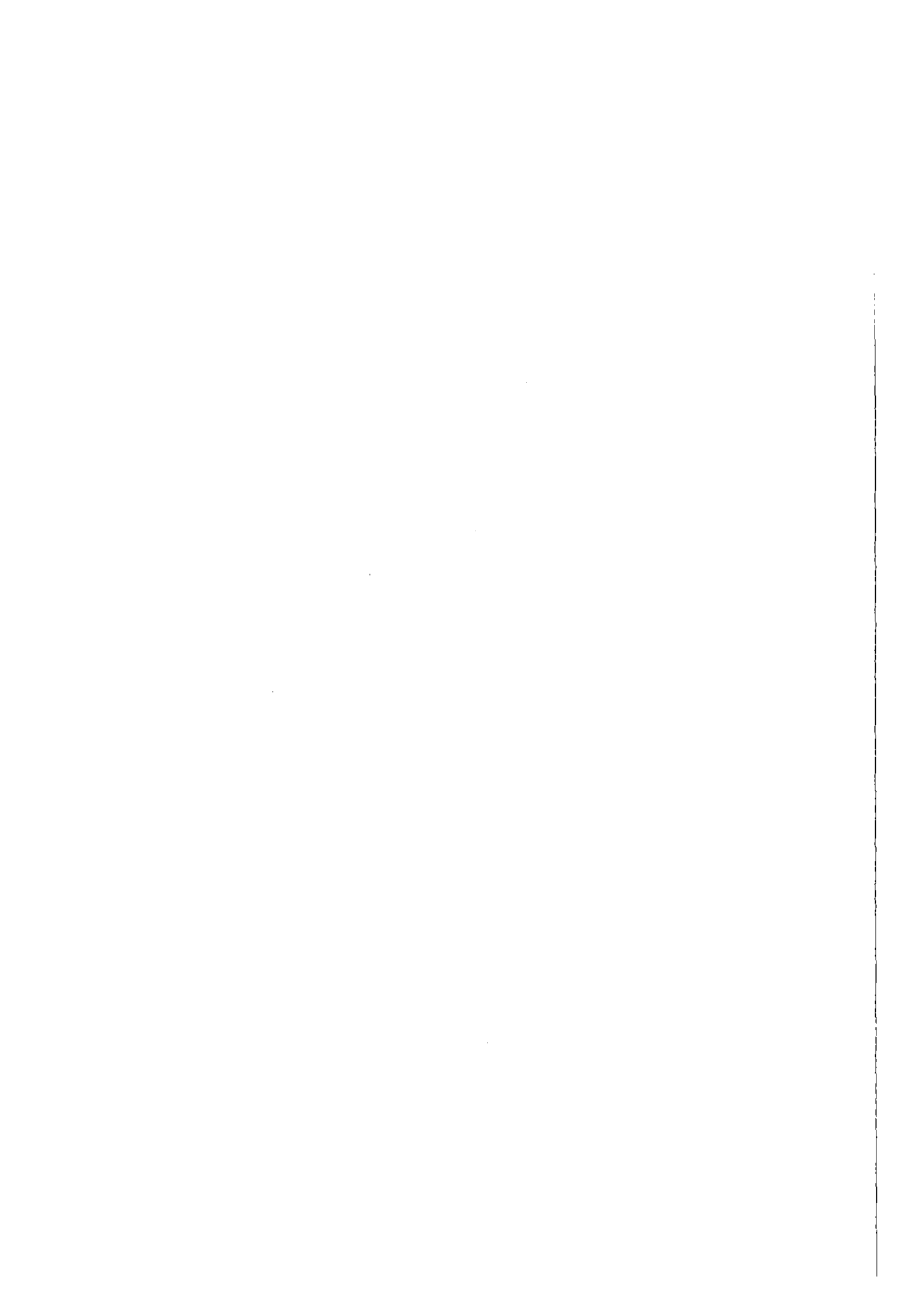
6. Year-round Research (Short scientific reports)

e) Genregulation

INDUCTION OF PROTEINS IN MAMMALIAN CELLS BY AGENTS WHICH ARREST

REPLICATION, AND BY TUMOR PROMOTORS

U. Mallick, H.J. Rahmsdorf, H. Ponta, P. Herrlich

Several arguments led us to assume that the immediate response of
mammalian cells to treatment with radiation or with tumor promotors
includes the induction of new proteins:

1) Bacterial cells react to agents which inhibit replication by
   the increased synthesis of a series of recombination and re-
   pair enzymes. This property may have been maintained in evolu-
   tion.

2) Mammalian cells, like bacteria, seem to acquire DNA repair
   capability following a first treatment with radiation.

3) Doses of radiation which do not cause significant cell death
   still elicit a uniform response in a majority of cells e.g.
   tumor virus induction or cell cycle changes.

4) The induction of synthesis of Epstein Barr Virus by the tumor
   promotor TPA (12-0-tetradecanoylphorbol-13-acetat) is prevented
   by the addition of aminoacid analogues immediately after TPA.

Our assumption has been confirmed since immediately after treatment
with various agents several new proteins are synthesized. The regula-
tion appears to be transcriptional. Human mutant cells have been
detected with altered expression of these proteins.

Arresting replication causes the induction of proteins in
lymphoid cell lines

The pattern of proteins synthesized is examined by pulse labeling
murine B cell lines with ^{35}S-methionine and subsequent analysis
by 2-dimensional resolution in gels. The pattern and the kinetics
of the synthesis of new proteins (fig. 1) was identical after
treatment of lymphoid cell lines with either UV, X-ray, mitomycin C
or hydroxyurea. The most pronounced new spot corresponded to a basic
protein of about 35 kd. In resting peripheral B lymphocytes, the
very same protein is synthesized at a constitutive rate but upon
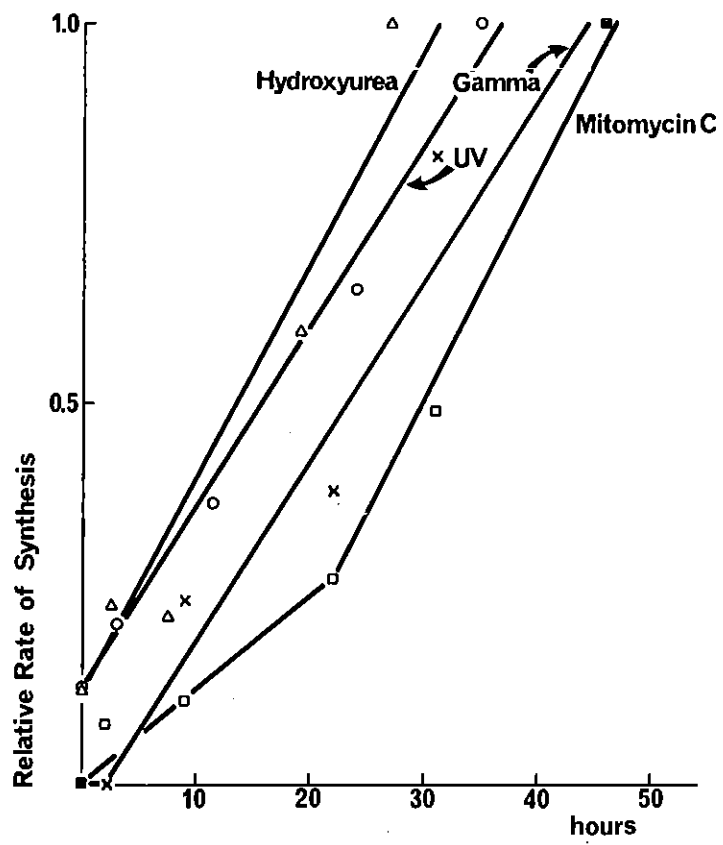
Fig. 1 Kinetics of protein induction.

polyclonal activation the rate is strongly reduced. The fast
response to all the agents tested suggests that only a few
molecular steps are involved in the induction. The activation
of genes occurs at the transcriptional level. Since all agents
have in common the ability to inhibit replication, it seems
likely that it is the arrest of DNA synthesis which mediates
the activation of the genes. At least one of the induced proteins
binds to DNA.

Fibroblast-specific pattern of radiation- and TPA-induced proteins

The induction of proteins in response to mutagens and TPA is not
specific for lymphoid cells. Rodent fibroblasts also respond to
the same agents in the same way. The pattern of proteins, however,
differed from that in lymphoid cells. The most pronounced basic
protein of 35 kd behaved differently on limited proteolysis to the
corresponding lymphocyte protein. Because of the monolayer growth
conditions, α-irradiation from a plane americium-241 source could
be included in the list of inducing agents.

Following the idea that the induced proteins may act in the chain
of events leading to tumor virus induction or to chromosomal
rearrangements, the effect of a tumor promotor was examined. Treat-
ment of human fibroblasts with TPA indeed led to the induction of
most proteins which were also induced by ultraviolet light. The
proteins appeared within two hours after TPA thus again suggesting
a direct action mechanism. Background synthesis in non-treated
control cells was barely, if at all, detectable. While retinoic
acid would block the effect of TPA on Epstein Barr Virus induction,
it did not inhibit protein induction nor could we see new proteins
in response to retinoic acid treatment. The antagonistic effect
of retinoic acid must therefore be established at a stage after
protein induction by TPA.

The induction by TPA also occurred in confluent cells or cells
which had been arrested by starvation. It thus seems that two
mechanisms feed into the same step of gene activation: Arrest of
DNA replication and the pleiotropic action of TPA.

