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COMMISSION OF THE EUROPEAN COMMUNITIES

RADIOTOXICITY OF TRITIUM IN MAMMALS

Critical analysis of the extrapolation to man of the results of tritium incorporation into animal tissues

by

G. SILINI, P. METALLI and G. VULPIS

1973

Report prepared by CNEN
Comitato Nazionale per l'Energia Nucleare
Centro di Studi Nucleari della Casaccia
Laboratorio di Radiobiologia Animale
Rome — Italy

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KEYWORDS

TRITIUM COMPOUNDS
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RBE
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MAMMALS
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CHROMOSOMAL ABERRATIONS
LET
RADIATION DOSES
RADIOECOLOGICAL CONCENTRATION
INGESTION
DRINKING WATER
RADIATION HAZARDS
MUTATION FREQUENCY
BIOSPHERE
GENETIC RADIATION EFFECTS
DELAYED RADIATION EFFECTS
REVIEWS
CRITICAL ORGANS
INTAKE
ANIMAL CELLS
BODY FLUIDS
EXCRETION
BIOCHEMICAL REACTION KINETICS
COSMIC RADIATION
ENVIRONMENT
DOSE-RESPONSE RELATIONSHIPS
TISSUES
THYMIDINE

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PREFACE

By the end of this century the quantities of tritium likely to be discharged to the environment are expected to be greater than those released at present. The pressing need to produce more electrical energy by nuclear power plants as well as the greater use of organic compounds containing tritium may result in increased contamination hazard of man and the environment.

Because of the complexity of its distribution in biological structures, the true nature of the risks the tritium represents is problematic and therefore it is relatively difficult to establish a dose-effect relationship which could serve as a basis in determining tolerance levels for the environment and maximum permissible doses for man. Numerous experiments have already been conducted on the contamination by tritium and its incorporation into animal tissues.

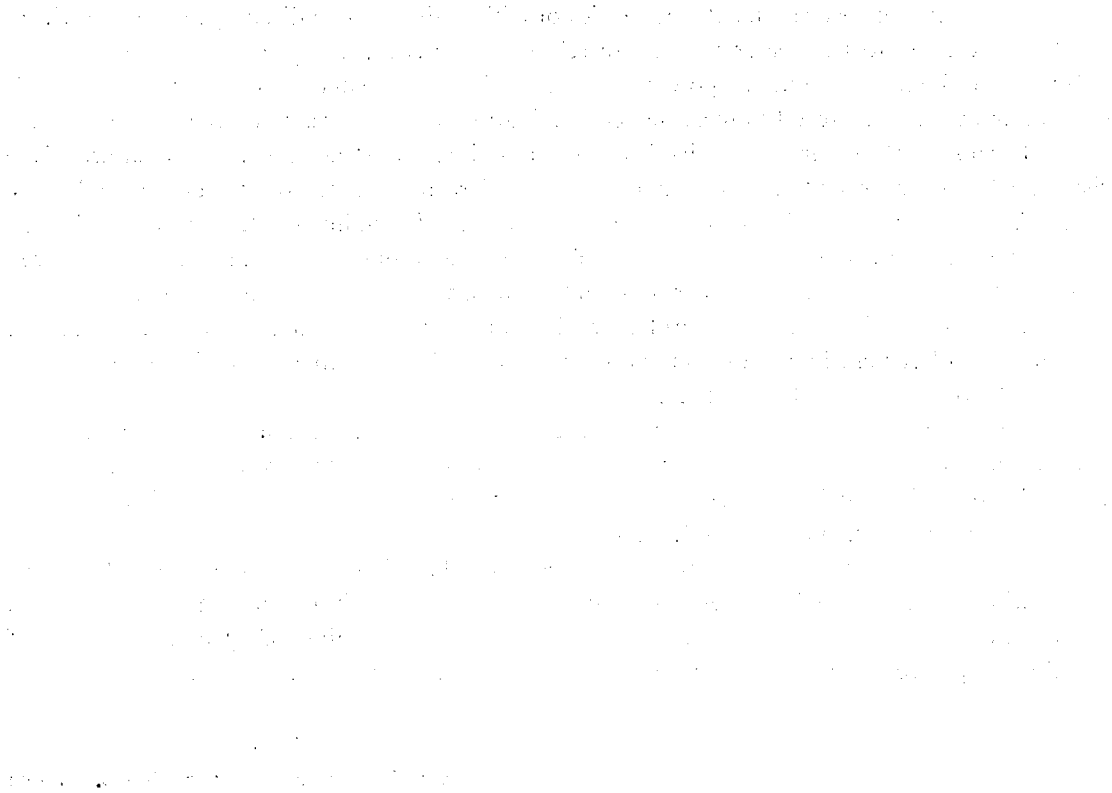
The Commission has asked Professor Silini and his team to prepare an analytical and critical study of current knowledge on tritium and to consider possible ways of extrapolating to man results of experimental toxicology.

Professor Silini has provided us with an excellent report which fulfils the proposed objective; it not only clarifies a number of previously obscure points on tritium metabolism, but also gives a list of topics which may serve as guide-lines for the new research projects which should be undertaken in this particularly complex field.

We wish to thank the authors of this report which, it is hoped, contains also the latest information on methods of dosimetry for organisms exposed to tritium contamination and thus constitutes an important contribution towards better understanding of specific problems of radiological protection connected with the presence of tritium in the environment.

Dr. P. RECHT

Director of the Health and Protection Directorate



1 — INTRODUCTION

Tritium has long been considered one of the less dangerous radionuclides because its radiation energy is relatively low so that the risk of external exposure is fairly remote and because the initial experiments on animals showed a relatively short biological half-life of the isotope and did not detect any obvious harmful effects in animals exposed to high doses of tritiated water. However, later experiments showed that an appreciable quantity of the isotope (a few per cent) administered under the form of tritiated water, was incorporated into the various components of the cell and subsequently eliminated with a half-life of the order of one-third (as a maximum) of the life of the animal. At present there is no clear proof that this tissue contamination can cause functional damage in exposed animals; but, there is likewise no justification for absolutely excluding the possibility of such damage. It is known that the presence of tritium into a number of nuclear constituents (although at much higher levels of incorporation) by the administration of tritiated thymidine can cause radiobiological lesions of a somatic or genetic nature and it is reasonable to expect that similar effects may be produced at lower concentrations (these effects then being proportionally less marked) or by the absorption of tritium through other forms.

Tritium is being produced in increasing quantities by power reactors and, although chronic or accidental contamination at present involves a relatively small number of people exposed to levels of less than the mCi down to the nCi, it is likely that the number of persons at risk will increase as a result of the presence of new nuclear reactors. Nuclear explosions are also a source of tritium contamination and this form of contamination could significantly increase if nuclear devices would be extensively used in civil engineering. Amongst the radionuclides having biological significance, tritium, like ^{14}C, is of particular importance because it is the isotope of one of the major constituents of organic substances, water and mineral salts, and this is reflected in the widespread use of tritium as a tracer in biology and medicine. The use of tritium labelled compounds for medical and research purposes is therefore another possible source of contamination, although at present this use appears to be adequately controlled.

Since it seems justifiable to assume that all these potential sources of contamination are likely to increase considerably in the future, it is important to examine the available data on the contamination by and toxicity of tritium, their significance for the protection of man and his environment and the need for further research in order to provide an accurate evaluation of the associated hazards. This, briefly, is the purpose of the present report.

2 — GENERAL DATA ON TRITIUM

2.1 — Physical data

Tritium is the radioisotope of mass 3 of hydrogen. Its nucleus, consisting of one proton and two neutrons, carries a positive charge which is balanced by the negative charge of the orbiting electron. The bond between the nuclear components in this isotope is relatively weak and the nucleus is therefore unstable. Tritium decays according to a typical beta decay pattern to the stable isotope ^{3}He by the emission of negative electrons of varying energy up to 0.018 MeV (mean energy: 0.0057 MeV). The physical half-life of tritium is 12.26 years. The maximum range of beta radiation from tritium is about 5 mm in air. In materials of unit density the maximum

range is about 6 μm (0.6 mg/cm^2), whereas the mean range is 0.56 μm (0.056 mg/cm^2). 50% of the beta radiation from tritium is absorbed in a layer of the same material about 0.4 μm thick. The energy of the bremstrahlung radiation in biological materials is 2.5×10^{-4} keV.

1 g of tritium is equivalent to about 9.7×10^3 Ci and 1 g of tritiated water is equivalent to 2.9×10^3 Ci. 1 cm^3 of gaseous tritium at normal temperature and pressure corresponds to 2.86 Ci and 1 mm^3 of tritiated water corresponds to about 2.92 Ci. 1 g of material uniformly labelled with tritium and having a specific activity of 1 mCi/g will receive a dose of about 12.14 rad/hr (see para. 5.1.1) (Feinendegen, 1967).

2.2 — Physiochemical behaviour

Since the mass of tritium is three times greater than that of hydrogen, there are distinct isotopic effects between the two nuclides, at least when the isotopic bond is directly involved in the chemical reaction (primary isotopic effect); these effects are less evident when the mass of the isotope indirectly influences the reaction through the steric conditions of the molecule (secondary isotopic effect).