Table. Induction of protein synthesis by TPA: Fibroblasts
from healthy individuals (1-3) and from Bloom's
patients (4-6) were treated with TPA for different
times, and pulse labeled with ^{35}S-methionine for
2 hours. The proteins were separated by 2-dimensional
gel electrophoresis (see front cover illustration).
The autoradiograms were evaluated by microdensitometry.
The effect of TPA on the synthesis of a 70 K protein
is shown. The values are normalized to a constitutively
synthesized protein.

		No TPA	Time after TPA treatment		
			4 h	8 h	24 h
1	NF Mathias	0.0	0.3	0.5	0.9
2	NF München	0.5		1.0	1.0
3	NF α_1	0.0		0.9	1.1
4	BS 2189	1.0	1.0	1.9	
5	BS Kiel	0.9		2.0	2.7
6	BS 1492	0.4	1.5	2.0	2.0

TPA-induced proteins are elevated in Bloom's syndrome

Because Mytomycin C, TPA and irradiation all lead ultimately to
chromosome interchanges and other chromosomal aberrations, we be-
came interested in examining cells from patients who suffer from
a spontaneous rate of chromosomal changes. Among these, all cells
from patients with Bloom's syndrome synthesized high background
levels of most or all of the proteins which are inducible by TPA
or UV (Table). Further TPA increased the rate to far-above-normal
levels. In a preliminary study, the level of synthesis correlated
well with the number of sister chromatid exchanges in these cells.
Our working hypothesis is that we are looking at a family of
proteins which work on DNA and are involved in the generation
of sister chromatid exchanges. Bloom's syndrome may be a pleiotropic
regulatory mutant which responds to external agents in excess to
normal.

6. Year-round Research (Short scientific reports)

f) Molecular Biology of Tumor Viruses

MOLECULAR BIOLOGY OF MOUSE MAMMARY TUMOR VIRUS

B. Groner, N.E. Hynes, U. Rahmsdorf, N. Kennedy, G. Knedlitschek,
L. Fabiani, P. Herrlich, H. Ponta

The expression and subsequent mammary tumor induction of a murine
retrovirus (mouse mammary tumor virus) is the subject of these
investigations. Recent advances in gene cloning techniques and
reintroduction of cloned genes into cultured cells have made
possible a crucial distinction in the classes of retroviruses.
Most replication competent viruses do not transform cultured cells
and therefore do not contain a transforming gene in their genome
(chronic viruses). Most transforming retroviruses, however, are
replication defective and a transforming gene has become part of
their genome by recombination of proviral DNA with a cellular gene
(acute viruses). A mechanism of action of transformation by chronic
viruses has been proposed to involve the integration site of
proviral DNA into the host genome. The structure of proviral MMTV
DNA contains a terminal redundancy of about 1.3 Kb which harbors
the signals responsible for control of proviral transcription. One
repeat unit (5') governs viral RNA synthesis whereas the second
(3') has been implicated to induce the synthesis of host RNA
sequences adjacent to proviral DNA. Since the integration site of
proviral DNA seems to be random a growth regulating host gene might
be affected, albeit with low frequency, through promotor insertion.
The DNA governing the expression of MMTV controls the crucial first
step in carcinogenesis since it is responsible for the transcription
of viral RNA which can in turn be reintegrated and responsible for
the transcription of a proto oncogene, the product of which might
transform the cell.

The molecular description of the events governing MMTV and oncogene
transcription and the hormonal control there of are being persued.

I. Molecular cloning of proviral MMTV genes

B.Groner, N.E. Hynes, N. Kennedy

Proviral MMTV genes are present in the germ line DNA and subsequently
in the somatic tissue DNA of all inbred mouse strains. Genetic experi-
ments have shown that the five copies of the proviral genes in the GR

mouse can be differentiated according to their potential to direct
synthesis of viral particles and induce mammary tumors.The various
biological properties, however, could be due to differential
regulation of equivalent genes by host factors or due to differences
in the biological potential of the genes based on their primary
structure. To distinguish these possibilities, and to identify the
DNA base sequence as well as the biological potential of proviral
DNA, we used recombinant DNA methods to isolate and amplify the
proviral genes of the GR mouse. Partial Eco RI digests of GR liver
DNA was size fractionated and 14 to 24 Kb fragments were integrated
into the λ Ch 4A vector. From the resulting gene library we
selected MMTV specific recombinants by plaque hybridisation.
Analysis of the MMTV positive recombinants revealed 5 types of
MMTV specific Eco RI fragments. Four correspond to the 3' fragments
of 4 different proviruses and one corresponds to the 5' fragment
of a single provirus. Four 5' specific Eco RI fragments and one
3' specific fragment could not be obtained although enough plaques
were screened to make it statistically probable.

II. DNA sequencing and proviral gene comparison

N. Kennedy, G. Knedlitschek, P. Herrlich, B. Groner

Many eukaryotic genes transcribed by RNA polymerase II have been
shwon to exhibit common features. These features include regulatory
signals 5' from the initiation of RNA synthesis as well as signals
involved in termination and processing of RNA transcripts. Most of
this information has been obtained from the analysis of the primary
DNA sequence of cloned genes. To sequence the MMTV DNA, obtained
as λ recombinants, we subcloned first the Eco RI fragments in the
plasmid vector pBr 322. Our main interest was initially the DNA
sequence constituting the large terminal repeats (LTR) of proviral
MMTV. These LTR contain the promotor and initiation sites for RNA
synthesis and we suspect also the regulatory signals mediating
glucocorticoid hormone control of transcription. Pst I fragments
from the 5' and 3' LTR sequences were subcloned. To obtain over-
lapping sequences for use in the Maxam Gilbert DNA sequencing method
further subclones containing deletions of defined size were obtained
by DNase I digestion, Eco RI linker ligation and size selection.
The sequence of the 3' and 5' LTR were obtained and compared.

A different approach was taken to compare the sequences of the
endogenous MMTV proviruses of the GR mouse. Heteroduplex forma-
tion between pairs of cloned proviral DNA and digestion of
heteroduplex with SI nuclease under increasingly stringent condi-
tions revealed that all copies of the endogenous proviruses are
very similar to each other but not identical. Only one copy of
the endogenous GR proviral genes was shown to be identical to the
exogenous proviral DNA obtained from GR tumor DNA. This result
is in agreement with the genetic data and supports the notion
that only a single specific MMTV copy is responsible for MMTV
production and mammary tumor induction in the GR mouse.

III. DNA mediated gene transfer into cultured cells

N.E. Hynes, U. Rahmsdorf

A recombinant clone containing an intact MMTV proviral gene was
investigated by restriction nuclease digestion and sequence
analysis. These methods did not reveal any striking differences
between the exogenous proviral DNA expressed in murine mammary
glands and tumors and the cloned provirus. A biological assay
was therefore used to test the coding potential and regulatory
signals included in the clone GR-40. $\lambda \cdot$MMTV recombinant DNA was
mixed with a cloned thymidine kinase gene from Herpes Simplex
Virus and the DNA was precipitated onto tK$^-$ mouse fibroblasts.
HAT selection medium was used to discriminate tK$^-$ from tK$^+$ cells
and 10 tK$^+$ revertants were obtained. These cell clones were analysed
for transfected GR-40 gene copies and we found that all cells with
a tK$^+$ phenotype had integrated into their genomic DNA several
copies of the cotransfected MMTV proviral gene. The stability of
the transfected DNA the LtK$^+$ cells was measured following culture
of cells under various conditions. The growth of cells in selective
medium (DMEM + HAT) resulted in a stable copy number of acquired
MMTV genes. When cells were cultured in non selective medium (DMEM)
the transfected and the cotransfected DNA were lost simultaneously
at a low frequency. The mechanism of acquisition and loss of
exogenously introduced DNA therefore involves large stretches of
DNA and possibly intracellular ligation and recombination events.

IV. Methylation and hormone regulated expression of transfected DNA

N.E. Hynes, B. Groner, U. Rahmsdorf

Methylation of DNA sequences in the 5 position of cytosine bases has long been suspected to influence DNA-protein interaction and thus gene regulation. We have investigated the state of methylation of active and inactive MMTV genes and found a correlation between DNA modification and MMTV transcription in transfected cells. Cells used for the above described transfection experiment (L tK⁻, mouse fibroblasts) contain four copies of the proviral MMTV DNA. Analysis of the proviral DNA of these cells reveals a high degree of CpG methylation and no transcription of proviral sequences in the absence or presence of glucocorticoid hormones. The proviral sequences introduced into these cells were amplified in bacterial cells and are not methylated. This nonmethylated state was maintained in the transfected cells after many rounds of DNA replication. The simultaneous presence of the MMTV gene in two different states of modification (methylated and nonmethylated) in the same cell supports the concept of semiconservative inheritance of DNA methylation patterns. Methylated DNA directs the methylation of its daughter strands.