The covalent C–^{3}H bond is energetically more stable than the corresponding bond between carbon and hydrogen. The secondary intermolecular H–H bonds are more stable when tritium is involved and the substitution of H–^{3}H bonds for H–H bonds may thus have a considerable effect on the tertiary structure and activity of proteins.

It is therefore clear that biochemical reactions directly or indirectly involving hydrogen bonds can be modified by the presence of tritium substituting for hydrogen. This explains the widespread distribution of tritium and deuterium in the various tissue components (Eidinoff *et al.*, 1953; Siri and Evers, 1962; Eidinoff, 1963).

2.3 — Sources of tritium

Tritium may be present in nature or produced as an artificial radionuclide.

2.3.1 — *Natural sources*

Tritium of cosmic origin is present in the atmosphere and is produced by the action of the various components of cosmic radiation, in particular high-energy protons (Fireman and Rowland, 1954), on nitrogen and hydrogen atoms in the upper atmosphere or by the action of neutrons of 4.4 MeV on ^{14}N, which is converted into ^{12}C according to the reaction

$$^{14}\mathrm{N}(\mathrm{n}, {}^3\mathrm{H})\,{}^{12}\mathrm{C} \qquad (2.1)$$

Atmospheric tritium is oxidized to tritiated water and is bound to aerosol particles (Perkins and Nielsen, 1965). It is carried into the biosphere by rain, snow or by deposition of dry or wet aerosols. Solar emmission may also be a source of atmospheric tritium (Craig, 1957). It has been calculated that the production of tritium of cosmic origin is 0.2 atoms/cm^2/s (Craig, 1957).

Natural tritium may also be produced on the earth's surface and this may explain the presence of ^{3}He which is its disintegration product (see para. 2.1) in the lower atmosphere. Tritium may be produced in rocks by reactions of the (α, n) type from natural uranium or thorium. The neutrons emitted by these reactions may, in turn, interact with lithium according to the reaction

$$^6\mathrm{Li}(\mathrm{n}, \alpha)^3\mathrm{H} \qquad (2.2)$$

to give rise to tritium (Kaufman and Libby, 1954). According to these authors, a bed of igneous

rock with a thickness of 1 km could provide 10^{-3} atoms of tritium per cm^2 per second, and this rate of production is therefore considerably lower than that due to cosmic radiation.

2.3.2 — *Artificial sources*

In addition to the reactions (2.1 and 2.2) mentioned above, tritium may be produced artificially by the following main reactions (Evans, 1966; Charamantieu, 1968)

$$^2H(d, p)^3H \quad (2.3)$$

$$^2H(n, \gamma)^3H \quad (2.4)$$

$$^3He(n, p)^3H \quad (2.5)$$

Little information appears to have been published on the release of and contamination by tritium as a result of nuclear explosions. It has been calculated, however, that the quantity of tritium produced under these conditions is of the order of 0.7 Ci per kiloton (Vaubert and Bittel, 1970). Recent estimates (Miskel, 1971) show the direct production of tritium by nuclear devices to be 1.4×10^{17} atoms of tritium per kiloton of fission and $1.4 \times 10^{23} - 1.0 \times 10^{24}$ atoms of tritium per kiloton of fusion, not including the production due to the interaction of neutrons or charged particles from the nuclear device with the environment. Nuclear explosions conducted under the Plowshare programme could be a further source of tritium, of the order of 12 Ci/kT (Lessler *et al.*, (1971).

Varying quantities of this isotope are produced by nuclear reactors, depending on the reactor type (Bramati, 1964, Jacobs, 1968; Weaver *et al.*, 1969). Power reactors produce about 50 mCi of tritium per MW per day by reactions of the (n, α) type on ^6Li contained as an impurity in fuels; the tritium thus produced remains however, in the fuel, provided that there is no leakage. Under normal conditions this tritium is subsequently released into the environment during the reprocessing of the fuel (Charamantieu, 1968), either under the form of gas or (in greater quantities) in solution. During these operations the tritium follows the same fate as nitric acid, in that the removal of the latter from the effluents also removes most of the tritium contamination. In the 1980's a number of reprocessing plants will be in operation, each of which will be capable of processing up to 0.6 MCi/year, and it is therefore important to pay close attention to these plants (Kouts and Long, 1971).

In reactors moderated and/or cooled with heavy water, tritium is produced by (n, γ) reactions on deuterium (see reaction 2.4). It is mostly combined with oxygen but may be present in the primary circuit as gaseous tritium or tritiated water vapour.