Transfected cells were analyzed for the synthesis of MMTV specific RNA. All 10 LtK⁺ revertant cell clones investigated were shown to produce MMTV specific RNA. The size of the RNA was indistinguishable from the MMTV RNA produced by cells after productive viral infection. We conclude that the acquired nonmethylated genes, introduced into L cells by DNA mediated transfection can be properly recognized by the cellular machinery and transcribed into RNA. Culturing transfected cells in the presence of 10^{-6} M dexamethasone resulted in a tenfold increase in MMTV RNA in these cells. We suggest that the DNA sequence which is responsible for the mediation of the hormone effect on MMTV RNA transcription is physically linked to the MMTV gene thus exerting a direct cis effect. L cell components, e.g. nuclear proteins, hormone receptor complex and RNA polymerase II probably place the transfected gene in a proper configuration to be hormone inducible. Hormone induction, however, was only observed on constitutively transcribed transfected genes. Endogenous silent MMTV genes could not be induced to be transcribed by hormone. This result suggests that hormone induction as a cis effect can only function on properly accessible transcriptionally active genes.

The accessibility may be influenced by activating factors such as tumor promotors and irradiation.

V. In vitro transcription of cloned MMTV DNA

L. Fabiani, N.E. Hynes

The reintroduction and proper transcription of the GR-40 gene in cultured cells shows that all the DNA sequence requirements for specific initiation of RNA synthesis are met. Molecular investigation of the control of the transcription process makes it desirable to obtain specific transcription in a cell free system. This would allow the isolation and characterisation of the cellular components which are involved in the initiation of transcription and eventually in hormonal regulation. The incubation of truncated gene fragments which include the promotor and initiation region of eukaryotic genes in extracts of HeLa cells has shown that specific initiation and synthesis of a 'run off' RNA product can be achieved. We prepared extract from HeLa cells and could observe specific RNA synthesis when the adenovirus late promotor fragment was offered as a template to the in vitro reaction. No specific RNA synthesis was so far observed when a MMTV promotor fragment was used as a template. The transcriptional stop signal immediately following the initiation site on the MMTV gene might be responsible for this result. We are investigating the possibility that the stop signal might be active in vitro but can be overread in vivo.

VI. Chimeric genes and deletion mutants

B. Groner, H. Ponta, N.E. Hynes, U. Rahmsdorf, P. Herrlich

If a specific DNA sequence mediates the hormonal response of MMTV RNA transcription it might be possible to identify this sequence via its biological activity. Two approaches are being taken: 1. Defined sequents of the MMTV proviral gene have been subcloned. These fragments include the MMTV promotor sequences contained in 5' and 3' LTRs. These subcloned fragments have subsequently been ligated to a 'crippled' thymidine kinase gene, i.e. a tK gene which still

contains the structural part coding for the enzyme but which is devoid of its promotor and initiation site. Transfection of these chimeric genes into LtK⁻ cells suggests that tK synthesis takes place under the MMTV promotor control. Hormone induction of this tK synthesis is being analyzed. 2. If the regulation of specific initiation of transcription of the MMTV gene is located in a different DNA sequence than the hormonal response it should be possible to dissociate the two biological functions. This is being attempted by deleting defined fragments of MMTV DNA from the 5' side preceeding the promotor region. Constitutive transcription uncoupled from hormonal induction of MMTV RNA is being analyzed. Both approaches, i.e. transfer of specific regulatory signals to other genes and site directed deletion mutants can be combined.

VII. Identification of oncogenes

N.E. Hynes, B. Groner

Since MMTV probably does not carry an oncogene the search for the molecular link between viral gene expression and malignant trans-formation is continuing. The promotor insertion model of chronic viruses postulates a stable change in genotype of transformed cells by recombination between a viral promotor and a cellular gene involved in growth control. This model implies a physical linkage between proviral sequences integrated into tumor tissue and the oncogene. We have·cloned proviral DNA copies from mammary tumor DNA and distinguished endogenous from acquired copies. We furthermore cloned the DNA of the proviral DNA in the mammary tumor induction locus mtv-2. Both proviral copies are candidates for a promotor insertion preceeding cellular oncogenes.

Transfection of NIH/3T3 cells with tumor DNA has been shown to cause transformation of these cells. This assay makes it possibile to score oncogenes not linked in cis to a proviral gene copy. We have shown that radiation and carcinogen induced mammary tumors do not necessarely integrate exogenous proviral DNA copies. It should be possible to ligate fragments of this tumor DNA to a cloned sequence of plasmid DNA and transfect NIH/3T3 cells. The resulting transformed

cell clones can be grown into mass culture and the introduced oncogene can be recovered using the ligated plasmid DNA as a probe. This approach is the most powerful to date to identify oncogenes, analyse their function and eventually unify the mechanisms by which viruses, radiation and carcinogens transform cells.

7. Publications of Coworkers of the IGT (1.1.1979-31.12.80)

7.1 Articles in Scientific Journals

M. Achtman, P.A. Manning, C. Edelbluth, P. Herrlich
Export without proteolytic processing of inner and outer
membrane proteins encoded by F sex factor tra cistrons in
Escherichia coli minicells
Proc. Natl. Acad. Sci. USA 76, 4837-4841 (1979)

H. Dertinger
Kinetics of regrowth of V79 spheroids after γ- and π^--irradiation
Strahlentherapie 155, 722-725 (1979)

H. Dertinger, A. Seiter
Erholungs- und Proliferationseffekte in multizellulären
Sphäroiden bei fraktionierter γ-Bestrahlung und kombinierter
Hyperthermiebehandlung
Strahlentherapie 155, 39-43 (1979)

B. Gabard
Removal of internally deposited gold by 2,3-dimercaptopropane
sodium sulphonate (Dimaval)
Br. J. Pharmac. 68, 607-610 (1980)

B. Gabard, F. Planas-Bohne, G. Regula
The excretion of trace elements in rat urine after treatment
with 2,3-dimercaptopropane sodium sulfonate
Toxicology 12, 281-284 (1979)

B. Gabard, R. Walser
Note on the metabolism of the mercury chelating agent sodium-
2,3-dimercaptopropane-1-sulfonate
J. Toxicol. and Environm. Health 5, 759-764 (1979)

C. Gärtner, E.A. Waldstein, U. Hagen
Mechanism of the ATP effect in the DNA repair synthesis of
γ-irradiated Escherichia coli cells
Biochim. Biophys. Acta 607, 247-255 (1980)

B. Groner, E. Buetti, H. Diggelmann, N.E. Hynes
Characterization of endogenous and exogenous Mouse Mammary Tumor
Virus proviral DNA with site specific molecular clones.
J. Virol. <u>36</u>, 734-745 (1980)

L. Guhl
Dekorporation von Radionukliden. Über die Eliminierung von inkorpo-
riertem ^{144}Cer mittels Zn-DTPA bei normaler und gestörter Nierenfunktion.
Strahlentherapie <u>155</u>, 216-219 (1979)

P. Herrlich, M. Schweiger, H.J. Rahmsdorf, H. Ponta, N. Spoerel,
M. Pfennig-Yeh, P. Wolfram, U. Rahmsdorf, B. Auer, E.F. Wagner
Virus T7: a probe of cellular control mechanisms.
In: Proc. 12th FEBS Meeting, Vol. 51 "Gene Functions",
Eds. S. Rosenthal, H. Bielka, Ch. Coutelle, Ch. Zimmer,
pp. 337-346, Pergamon Press Oxford and New York 1979

S. Herrmann
Die kombinierte Wirkung von Strahlung und chemischem Agens
auf die Mutationsinduktion bei E.coli
Dissertation, Universität Karlsruhe 1980

J. Hilkins, J. Hilgers, R. Michalides, M. van der Valk,
B. van der Zeijst, A. Colombatti, N.E. Hynes, B. Groner
Analysis of murine retroviral genes and genes for viral membrane
receptors by somatic cell genetics. In: Viruses in Naturally
Occuring Cancers, Cold Spring Habour Conferences on Cell Prolifera-
tion, Vol. 7, 1033-1048 (1980)

G. Hotz
Die Gefährdung des Menschen durch Röntgenstrahlen
Urologe B <u>19</u>, 103-107 (1979)

J. Lohbreier-Dörr, G. Hotz
Influence of diethylenetriamine pentaacetic acid (DTPA) on the
synthesis of DNA, RNA, and protein in Escherichia coli
Biochem. Pharmacol. <u>28</u>, 1679-1680 (1979)

C. Lücke-Huhle, E.A. Blakely, P.Y. Chang, C.A. Tobias
Drastic G_2-arrest in mammalian cells after irradiation with
heavy-ion beams
Radiat. Res. 97-112 (1979)

C. Lücke-Huhle, H. Schlag
Differential radiosensitivity of two mammalian cell lines
after hyperthermic pretreatment
Strahlentherapie 155, 649-654 (1979)

C. Lücke-Huhle, E.A. Blakely, F.Q. Ngo, P.Y. Chang, C.A. Tobias
Survival and kinetic response of V79-spheroids after exposure
to heavy ion beams
Int. J. Radiat. Biol. 37, 483-492 (1980)

U. Mallick, P. Herrlich
Regulation of synthesis of a major outer membrane protein:
cyclic AMP represses Escherichia coli protein III synthesis.
PNAS 76, 5520-5523 (1979)

H. Martin-Bertram, U. Hagen
Genomic integrity of T1 DNA after γ- and ultraviolet irradiation
Biochim. Biophys. Acta 561, 312-323 (1979)

F. Planas-Bohne
Influence of several chelating agents on the excretion
and organ concentration of copper in the rat
Toxicol. Appl. Pharmacol. 50, 337-345 (1979)

F. Planas-Bohne
Chelate treatment in acute cadmium poisoning
Experientia 36, 1001-1002 (1980)

F. Planas-Bohne, B. Gabard, E.H. Schäffer
Toxicological studies on sodium 2,3-dimercaptopropane-1-sulfonate
in the rat
Arzneimittelforschung 30, 1291-1294 (1980)