In reactors which are moderated and cooled with natural water there is a low rate of production of the isotope by reactions 2.2 and 2.4, which occur with the lithium impurities and the natural deuterium in the water, respectively. The introduction of lithium or boron compounds to control the neutron flux or to avoid corrosion may lead to an increase in tritium production.

In graphite-moderated reactors cooled with CO_2 or air, there are considerable quantities of the isotope in the graphite. A tabulation produced by Kouts and Long (1971) showed that the annual production of tritium from reactors of industrial significance (either in operation or in the design stage) is 16-45 kCi/1000 MW, except in the case of liquid-sodium breeder reactors in which annual production is increased by a factor of 10. Thermonuclear reactors will produce 10^5 times more tritium (per MW) than fission reactors, which will pose more problems with containment.

Tritium can also be prepared intentionally on a large scale in reactors by the action of neutrons on alloys of lithium and light metals, from which the isotope can subsequently be separated by acid treatment and used as a raw material for thermonuclear experiments (Evans, 1966).

Contamination measurements carried out in the vicinity of nuclear reactors have indicated the presence of low, but nevertheless significant, level of tritium contamination (Haney *et al.*,

1962; Evans, 1967; Hatch *et al.*, 1970; Aurand *et al.*, 1971). Uniform contamination of plant and animal tissues at the site of nuclear explosions has also been demonstrated (Koranda, 1967; Martin and Koranda, 1971).

Tritium may also be produced in the vicinity of accelerators, especially neutron generators of the Cockroft-Walton type, in which tritiated targets (metallic or gaseous) are bombarded with protons or deutons. With metal targets tritium has been produced in the gaseous state at rates of the order 1 Ci/hr of operation. The problem of contamination appears to be less serious, however, in accelerators of the Van de Graaf type (Nellis *et al.*, 1967).

The quantity of tritium present on the earth in 1969 was estimated at around 1700 MCi, of which 70 MCi was attributed to natural sources and the remainder to artificial reactions, in particular thermonuclear experiments (Weaver *et al.*, 1969). According to a study published in 1968 (Cowser and Tadmor, 1968) of the likely increase in major sources of tritium up to the year 2000, it is estimated that the total activity of natural tritium may by then have reached a level of the order of $10^{7 \cdot 5}$ Ci (this estimate is comparable with the estimate mentioned above), the activity due to nuclear tests may be of the order of 10^8 Ci and that due to industrial sources 10^9 Ci. These figures are, of course, subject to variations owing to the actual increase in the number of power reactors in the future and to the development of thermonuclear energy sources (Rose, 1970), which are still difficult to forecast.

2.4 — Tritium transfer paths

In the first place, it is necessary to take into account that the slight temperature gradient in the stratosphere gives the masses of air a high degree of stability, whereas in the troposphere the steep gradient gives rise to strong currents. Considering further that the concentration of water vapour is relatively high and the precipitation-evaporation cycle is short, there is a high degree of dilution and rapid precipitation of the radioactive residues on the ground. The exchange mechanisms of the tritium are, however, essentially different in the two zones.

In the *atmosphere* the transfer paths of tritium have been studied by tracing the pollution from the stratosphere in which tritium is accumulated following H-bomb explosions (Suess, 1969). The movement of tritium from the stratosphere to the troposphere was found to be sufficient to maintain in the latter a fairly constant concentration of the isotope, although there has been a tendency for this concentration to fall in recent years (Stewart and Wyerman, 1970), in spite of the deposition on the soil by rain-out. In the northern hemisphere tritium rain-out exhibits a peak in spring coinciding with the increase in rainfall. The concentration of atmospheric tritium is greater over the continents than over the oceans, since the atmospheric humidity is more stable over the land than over the sea. The exchange of tritium following its release from gaseous effluents has been dealt with exhaustively in the literature (Chamberlain and Eggleton, 1962).

Tritium concentrations in continental and oceanic *waters*, both at the surface and at depth, are much lower than rainfall concentrations (Theodorsson, 1967). The problem of transfer of tritium in *continental waters* as a function of their temperature requires, has been studied by Roether (1967) and the problems of its accumulation in lakes (Peterson *et al.*, 1969) and rivers following the immission by contaminated effluent (Parker, 1958; Jacobs, 1968) have also been dealt with in detail. Since 1970 a tritium contamination monitoring system has been in operation in the United States. Data relating to concentrations in atmospheric precipitations, continental surface waters and drinking water are available in Radiation Data and Reports (1972). As regards *oceanic waters*, studies of tritium movements as a function of temperature gradients have shown that tritium circulation below the thermocline is extremely slow (Libby, 1962; Bainbridge, 1963; Suess, 1969). Tritium levels in marine organisms in contaminated areas have also been studied (Koranda, 1965).