E. Polig
Minidosimetry of alpha-radiation from ^{239}Pu in the skeleton.
In: Proc. 5th Intern. Congress of the International Radiation
Protection Association, Vol. III, pp. 125-128, Jerusalem, 1980

H. Ponta, M.-L. Pfennig-Yeh, E.F. Wagner, M. Schweiger,
P. Herrlich
Radiation sensitivity of messenger RNA
Molec. Gen. Genet. 175, 13-17 (1979)

H.J. Rahmsdorf
Protein kinase action as a mediator of chemotaxis and
differentiation in the cellular slime mold, Dictiostelium
discoideum. In: Proc. 12th FEBS Meeting, Vol. 54 "Cyclic
Nucleotides and Protein Phosphorylation in Cell Regulation",
Eds. E-G. Krause, L. Pinna, A. Wollenberger, pp. 199-210,
Pergamon Press Oxford and New York 1979

H.J. Rahmsdorf, D. Malchow, G. Gerisch
Cell surface protein kinases in Dictyostelium: are they artifacts?
Cell Biology International Reports 3, 237-245 (1979)

H.J. Rahmsdorf, S.H. Pai
Protein kinases of Dictyostelium discoideum, strain AX-2.
Biochim. Biophys. Acta 567, 339-346 (1979)

G. Schäfer, P. Haas, Th. Coquerelle, U. Hagen
Properties of an endonuclease activity in Micrococcus luteus
acting on γ-irradiated DNA and on apurinic DNA
Int. J. Radiat. Biol. 37, 11-18 (1980)

A. Seidel, R. Winter, C. Jentzsch, R. Gruner, H.-G. Neumann,
S. Hanke
The biochemical mechanism responsible for differences in the
half-life of monomeric ^{239}Pu in rat and Syrian hamster liver.
In: "Biological Implications of Radionuclides Released from
Nuclear Industries", Vol. I, pp. 401-415, I.A.E.A., Wien, 1979

A. Seidel, R.M. Flügel, D. Komitowski, G. Darai
Retention and effects of ^{239}Pu in the tree shrew (Tupaia belangeri).
In: Proc. 5th Intern. Congress of the International Radiation
Protection Association, Vol. II, pp. 123-126, Jerusalem, 1980.

A. Seidel, D.M. Taylor
Inorganic biochemistry in environmental research: methods, results,
perspectives. In: "Management of Environment", Ed. B. Patel,
pp. 203-216, Wiley Eastern Ltd., New Delhi, 1980.

W. Sontag
Quantitative studies on bone-remodelling rates intended to
improve estimates of radiation dose caused by bone-seeking
radionuclides. In: Biological Implications of Radionuclides
Released from Nuclear Industries", Vol. I, pp. 417-428,
I.A.E.A. Wien, 1979

W. Sontag
An automatic microspectrophotometric scanning method for
the measurement of bone formation rates in vivo
Calcif. Tissue Int. 32, 63-68 (1980)

N. Spoerel, P. Herrlich
Colivirus T3-coded S-Adenosylmethionine hydrolase.
European Journ. Biochem. 95, 227-233 (1979)

N. Spoerel, P. Herrlich, Th.A. Bickle
A novel bacteriophage defence meachnism: the antirestriction
protein
Nature 278, 30-34 (1979)

D.M. Taylor
Late biological effects of internally deposited Curium-244
in rats. In: "Biological Implications of Radionuclides Released
from Nuclear Industries", Vol. I, pp. 153-161, I.A.E.A. Wien,
1979

D.M. Taylor
Mobilization of internally deposited plutonium from the rat
by pregnancy and lactation
Int. J. Radiat. Biol. 38, 357-360 (1980)

D.M. Taylor, V. Volf
Oral chelation treatment of injected ^{241}Am or ^{239}Pu in rats
Health Physics 38, 147-158 (1980)

V. Volf
Removal of incorporated radionuclides: methods, benefits and
risks. In: "Radiation Research", Proc. of the 6th Intern. Congress
of Radiation Research, Eds. S. Okada et al., pp. 913-921,
Maruzen Co. Ltd., Tokio, Japan 1979

V. Volf, G. Möhrle
Möglichkeiten der Behandlung nach Inkorporation von Radio-
nukliden. In: "Strahlenschutzkurs für ermächtigte Ärzte.
Spezialkurs", Hrsg. F.E. Stieve, G. Möhrle, pp. 228-242,
Hildegard Hoffmann Verlag, Berlin 1979

V. Volf, E. Polig, E. Gemenetzis
Prevention of late effects by removal of radionuclides from
the body? In: "Biological Implications of Radionuclides Released
from Nuclear Industries", Vol. II, pp. 41-51, I.A.E.A. Wien, 1979

V. Volf
Influence of Cadmium and Copper on the distribution pattern
of Plutonium in the rat
J. Toxicol. Environ. Health 6, 493-501 (1980)

V. Volf
Prophylaxis and Therapie der Inkorporation von Radionukliden
Schweiz. Z. Milit.-Med. 57, 95-101 (1980)

V. Volf
Can mixed ligand therapy completely remove Plutonium from the body?
Health Physics 39, 364-366 (1980)

K.F. Weibezahn, H. Dertinger, H. Schlag, C. Lücke-Huhle
Biological effects of negative pions in monolayers and
spheroids of Chinese hamster cells
Rad. and Environm. Biophys. 16, 273-277 (1979)

K.F. Weibezahn, C. Sexauer, T. Coquerelle
Negative pion irradiation of mammalian cells. III. A comparative
analysis of DNA strand breakage, repair and cell survival after
exposure to π^-mesons and X-rays
Int. J. Radiat. Biol. 38, 365-371 (1980)

R. Winter
Die subzelluläre Bindung von ^{239}Pu in der Leber ausgewählter
Nagetierspezies
Dissertation. Universität Karlsruhe 1980

R. Winter, A. Seidel
Subcellular distribution of ^{239}Pu in the liver of rat, mouse,
Syrian and Chinese hamster. In: Proc. 5th Intern. Congress of
the International Radiation Protection Association, Vol. II,
pp. 135-138, Jerusalem, 1980

H. Appel, T. Krüger, W.-G. Thies
Messung der Spektrumsform der β-Übergänge $Cl^{34m}(3^+-2^+)S^{34}$ und
$Cl^{34}(0^+-0^+)S^{34}$
Verhandlungen der Deutschen Physikalischen Gesellschaft,
R. 6, Bd. 15, 1202-1203 (1980)

H. Dertinger, U. Hagen, C. Lücke-Huhle, H. Schlag, K.F. Weibezahn
Molekulare Mechanismen der Inaktivierung von Säugetierzellen durch
negative Pionen
SIN Jahresbericht 1978, C63-C64

H. Dertinger, D. Hülser, G. Hinz
Survival and chromosome injury of spheroid cells after irradiation
studia biophysica 76, 31-32 (1979)

H. Dertinger, C. Lücke-Huhle, H. Schlag, K.F. Weibezahn
Biologische Wirkungen von π^--Mesonen auf Zellkulturen
KfK-Nachrichten 11, 8-11 (1979)

H. Dertinger, G. Hinz, D. Hülser
Cell coupling and radiosensitivity of cells under tissue-
equivalent organization (spheroids)
Rad. and Environm. Biophys. 17, 316 (1980)

B. Groner, N. Hynes, N. Kennedy, R. Michalides
Mouse Mammary Tumor Virus: isolation of the endogenous
proviral genes of the GR mouse and their molecular comparison
Europ. J. Cell Biol. 22, 5 (1980)

B. Groner, N. Hynes, H. Diggelmann, R. Michalides, N. Kennedy
Characterization of endogenous and exogenous MMTV proviral copies
and their flanking host DNA. In: Abstractband "12th meeting on
mammary cancer in experimental enimals and man", Maastricht,
May 11-14, 1980

R. Gruner, A. Seidel
Subcellular binding of ^{239}Pu in rat liver as determined by
Triton WR 1339 injection
Europ. J. of Cell Biol. 20, 121 (1979)

P. Herrlich, M. Hirsch-Kauffmann, L. Beutin, M. Schweiger
Schädigung des Genoms durch Nitrofuran-Derivate
Archiv f. Genetik 51, 10 (1978)

P. Herrlich
Gen-Manipulation
KfK-Nachrichten 11, No 4, 49-56 (1979)

N. Hynes, B. Groner, E. Buetti, N. Kennedy
Isolation of an intact MMTV provirus endogenous to the GR mouse.
In: Abstractband "12th meeting on mammary cancer in experimental
animals and man", Maastricht, May 11-14, 1980

N. Hynes, B. Groner, R. Michalides, N. Kennedy
Isolation of the endogenous MMTV proviruses of the GR mouse
and their molecular comparison. In: "Abstracts of papers pre-
sented at the meeting on RNA TUMOR VIRUSES", Cold Spring Harbor
Laboratory, p. 37, Cold Spring Harbor, New York, May 21-25, 1980

W. Kündig, E. Holzschuh, P.F. Meier, B.D. Patterson, K. Rüegg,
J.P.F. Sellschop, M. Stemmet, H. Appel
The anomalous muonium state in diamond
SIN Newsletter No. 13, 32-33 (1980)