The absorption (Charreau and Jacquinot, 1967), diffusion (Stewart, 1965) and rate of migration of tritium in the *soil* in relation to similar parameters for water have been studied for a long time, in terms of the chemical composition of the soil (mainly acid clays) (Corey and Horton, 1968; Stewart, 1965), under experimental laboratory conditions and in field experiments. An up-to-date critical bibliography of publications on the movement of tritium in ecological systems can be found in Koranda and Martin (1971). A compilation of data relating to tritium concentrations, estimates of production, dose to the population, etc., which are of importance for the purpose of environmental protection, has been prepared by Terpilak *et al.* (1971).

3 — TRITIUM METABOLISM IN MAMMALS

3.1 — Intake routes

Tritium can be absorbed by experimental animals and by man under the form of a) gas or b) tritiated water or c) by the absorption of artificially labelled compounds or d) by the absorption of radioactivity in contaminated environments. The first two forms of exposure (gas or tritiated water) may be suffered as a result of accidents during the operation of nuclear reactors, during the reprocessing of fuel or during the preparation of labelled compounds. The number of persons at risk in these cases might total some tens of thousands, but the contamination levels can be, and have been, fairly high (up to 1 mCi of tritium/kg).

3.1.1 — *Absorption of gaseous tritium*

Since tritium is easily adsorbed onto metallic surfaces which become contaminated, it can be absorbed as a gas through the *skin* by contact with such surfaces. This route of contamination has been studied on animals (Hutchin and Vaughan, 1965) in which the tritium can be recovered initially as free tritiated water. Within 24 hours about 10% of the absorbed radioactivity has been incorporated in the tissues and is therefore recoverable only as catabolic water. Within about one month the level of tritium in the skin has fallen to about 1% of the initial value and appears to be bound to cellular components. Tritiated water released by the skin is eliminated with the urine.

This mode of contamination is not a very widespread or dangerous one, since it cannot give rise to the absorption of high levels of radioactivity.

Gaseous tritium may also be inhaled through the *lungs* and a small proportion of this, of the order of 10^{-3}, may be absorbed and oxidized to tritiated water (Pinson and Anderson, 1950; Rarrick, 1967).

3.1.2 — *Absorption of tritiated water*

The absorption of the isotope under this form is extremely rapid and takes place through the skin, lungs or gastrointestinal tract (Pinson and Langham, 1957; Osborne, 1966).

Tritiated water may be absorbed through the *skin* in the form of water vapour or liquid water. In the first case (Pinson and Langham, 1957) the exposure of the hands or forearms results in a level of contamination which is a function of the skin temperature and the rate of transpiration, in that a high temperature increases the absorption and perspiration decreases it. The absorption of tritium through the skin as tritiated water depends on the same parameters (Pinson and Langham, 1957).

The absorption of tritiated water vapour through the skin is so effective that, when a man is exposed to an atmosphere containing HTO, the absorption by this route is equivalent to the absorption through the lungs and is of the order of 10 μCi/min. for each μCi/litre of air for each

route (Pinson and Langham, 1957). Percutaneous absorption corresponds to a volume of water equal to the volume of normal transpiration. In contrast with the process in which the water is actively excreted by the sudoriferous glands, the elimination of water by normal transpiration is a reversible diffusion process and the absorption of HTO vapour represents the intake phase of this process.

The absorption of tritiated water through the *lungs* is also to a reversible diffusion process and, considering that the surface area for pulmonary exchange is about 50 times greater than that for cutaneous exchange (Werbel, 1963), it is possible to calculate that percutaneous absorption is considerably more efficient. This is probably due to the fact that the alveolar surface is covered with water in which the inhaled vapour is diluted and this reduces the specific activity of the absorbed water. This phenomenon does not occur on the dry skin and therefore the reduction in specific activity in the lungs counterbalances the difference in area through which the absorption occurs (De Long *et al.*, 1954).

The ingestion of tritiated water through the *intestinal tract* (Pinson and Langham, 1957) leads to a rapid movement of the isotope into the blood with equilibrium being reached in less than an hour, even for quantities of ingested liquid of the order of one litre. The activity eliminated through the urine varies with blood concentration. The ingestion of non-tritiated water after contamination leads to a reduction of the biological half-life, which is therefore a function of the ingested water. For an uptake of water *ad libitum* in man, the half-life varies from 9-13 days.