C. Lücke-Huhle
Wirkung dicht-ionisierender Strahlen auf den Zellvermehrungs-
Rhythmus
KfK-Nachrichten 11, No. 4, 28-31 (1979)

C. Lücke-Huhle, E.A. Blakely and C.A. Tobias
The influence of intercellular contact on mammalian cell
survival after heavy-ion irradiation. In: "High LET Radiations
in Clinical Radiotherapy", Suppl. to the Europ. Journ. of
Cancer, pp. 227-228, Pergamon Press 1979

C. Lücke-Huhle
Efficient G2-delay after very low doses (0.03-1 Gy) of alpha
particles
Rad. and Environm. Biophys. 17, 365 (1980)

C. Lücke-Huhle
Zellzyklusveränderungen und Zellabtötung nach Bestrahlung
mit beschleunigten schweren Ionen. In: "Bestandsaufnahme
Krebsforschung in der Bundesrepublik Deutschland", 1979, DFG,
Band II Dokumentation, E.A. Boedefeld, Hrsg., S. 151, H. Boldt
Verlag, Boppard 1980

C. Lücke-Huhle, M. Pech
Radiation-induced G2-delay, a DNA repair period?
Europ. J. Cell Biol. 22, 98 (1980)

C. Lücke-Huhle, H. Schlag
Differences in cell cycle perturbation after irradiation
depending on radiation ionisation density
Cell Tissue Kinet. 13, 204 (1980)

U. Mallick, P. Herrlich
Regulatory role of cGMP in Escherichia coli.
Hoppe Seyler's Z. physiol. Chemie 360, 321-322 (1979)

U. Mallick, P. Herrlich
cAMP represses synthesis of major outer membrane protein.
Hoppe Seyler's Z. physiol. Chemie 360, 322 (1979)

U. Mallick, H.J. Rahmsdorf, H. Ponta, T. Coquerelle, R. Eife,
P. Herrlich
Stop of replication induces and represses the synthesis of
specific proteins in mammalian cells
Europ. J. Cell Biol. 22, 102 (1980)

H.J. Rahmsdorf
Wir lernen von einem Schleimpilz Mechanismen zellulärer
Differenzierung und Bewegung
KfK-Nachrichten 11, No. 4, 45-48 (1979)

H.J. Rahmsdorf, H. Ponta, P. Herrlich, Ch. Heusser
Primary transcripts of H and L immunoglobulin gens.
Abstract XIth International Congress of Biochemistry, Toronto 1979

H.J. Rahmsdorf, H. Ponta, U. Mallick, K.F. Weibezahn, P. Herrlich
Different DNA repair capacity in various differentiated cells.
Europ. J. Cell Biol. 22, 98 (1980)

W. Sontag
Verteilung von Radionukliden im Knochen unter Berücksichtigung
der Umbaudynamik
KfK-Nachrichten 11, No. 4, 32-37 (1979)

N. Spoerel, T.A. Bickle, P. Herrlich
Antirestriction proteins.
Hoppe Seyler's Z. physiol. Chemie 360, 234 (1979)

N. Spoerel, F. Litfin, P. Herrlich
Two mechanisms of antirestriction. In: Proc. EMBO Workshop
on Protein-DNA Interactions in Bacteriophages, Salamanca/Spain,
June 30 - July 5, 1980

D.M. Taylor
Biochemische Untersuchungen mit Transuranelementen - ihre Rolle
in der Radiotoxikologie
KfK-Nachrichten 11, No. 4, 3-7 (1979)

K.F. Weibezahn, T. Coquerelle
Rejoining kinetics of DNA double strand breaks in mammalian cells
Rad. and Environm. Biophys. 17, 351 (1980)

8. In-House-Seminars

1979

Januar

N. Spoerel
MPI f. Molek. Genetik
Berlin
— Der Mechanismus, wie bakterielle Viren den Restriktionsenzymen entkommen

T. Schröder
Inst. f. Humangenetik
Heidelberg
— Untersuchungen zu spontan erhöhter Chromosomeninstabilität beim Menschen

W. Schmatz
IAK I, Kernforschungszentrum
Karlsruhe
— Streuung langsamer Neutronen als Methode zur Untersuchung biologischer Substanzen

Februar

K. Geider
MPI f. Medizin.Forschung
Heidelberg
— DNA-Replikation des Phagen fd in vitro

K. Keck
Inst. f. Immunologie
Konstanz
— Die genetische Kontrolle der Erkennung von immunogenen Determinanten

D. Richter
Physiol.-chem.Inst. d.Univ.
Hamburg
— Metabolismus und Funktion des Regulationsnukleotids Guanosin-Tetraphosphat auf Translationsebene in E.coli

H. Esche
Inst.f.Genetik der Univ.
Köln
— In vitro Translation von Ad 12-spezifischer mRNA

März

B. Groner
Schweiz.Krebsforschungsinst.
Lausanne
— Integration and expression of mouse mammary tumor virus genes in mouse strains with high and low tumor incidence

April

K. Bauer
Max-Volmer-Inst.f.Physikal. Chemie, TU
Berlin
— Metabolismus von Gehirnpeptiden

E. Jost
EMBL
Heidelberg
— Struktur und Superstruktur von Chromatin

H.H. Grünhagen
Inst. f. Physiolog.Chemie
Univ. d. Saarlandes
Homburg
— Biochemische und physikalisch-chemische Untersuchungen der erregbaren Membran in Nerv und Muskel

H.-J. Bohnert
Botan. Inst. I, Univ.
Düsseldorf
— Lokalisierung von Genen auf der Plastiden-DNA

Mai

G. Kümmel
Zoologie II, Univ.
Karlsruhe
— Funktionsmorphologie. Eine Arbeitsmethode

G. Nass
Zentrallab.f.Mutagenitätsprüf.
Freiburg
— Die Beteiligung von tRNA an Replikationsprozessen in Pro- und Eukaryonten

Juni

R. Devoret
Centre Nat. de la Recherche Scientif.,Dept. of Enzymology
Gif-sur-Yvette
— Lysogenic induction and mutagenesis as a consequence of DNA lesions produced by carcinogens and anti tumor agents

H. Dertinger
IGT, Kernforschungszentrum
Karlsruhe
— Mikroverteilung der Energie beim Durchgang schneller geladener Teilchen durch biologisches Material

S. Schäfer
Inst. f. Pharmakol.u.Toxikol.
Bochum
— Intestinale Sekretion von Metallen bei Säugetieren

Juli

T. Norseth
Inst. of Occupational Health
Oslo
— Toxicity and interactions of heavy metals

H.P. Bertram
Inst. f. Pharmakol.u.Toxikol.
Münster
— Dekorporation von Quecksilber beim Menschen

P. Tulkens
Internat.Inst. of Cell and Mol. Pathol.
Brüssel
— Drug binding to lysosomes: Concepts and consequences

Oktober

C. Gualerzi
MPI f. Molek. Genetik
Berlin
— The role of the initiation factors in initiation of protein synthesis in E.coli

F.W. Studier
Brookhaven Nat. Lab.
— Gene expression after bacteriophage T7 infection

M. Wabl
Miescher Laboratorium
Tübingen
— Die Strategie des Immunsystems

J. Rétey
Inst. f. Biochemie
Karlsruhe
— Steriospezifität von Enzymreaktionen

G. Bornkamm
Inst. f. Virologie
Freiburg
— Struktur des Genoms von Epstein-Barr-Viren

R.E. Durand
Johns Hopkins Oncology Center
Baltimore
— Combined modality studies in multicell spheroids

November

H. Schuster
MPI f. Molek. Genetik
Berlin
— DNA Replikationsproteine von Plasmiden: Isolierung und Eigenschaften

K. Sperling
Inst. f. Humangenetik
Berlin
— Zellzyklus und Chromosomenzyklus

M. Radman
Lab. de Biologie Moleculaire
Brüssel
— On the relationship of DNA repair on mutagenesis and recombination in bacterial and mammalian cells

A.-E. Harmuth-Hoene
ISTL, Kernforschungszentrum
Karlsruhe
— Der Einfluß von Ballaststoffen in Lebensmitteln auf die Mineralstoffresorption bei Mensch und Tier

Dezember

S. Herrmann
IGT, Kernforschungszentrum
Karlsruhe
— Untersuchungen zur Wechselwirkung von UV und EMS bei der Induktion von Mutationen

D. Green
MRC Radiobiol. Unit
Harwell
— Studies on the microdistribution of plutonium-239 in mouse bone relevant to tumor induction

R. Winter
IGT, Kernforschungszentrum
Karlsruhe
— Die subzelluläre Verteilung von ^{239}Pu in der Leber von vier Nagetierspezies

1980

Januar

C. Ackermann
Botanisches Institut
Heidelberg
— Erbliche Pflanzentumore

K. Decker
Inst. f. Biochemie
Freiburg
— Mechanismen der Hepatotoxizität von d-Galaktosamin

V. Volf
IGT, Kernforschungszentrum
Karlsruhe
— Metall-Wechselwirkung in vivo

E. Polig
IGT, Kernforschungszentrum
Karlsruhe
— Dosimetry of alpha-emitters in bone

W. Sontag
IGT, Kernforschungszentrum
Karlsruhe
— Quantitative aspects of bone structure and bone remodelling

Februar

D. Williams
Dept. Chem.,Univ.Wales
Cardiff
— Trace Element Drugs

April

D. Bootsma
Dept.Cell Biol. and Genet.
Erasmus Universität
Rotterdam
— Genetic analysis of DNA repair in eucaryotic cells

H. Appel
IGT, Kernforschungszentrum
Karlsruhe
— Die Beobachtung der Hyperfeinwechselwirkung als Hilfsmittel beim Studium von Biomolekülen

Speaker / Affiliation	Title
P. Wrede Inst. f. Genetik Heidelberg	Strukturuntersuchungen von t-RNA mit Nukleasen
A. Hinnen Friedrich-Miescher-Institut Basel	Cloning in yeast
Mai M. Lehmann IGT, Kernforschungszentrum Karlsruhe	Regulation der Glykogen-Synthese bei S.typhimurium
R. Jaenisch Pette-Institut Hamburg	RNA-Tumorviren, zelluläre Differenzierung und Embryogenese
M. Beato Inst. f. physiol. Chemie Marburg	Hormonelle Steuerung des Uteroglobin-Gens
Juni U. Zimmermann Inst. f. Medizin, KFA Jülich	Elektrischer Durchbruch von Zellmembranen: Anwendung in Biologie, Medizin und Technik
W.-G. Thies IEKP, Kernforschungszentrum Karlsruhe	Was kann man mit der Methode der gestörten Winkelkorrelationen über Biomoleküle lernen?
V. Schirrmacher Inst. f.Immunologie, DKFZ Heidelberg	Untersuchungen über die Expression von Histokompatibilitätsantigenen und von Tumorantigenen auf chemisch induzierten Maus-Tumoren
F. Gannon Med. Fakultät d. Univ. Strasbourg	Chicken ovalbumin gene: Cloning and Analysis
H.P. Vosberg MPI f. Med. Forschung Heidelberg	DNA-Topoisomerasen: Ihr Wirkungsmechanismus und ihre Funktion
H.V. Rickenberg Div. Molec. and Cellular Biol Research Center Denver, USA	Aspects of Regulation by Cyclic AMP in E. coli and Dictyostelium discoideum
Juli A. Seidel IGT, Kernforschungszentrum Karlsruhe	Transurane in der Leber: Fakten, Probleme, Spekulationen
K. Eichmann Inst. f. Immun.u.Genetik, DKF Heidelberg	Funktionelle Analyse von T-Zellrezeptoren
August A. Sippel Inst. f. Genetik Köln	Strukturaufklärung des Hühner-lysozym-Gens
Oktober J. Hirschberg Dept. of Genetics Jerusalem	Cell Cycle Investigation Using Cell Cyle Mutants
U. Schibler ISREC Lausanne	Tissue Specific Expression of Mouse α-Amylase Genes
D.M. Taylor IGT, Kernforschungszentrum Karlsruhe	The Toxicology of Radioiodine
T. Hohn Friedrich-Miescher-Inst. Basel	Cauli-flower-mosaic Virus ein zukünftiger Vektor für Pflanzen-DNA?
A. Trautwein Univ. d.Saarlandes,Fachbereich 12-1 Saarbrücken	Einige bio-anorganische Anwendungen der Mößbauer-Spektroskopie
November H.P. Beck Inst. f. Biophysik Hamburg	Zellkinetische Analyse mit Hilfe der BUdR/33258-Hoechst-Technik
V. Volf IGT, Kernforschungszentrum Karlsruhe	Jodtabletten als Schilddrüsenschutz nach Reaktorunfall
C. Moroni Friedrich-Miescher-Inst. Basel	Expression of Endogenous C-type Viruses in Stimulated Mouse Lymphocytes

Speaker / Affiliation	Title
C.S.P. Iyer Inst.f. Chemie, KFA Jülich	Toxic Heavy Metals in Nature
H.G. Schweiger MPI f. Zellbiologie Ladenburg	Zirkadianer Rhythmus und Genexpression
K.-H. Klempnauer Fachbereich Biologie Konstanz	Reifung von nurepliziertem SV40 Chromatin
Dezember P. Traub MPI f. Zellbiologie Ladenburg	Zur Biochemie des Intermediär-Filamentproteins Vimentin in Mammaliazellen
D.M.Taylor IGT, Kernforschungszentrum Karlsruhe	Platin Zytostatika
J.R. Sheppard Pharmakol. Inst. Heidelberg	In vitro studies of central nervous system development
G. Howells MRC Radiobiol. Unit Harwell	^{239}Pu metabolism and effects on reproduction in mouse

9. Workshops

"Workshop on Tumorviruses"
(November 20, 1980)

This workshop was organized jointly under the auspices of the
Institute for Genetics and Toxicology (KfK) and Institute for
Genetics (University of Karlsruhe) to bring together about 50
scientists essentially from AGF institutions to discuss important
advances in the field of tumor virology.

Organizing Committee: P. Herrlich (IGT)
                         B. Groner   (IGT)
                         G. Kammerer (IGT)

Speakers:

T. Graf (Heidelberg)   "Modellstudien zur Virus-induzierten
                                  Leukämogenese"

V. Erfle (München)   "Die Beteiligung von endogenen
                                  Retroviren bei der strahleninduzier-
                                  ten Carcinogenese"

B. Groner (Karlsruhe)   "Hormon-abhängige Expression eines
                                  endogenen m-mtv Provirus nach Klonie-
                                  rung und Transfer des Gens in Kultur-
                                  zellen"

R.M. Flügel (Heidelberg)   "Eigenschaften des Tupaia Adenovirus =
                                  TAV und seines Genoms"

G. Sauer (Heidelberg)   "Die Biologie latenter Papova Tumor-
                                  viren"

10. Teaching Activities at the Universities of Karlsruhe and Heidelberg

Several members of the staff are involved in teaching the student programmes (lectures, seminars) and organizing special courses.

H. Appel,
F. Planas-Bohne,
A. Seidel,
D.M. Taylor

Biochemie umweltrelevanter Schwermetalle

H. Dertinger

Thermodynamik biologischer Prozesse

Spezielle Probleme der Biophysik

Physikalische Methoden in der Biologie

Statistische Methoden in der Biologie

B. Groner

Gentechnologisches Praktikum

B. Groner,
P. Herrlich,
N. Hynes,
N. Kennedy,
H. Ponta,
H.J. Rahmsdorf

Regulation der Gene

P. Herrlich

Einführung in die Immungenetik

Humangenetik I

Humangenetik II

Genetische Seminare für Anfänger und
   Fortgeschrittene

Literaturseminare für Fortgeschrittene

P. Herrlich,
G. Hotz,
U. Mallick,
H. Ponta,
H.J. Rahmsdorf

Seminare zu den Genet.Kursen I u. II

Genet. Kurse I u. II

P. Herrlich,
C. Lücke-Huhle,
H. Ponta,
H.J. Rahmsdorf

Genet. Kurs III

P. Herrlich,
U. Mallick,
H. Ponta,
H.J. Rahmsdorf

Vorbereitungsseminar
für die Genet. Kurse

P. Herrlich, G. Hotz, H. Ponta, H.J. Rahmsdorf, A. Seidel, V. Volf	Anleitung zu wissenschaftlichen Arbeiten
G. Hotz	Strahlen- und molekularbiologisches Praktikum
C. Lücke-Huhle	Einführung in die molekulare Strahlenbiologie Einführung in die zelluläre Strahlenbiologie Seminar über Strahlenbiologie
U. Mallick	Einführung in die Genetik
H. Ponta	Einführung in die Biochemie Biochemie für Lehramtskandidaten (Seminar)
A. Seidel	Biologische Wirkung kerntechnischer Schadstoffe, Dosis-Effektbeziehungen u. Risikoanalyse
A. Seidel, V. Volf	Radiotoxikologisches Seminar
D.M. Taylor	Einführung in die Radiotoxikologie Grundlagen der Chemotherapie maligner Tumoren
V. Volf	Biologisches Verhalten und Toxizität der Radionuklide Allgemeine Toxikologie der Radionuklide I

11. Extramural Activities of Institute Members

Januar 1979

Zentrallaboratorium für Mutagenitätsprüfung, Freiburg

    U. Mallick, "Negative Regulation der Proteinsynthese
durch cAMP"

März 1979

I.S.R.E.C. Epalinges, Lausanne, Schweiz

    P. Herrlich, "F-factor coded tra-gene expression"

Mai 1979

6th. International Congress of Radiation Research, Tokyo

    T. Coquerelle, "Endonuclease activity against γ-irradiated
DNA and apurinic DNA in extracts of
Micrococcus luteus"

    C. Lücke-Huhle, "Cell cycle alterations after high and
low LET irradiation of mammalian cells
in vitro"

Juni 1979

Second Annual Symposium of the Molecular Biology Subgroup.
(Funded by the European Communities Commission)
CSN-Casaccia, Rom

    K.F. Weibezahn, "X and π^- irradiation compared at DNA and
survival levels"

        "A physical method for cell permeabilisation"

Juli 1979

Institut für Biologie, GSF, Neuherberg (München)

    C. Lücke-Huhle, "Zellzyklusveränderungen und Zelltod nach
dicht-ionisierender Bestrahlung"

Strahlenbiologisches Institut der Universität, Zürich

    H. Dertinger, "π^--Bestrahlungsexperimente an Multizell-
Sphäroiden"

September 1979

Workshop in Liquid Scintillation Counting Chemical Society
of London, London

    D.M. Taylor, Lecturer

Oktober 1979

I.S.R.E.C. Epalinges, Lausanne, Schweiz

    T. Coquerelle, "Endonuclease activities in extracts of
Micrococcus luteus acting on γ-irradiated DNA
and on apurinic DNA"