3.1.3 — *Absorption under other forms*

The uptake of tritium-labelled compounds by laboratory staff by ingestion, injection, inhalation or accidental contamination of the skin represents a possible hazard, even if this is fairly remote. The number of persons at risk (particularly young adults) is estimated at present to be of the order of the tens of thousands and is bound to increase with the increasing use of labelled compounds in medicine, biology and other industrial applications. This route of contamination does not present special problems as regards the mechanisms of absorption. It should be mentioned, however, that the injection of water or other tritiated compounds in animal experiments is the most widespread form of contamination since it is independent of absorption mechanisms and allows accurate dosage schedules and balances.

The possible risk of chronic uptake of tritium, especially through the ingestion of food or water (Mogissi and Liebermann, 1972) originating from a contaminated environment, has not been the subject of special studies. However, in view of the efficiency with which tritium is bound in the photosynthesis process (see para. 3.4.2), this is a route of contamination which may be significant for the ingestion of tritium in higher animals and in man in special cases. Actually, although the body burden could not be much higher than one nCi/kg, the number of persons at risk in this case could be very large, perhaps of the order of hundreds of thousands of individuals in all age groups.

3.2 — Movement of tritium through the body fluids

3.2.1 — *Plasma*

Regardless of the form under which the tritium is taken in, it is rapidly oxidized to tritiated water (HTO) and is first diluted uniformly in the blood plasma within a maximum time of 10 minutes after, for example, intravenous injection. It may however be assumed that the time required to reach maximum concentration in the blood may vary according to the intake route (corresponding to various rates of absorption) and on the exposure time. It may also be assumed that, for a route of entry other than intravenous injection, the concentration reaches an extended

plateau rather than a peak value. If equilibrium is rapidly reached between intravascular, extracellular and intracellular water, one may also assume that the tritium concentration in the urine is a reasonable indicator of its concentration in the blood; on this basis, studies of tritium concentrations in the urine indicate that the plateau value for concentration in the blood is reached within a maximum of 2 hours after, for example, percutaneous absorption (Osborne, 1966).

3.2.2 — *Extracellular and intracellular fluids*

Being a reversible process, the passage of tritiated water from the blood plasma to the extracellular liquids is an extremely complex phenomenon (Moore, 1962), at least in the case of intestinal intake. In spite of the large pool of extracellular fluids including, in this particular case, the intestinal content, the lymph, the intestinal fluids, the cerebrospinal fluid, etc., the passage is rapid and has an apparent half-life of the order of 12 minutes (Pinson and Langham, 1957). The passage from the extracellular fluids and to intracellular water is also rapid, but is even more complex since it requires active and/or passive transfer mechanisms, varying from one tissue to another. In a particularly simple case, such as the passage from the plasma to the intracellular water in erythrocytes, equilibrium is achieved within times of the order of 10 minutes (Moore, 1962); in tissues where the rate of exchange is low (bone, cartilage), however, the process probably requires much longer times. It would appear that, after exposure of the order of one hour, the radioactivity is in most cases uniformly distributed throughout the organism within the total free water within the first 24 hours (Woodard, 1970).

3.2.3 — *Cellular components*

A small but significant proportion of the tritium originating from tritiated water is incorporated into relatively stable biochemical components of the cell and is excreted with a half-life which in some cases can be extremely long. Refer to para. 3.3 and 3.4 for a discussion of these phenomena.

3.3 — Excretion paths

Most of the tritium absorbed by mammals and oxydized to tritiated water is excreted with half-lives of the order of a few days, mainly through the urine. Other excretion routes may be perspiration, skin desquamation and hair loss, faeces, water vapour in the expired air, milk, etc.

3.3.1 — *Excretion kinetics in laboratory animals*

A large number of studies have been conducted on various laboratory animals (guinea pigs rabbits, goats, cows), but mainly on the rat and the mouse, since 1951 (Thompson, 1951, 1952, 1953; Pinson, 1952; Thompson and Ballou, 1954 a) and b), 1956; Lambert and Clifton, 1967; Vennart, 1969; Sanikidze and Romanovskaya, 1970; Wheeler *et al.*, 1972). Analyses of tritium present in the animal under the form of free tritiated water, carried over a sufficient period of time after a single injection of tritiated water, have shown that the radioactivity disappears according to very complicated kinetic patterns. In the mouse and the rat it has been possible to demonstrate three main kinetics lasting 1-3 days, about 10 days and about 70 days, respectively. The specific activity of tritium in the free water and the urine between 10 and 20 days is higher than the specific activity of the tritium present in tissues. This indicates that the slow reduction in tissue specific activity is related to the renewal time of the biological compounds which the tritium has been incorporated and from where it is released by catabolism of the labelled molecules. Following chronic administration of tritiated water (Thompson and Ballou, 1956)

the tritium is excreted with half-lives the order of 3, 22 and 130 days; the tissues which take up the isotope most slowly are also those which eliminate it most slowly, thus confirming the previous conclusion. For a complete tabulation of the tritium excretion constants under various conditions and in various conditions and in various animals, see Elwood (1971) and Bennett (1972).

3.3.2 — *Excretion kinetics in man*

Studies conducted on people who had suffered accidental contamination have shown that in man the rate of excretion in the urine which, as we have seen, is reasonably representative of the concentration in the body fluids (see para. 3.2) follows an exponential pattern immediately after intake. The half-lives are about 9 days, with wide variations between individuals (between 2.4 and 18 days); among the same individuals there are also variations other than those relating to time. Excretion in the urine is increased when diuresis is stimulated, when the subject is kept at high ambient temperatures but in good fluid balance, and when the subject is young (Pinson and Langham, 1957; Butler, 1962; Wylie *et al.*, 1963; Snyder *et al.*, 1964; Butler and Leroy, 1965; Sadarangani *et al.*, 1971).

Where the degree of contamination has been less than 1 mCi, it has been possible to trace the tritium concentration in the urine for about one month without detecting any variation in the slope of the excretion curve. In other cases in which larger amounts of radioactivity have been involved, bi-phasic excretion curves have been found (Snyder *et al.*, 1964) with half-lives of about 9 and 34 days, or even tri-phasic curves with half-lives of 8, 20 and 400 days, respectively (Sanders and Reinig, 1967). In two cases described by Moghissi *et al.*, (1971) the half-lives of the intermediate phase and the long phase were 21-33, and 280-2020 days, respectively. The long excretion phases are presumably due to the loss of tritium initially incorporated into slowly renewing cellular components and released subsequently by the metabolism of these tissues.

Considering the wide variety of mechanisms by which the tritium in tritiated water can first be incorporated into and then lost from the various tissues, it is reasonable to assume that the half-lives given above for the various components of the excretion curves do not represent simple phenomena but are the result of a large number of different mechanisms related to the metabolism and to the cell and tissue kinetics, the average rate of these processes being approximately within the indicated limits. In addition, it is interesting to note that the mean excretion constants found in man compare fairly well with those in experimental animals, bearing in mind the more rapid renewal of cellular components in the shorter-lived animals.

3.4 — Incorporation of tritium into cellular components

Since the tritium is distributed as tritiated water in the free water compartment which represents about 60% of the weight of a mammalian organism, and since this tritiated water is excreted very rapidly (1-3 days and about 10 days for rodents and man, respectively, are the mean half-lives of the initial component of the excretion curves, see para. 3.3), the ICRP has tended to regard the water in the body as the only significant compartment and thus to recommend permissible concentrations based on these assumptions (ICRP, 1959). Nevertheless, the studies referred to in para. 3.3.1 have shown that a proportion, though small, of the radioactivity is distributed and incorporated into various tissues from which it is released according to complex kinetic patterns and in some cases with very low rates. A complete tabulation of the mean biological lives of tritium in body water and tritium bound to organic compounds in various animals is given by Elwood (1971). An up-to-date critical discussion of the metabolic exchanges of tritium in man (mainly under the form of tritiated water) was included in a recent review by Osborne (1972).

3.4.1 — *Experimental data*

Studies of the incorporation into tissue of tritium from tritiated water, are carried out by endoperitoneal or intravenous injection in single or multiple doses or by administration in drinking water. The maximum doses used in these studies were 86 μCi/g of body weight for injection and 4 μCi/ml in drinking water. The contaminated animals are then venally sacrificed, samples are taken of various tissues and dried; the distillation water collected and its tritium content is a measure of the radioactivity contained in the free water in that tissue. The various samples of dried tissue are then incinerated and new radioactivity measurements carried out on the combustion water. This radioactivity is representative of the tritium combined with the various organic molecules. A similar procedure is also followed for chronic administration. The data currently available on various experimental animals can be summarized as follows (Thompson, 1951, 1952, 1953; Thompson and Ballou, 1954 a) and b); 1956; Lambert and Clifton, 1967; Vennart, 1969; Hatch and Mazrimas, 1972):

(1) 24 hours after a single injection of tritiated water, the amount of tritium incorporated in the tissues by exchange or by the normal metabolic pathways is of the order of 7% of the injected dose, of which 1.5% is metabolically bound (Siri and Evers, 1962). The liver contains radioactivity mainly under the form of glycogen and lipids.

(2) In the mouse after about 10 days the levels of specific activity of tritium in the free water and of the tritium incorporated into the tissue components are usually inverted, in that the level of tritium present in the tissue components is higher than that bound to water (Thompson 1952).