10th Meeting of the European Study Group for Cell Proliferation,
Knokke, Belgien

    C. Lücke-Huhle, "Differences in cell cycle perturbation
after irradiation depending on the ionisation
density"

Symposium of the AGF (Arbeitsgemeinschaft der Großforschungs-
einrichtungen) on "Chemicals in the environment", Bonn

    F. Planas-Bohne, " Dekorporation von Schwermetallen -
insbesondere Quecksilber"

Neubrandenburgisches Symposium, Neubrandenburg (Berlin)

    H. Dertinger, "Survival and chromosome injury of spheroid
cells after irradiation"

November 1979

Institut für Biophysik der Universität, Hamburg

    H. Dertinger, "Interzelluläre Kommunikation und Strahlen-
resistenz in Sphäroiden"

Batelle Pacific Northwest, Richland, Washington

    H. Appel, "The Application of hyperfine interaction methods
to biological molecules"

Single photon radiopharmaceuticals and labels - course "Advances
in Nuclear Medicine" - Royal Postgraduate Medical School, London

    D.M. Taylor, Lecturer

Fakultät für Bio- u. Geowissenschaften der Univ., Karlsruhe

    P. Herrlich, "Genmanipulation" (Ringveranstaltung)

Dezember 1979

124. Tagung der Vereinigung Nordwestdeutscher Chirurgen, Hamburg

    V. Volf, "Prophylaxis und Therapie der Inkorporation
von Radionukliden"

Molekularbiol. Kolloquium der Naturwiss.-med. Arbeitskreise
der Univ., Frankfurt

    P. Herrlich, "Das Oberkommen der Wirtsrestriktion
durch Bakteriophagen"

Januar 1980

Institut für Humangenetik der Universität, Bremen

    C. Lücke-Huhle, "Zellzyklusveränderungen nach ionisierender
Strahlung"

Institut für Humangenetik der Univ., Kiel

    P. Herrlich, "Protein X induction in mammalian cells"

Zentrum f. Humangenetik der Univ., Bremen

    P. Herrlich, "Antirestriktion. Untersuchungen an
Restriktionsenzymen bei Virusinfektionen
von E.coli"

Februar 1980

EULEP Symposium on Bone and Bone-Seeking Radionuclides: Physiology,
Dosimetry and Effects, Reisensburg

    E. Polig, "Dosimetry of alpha-emitters in bone"

    W. Sontag, "Quantitative aspects of bone structure
and bone remodelling"

12th Annual Meeting of the Union of Swiss Society of Experimental
Biology, Basel
    B. Groner, "Mouse Mammary Tumor Virus genes can be integrated
        adjacent to unique and reiterated host DNA
        sequences"

    B. Groner, "Molecular cloning of cellular unintegrated DNA
        forms of mouse mammary tumor virus (MMTV)"
        (Poster)

    B. Groner, "Integration and expression of exogenously
        acquired mouse mammary tumor virus sequences not
        normally expressing MMTV"
        (Poster)

April 1980

Institut für Anthropologie und Humangenetik, Heidelberg

    T. Coquerelle, "Repairuntersuchungen mit der alkalischen
        und neutralen Elutionsmethode",

Seminar Umweltverschmutzung und Mikrobiologie: Prinzipien und
neue Aspekte, Freie Universität, Berlin

    D.M. Taylor, "Toxikologie von Umweltschadstoffen, insbesondere
        von Stoffen aus dem Kernbrennstoff-Zyklus"

Annual Congress British Institute Radiology, London

    D.M. Taylor, "Tumour localization with Gallium-67 and
        Indium-111 radiopharmaceuticals"

Mai 1980

South Utah Chapter, Health Physics Society, Salt Lake City

    D.M. Taylor, "Some radiotoxicological problems with the
        actinide elements"

Inhalation Toxicology Research Institute, Lovelace Foundation,
Albuquerque, New Mexico

    D.M. Taylor, "The subcellular distribution of the actinides -
        results and reflections"

International Symposium on Penicillamine: Scope and Clinical
Perspectives, Miami, USA

    F. Planas-Bohne, " Pharmacokinetics and metabolism of
        penicillamine in rats - An overview"

Hospital of Special Surgery, Department of Reumatology,
Cornell University, New York

    F. Planas-Bohne, "Pharmacokinetics of Penicillamine
        in rats"

12th Meeting on Mammary Cancer in Experimental Animals and Man,
Maastricht, The Netherlands

    B. Groner, "Characterisation of endogenous and exogenous
        MMTV proviral copies and their flanking
        host DNA" (Poster)

    B. Groner, "The molecular cloning of an intact MMTV
        proviral copy endogenous to the GR mouse:
        Characterisation of proviral and flanking
        genomic DNA and gene transfer experiments"
        (Poster)

Heidelberger DNA-Tagung

    B. Groner, "Mouse Mammary Tumor Virus: Isolation of the
        endogenous proviral genes of the GR mouse
        and their molecular comparison"

Nato Advanced Study Institute EMBO Lecture Course on "Chromosome
Damage and Repair", Bergen, Norwegen

    H.J. Rahmsdorf, "Stop of replication induces and represses
        the synthesis of specific proteins in mammalian
        cells"

12th Meeting on Mammary Cancer in Experimental Animals and Man,
Maastricht, The Netherlands
    N. Hynes, "Isolation of an intact MMTV provirus endogenous
        to the GR mouse"

Cold Spring Harbor Meeting on RNA Tumor Viruses, Cold Spring Harbor

    B. Groner, "Isolation of the endogenous MMTV proviruses of
        the GR mouse and their molecular comparison"

    N. Hynes, "Isolation of the endogenous MMTV proviruses
        of the GR mouse and their molecular comparison"

Frederick Cancer Research Center, Frederick, Maryland, USA

    N. Hynes, "Isolation of the endogenous MMTV proviruses
        of the GR mouse and their molecular comparison"

Juni 1980

Radiologisches Kolloquium der Universität, Essen

    C. Lücke-Huhle, "Wirkung dichtionisierender Strahlen auf
        den Mitosezyklus"

Institut für Biophysik und Strahlenbiologie der Universität,
Hamburg

    C. Lücke-Huhle, " Strahleninduzierte G2-Verzögerung und
        Oberleben von Zellen"

Conference on "Spheroids in Cancer Research", Rochester, USA

    H. Dertinger, "On the mechanism of contact resistance in
        in multicell spheroids"

Radiobiological Institute, SCK/CEN Mol, Belgium

    D.M. Taylor, "The subcellular distribution of the Actinides:
        "Results and Reflections"

Symposium on Physical Aspects of Medical Imaging, University of
Manchester

    D.M. Taylor, "The radiopharmaceutical and its interaction
        with the patient"

Juli 1980

Zentrum für Hygiene, Universität Freiburg
    U. Mallick, "Protein "X" synthesis in mammalian cells"

Gordon Conference on Hormone Action, Plymouth, USA

    B. Groner, "Hormone responsive expression of an endogenous
        proviral gene of mouse mammary tumor virus
        after molecular cloning and gene transfer
        into cultured cells"

August 1980

2nd International Congress on Cell Biology, Berlin

B. Groner, "Mouse Mammary Tumor Virus: Isolation of the
endogenous proviral genes of the GR mouse and
their molecular comparison"

Meeting of the Study Group of the CEC on Primary Effects of
Radiation on Nucleic Acids, Rotterdam

T. Coquerelle, "Rejoining of DNA double strand breaks in
chromosome breakage syndromes"

15th Annual Meeting of the European Society for Radiation Biology,
Rotterdam

C. Lücke-Huhle, "Efficient G2 delay after very low doses
(0.03-1 Gy) of alpha particles"

H. Dertinger, "Cell coupling and radiosensitivity of cells
under tissue-equivalent organization (spheroids)"

V. Volf, "Bone and bone-seeking radionuclides: Physiology,
dosimetry and effects"

September 1980

2nd International Congress on Cell Biology, Berlin

H. Hynes, "Mouse Mammary Tumor Virus: Isolation of the
endogenous proviral genes of the GR mouse
and their molecular comparison"

C. Lücke-Huhle, "G2 delay - a period of DNA repair" (Poster)

K.F. Weibezahn, "Different DNA repair capacity in various
differentiated cells" (Poster)

International Symposium on Medical Radionuclide Imaging, IAEA,
Heidelberg

D.M. Taylor, "Evaluation of new radiopharmaceuticals for
tumor localization: The value of the human
tumor xenograft"

International Symposium on Radioiodines, Banff, Canada

D.M. Taylor, "The radiotoxicology of Iodine"

Oktober 1980

Wallenberglaboratorien, Stockholm

K.F. Weibezahn, "Rejoining kinetics of DNA double strand
breaks in mammalian cells"

Meeting of the Subgroup of the Biology of the Commission of the
European Communities, Stockholm

T. Coquerelle, "Repair of DNA double strand breaks in
Ataxia Telangiectasia and Fanconi fibroblasts"

Nato International School of Pure and Applied Biostructure,
Erice/Sizilien

C. Lücke-Huhle, "The target of radiation-induced G2 delay"

Institut für Medizinische Virologie der Universität, Heidelberg

D.M. Taylor, "Problems with cultured cells in radio-
toxicological studies"

Institut "Gustave Roussy", Villejuif

H. Dertinger, "Cell interaction and radiosensitivity of
multicell spheroids"

Nucl. Phys. Res. Unit, University of the Witwatersrand,
Johannesburg

H. Appel, "Mössbauer techniques and their comparison to
other nuclear hyperfine interaction methods"

November 1980

Royal Society of Chemistry at University of Wales Institute
of Science and Technology, Wales

D.M. Taylor, "The biochemistry of the Actinides"

Symposium on Dosimetry and Risks to Patients in Radiopharma-
ceutical Investigations, British Institute of Radiology, London

D.M. Taylor, "The ten day principle and radiopharmaceutical
investigations"

Dezember 1980

Biologisches Institut der Universität, Stuttgart

H. Dertinger, "Zellinteraktion und Strahlenresistenz in
Multizell-Sphäroiden"

5th Internat. Symposium on Flow Cytometry, Rom

L. Hieber, " Analysis of a drastic G2 delay after irradiation
with alpha particles by the BUdR H-technique"
(Poster)

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Additional Year-round-activities:

Th. Coquerelle: Member of the "Study group on primary effects of
radiation on nucleic acids", Commission of the European Communities,
Brussels.

P. Herrlich: Beiratsmitglied der Deutschen Forschungsgemeinschaft
(DFG) für das Zentralinstitut für Mutagenitätsforschung, Freiburg.

G. Hotz: Member of the "Study group on biochemistry and genetics
of DNA repair", Commission of the European Communities Brussels.
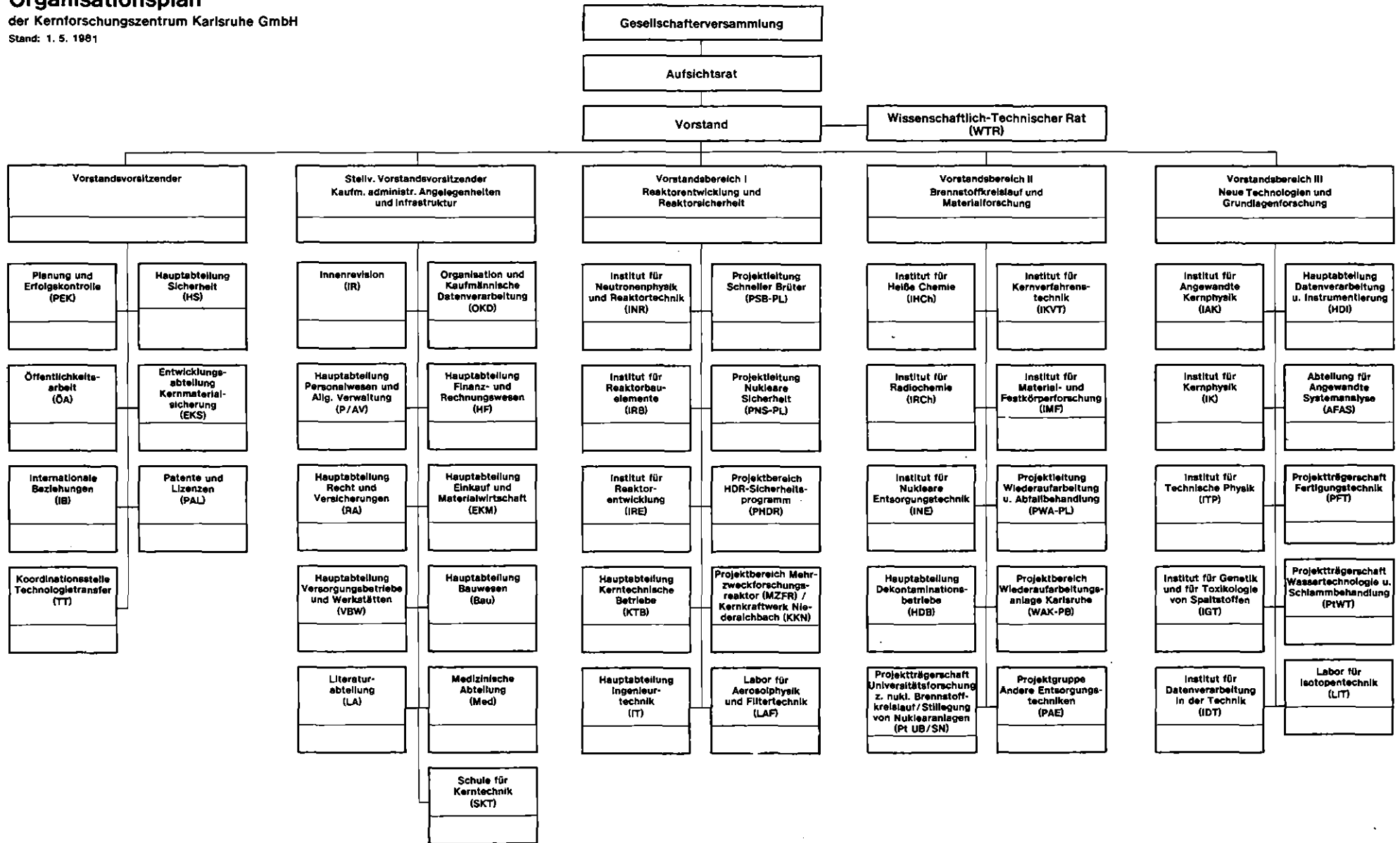
E. Polig, A. Seidel, W. Sontag, D.M. Taylor, V.Volf: Members of the
"European Late Effects Project Group (EULEP)", Brussels.

V. Volf: Mitglied des Arbeitskreises "Ärztliche Hilfe bei Kern-
energie-Katastrophen" des Wissenschaftlichen Beirats der Bundes-
ärztekammer, Köln. Mitglied des Ausschusses "Strahlenmedizin" des
Hauptverbandes der Gewerblichen Berufsgenossenschaften, Köln.

Organisationsplan

der Kernforschungszentrum Karlsruhe GmbH

Stand: 1. 5. 1981

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                        │   Gesellschafterversammlung     │
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                        │          Aufsichtsrat           │
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            │      Vorstand       │───│ Wissenschaftlich-Technischer Rat  │
            └─────────────────────┘   │              (WTR)                │
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Vorstandsvorsitzender		Stellv. Vorstandsvorsitzender Kaufm. administr. Angelegenheiten und Infrastruktur		Vorstandsbereich I Reaktorentwicklung und Reaktorsicherheit		Vorstandsbereich II Brennstoffkreislauf und Materialforschung		Vorstandsbereich III Neue Technologien und Grundlagenforschung	
Planung und Erfolgskontrolle (PEK)	Hauptabteilung Sicherheit (HS)	Innenrevision (IR)	Organisation und Kaufmännische Datenverarbeitung (OKD)	Institut für Neutronenphysik und Reaktortechnik (INR)	Projektleitung Schneller Brüter (PSB-PL)	Institut für Heiße Chemie (IHCh)	Institut für Kernverfahrenstechnik (IKVT)	Institut für Angewandte Kernphysik (IAK)	Hauptabteilung Datenverarbeitung u. Instrumentierung (HDI)
Öffentlichkeitsarbeit (ÖA)	Entwicklungsabteilung Kernmaterialsicherung (EKS)	Hauptabteilung Personalwesen und Allg. Verwaltung (P/AV)	Hauptabteilung Finanz- und Rechnungswesen (HF)	Institut für Reaktorbauelemente (IRB)	Projektleitung Nukleare Sicherheit (PNS-PL)	Institut für Radiochemie (IRCh)	Institut für Material- und Festkörperforschung (IMF)	Institut für Kernphysik (IK)	Abteilung für Angewandte Systemanalyse (AFAS)
Internationale Beziehungen (IB)	Patente und Lizenzen (PAL)	Hauptabteilung Recht und Versicherungen (RA)	Hauptabteilung Einkauf und Materialwirtschaft (EKM)	Institut für Reaktorentwicklung (IRE)	Projektbereich HDR-Sicherheitsprogramm (PHDR)	Institut für Nukleare Entsorgungstechnik (INE)	Projektleitung Wiederaufarbeitung u. Abfallbehandlung (PWA-PL)	Institut für Technische Physik (ITP)	Projektträgerschaft Fertigungstechnik (PFT)
Koordinationsstelle Technologietransfer (TT)		Hauptabteilung Versorgungsbetriebe und Werkstätten (VBW)	Hauptabteilung Bauwesen (Bau)	Hauptabteilung Kerntechnische Betriebe (KTB)	Projektbereich Mehrzweckforschungsreaktor (MZFR) / Kernkraftwerk Niederaichbach (KKN)	Hauptabteilung Dekontaminationsbetriebe (HDB)	Projektbereich Wiederaufarbeitungsanlage Karlsruhe (WAK-PB)	Institut für Genetik und für Toxikologie von Spaltstoffen (IGT)	Projektträgerschaft Wassertechnologie u. Schlammbehandlung (PtWT)
		Literaturabteilung (LA)	Medizinische Abteilung (Med)	Hauptabteilung Ingenieurtechnik (IT)	Labor für Aerosolphysik und Filtertechnik (LAF)	Projektträgerschaft Universitätsforschung z. nukl. Brennstoffkreislauf/Stillegung von Nuklearanlagen (Pt UB/SN)	Projektgruppe Andere Entsorgungstechniken (PAE)	Institut für Datenverarbeitung in der Technik (IDT)	Labor für Isotopentechnik (LIT)
			Schule für Kerntechnik (SKT)						

13. Address of the Institute

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