(3) The biological half-lives of the two fractions are different, since the tritium present in the free water decreases to 50% within little more than one day, whereas the tritium bound in tissue components decreases in two stages with half-lives of 9 and 90 days, respectively (Thompson, 1952).

(4) Between 30 and 50 days after acute or chronic exposure, the amount of tritium incorporated into the tissues is relatively small (of the order of 1.4% to 1.1%, respectively) and most of this is contained in the slow-reversing tissues (brain, muscle, skin) (Pinson and Langham, 1957). Similar values have also been found in larger animals (Potter *et al.*, 1971).

(5) The tritium incorporated after chronic exposure is excreted according to three rates with mean lives of about 3, 22 and 130 days. Excretion is slowest for tissues in which incorporation is slow (Thompson and Ballou, 1956).

(6) Biopsies on skin and fat tissue taken from a man who was chronically exposed to tritiated water for 8 months showed a stable incorporation of tritium into cellular components (Pinson *et al.*, 1952).

(7) In all the observed cases no radiobiological damage attributable to tritium, either immediate or delayed, has been observed in spite of the relatively high doses (86 μCi/g of body weight for injection, or 4 μCi/ml in drinking water).

3.4.2 — *Incorporation mechanisms*

After the administration of tritiated water, the tritium may replace hydrogen bound to tissue components in two ways: by *exchange* between hydrogen and tritium in an existing compound which is therefore unstably and reversibly labelled; or by stable *incorporation* into a molecule during its biosynthesis.

The exchange between tritium in water and the unstable hydrogen of the organic compounds takes place very rapidly and depends, since the reaction is reversible, on the specific activity of the tritium in the water and the solute, respectively. Many of the reactions used for the preparation of labelled organic molecules, including the Wilzbach reaction, are not relevant to *in vivo* systems (Evans, 1966; Feinendegen, 1967). In general it is considered that the hydrogen

atoms bound to oxygen, nitrogen, sulphur or phosphorus exchange readily with tritium present in the water where the compound is dissolved, whereas hydrogen bound to carbon is not exchangeable (Jones, 1965; Von Hippel and Printz, 1965; Feinendegen, 1967). The hydrogen bonds in DNA or RNA molecules and the covalent bonds in the peptide group are considered to be unstable and therefore readily replaceable.

As regards the *incorporation* into organic molecules at the time of their synthesis, it is possible to distinguish two mechanisms. The first is the incorporation of tritium into *plant tissues* by normal processes of photosynthesis in which the water is the only source of hydrogen. In this case it is relatively simple to identify the reactions which bring about the incorporation of tritium and it has been shown that the subsequent movement of the isotope along the metabolic chain is very rapid with the appearance of labelled amino acids only a few minutes after exposure to tritium (Moses and Calvin, 1959).

The incorporation of tritium into organic molecules of higher *animals* is considerably more complex and has been discussed in detail by Feinendegen (1967) and Smith and Taylor (1969). There are generally a large number of metabolic pathways by which the tritium can be incorporated into proteins, lipids, carbohydrates and nucleic acids, starting from a medium containing tritiated water. Many of these reactions have been shown to occur *in vitro* and *in vivo*. It should be noted, however, that these reactions do not all have equal significance for radiation protection, since it is likely that tritium incorporated into a compound with a short half-life (such as a dextrose molecule) will be excreted more rapidly, whereas tritium incorporated into ribose, and especially into desoxyribose of nucleic acids, will exchange more slowly and therefore is more likely to give rise to radiobiological effects. The same can be said of the lipids, in which the rate of exchange depends to a great extent on the state of nutrition.

As already stated in para. 3.3.2, the complexity of these exchanges and reactions must be borne in mind in interpreting the kinetics of incorporation and excretion of tritium in the body.

From the point of view of environmental protection, it is interesting to note that the available data on concentration factors in food chains, which are also of interest for man, are relatively few. Hatch and Mazrimas (1972) found ratios of specific activity between tritium bound to organic molecules and tritium in free water which varied between 1.1 and 1.9. Bogen *et al.*, (1971) found that the same ratio could vary from 1.2 and 5.6 in a series of popular foods.

4 — RADIOTOXICITY OF TRITIUM IN MAMMALS

4.1 — Premises

The first studies on the radiotoxicity of tritium were conducted in the 1950's when the development of reactors made it necessary to investigate the somatic risk of tritiated water. When widespread use was later made of a number of tritiated precursors of DNA as tracers, the problem of the toxicity of tritium also extended to these compounds and it became possible to investigate harmful genetic effects.

In the interest of clarity, this report subdivides the treatment of the two categories of tritiated compounds considered (tritiated water and tritiated thymidine) into different levels of biological organization: cell, tissue, organ and organism. This subdivision, of course, only represents a system of ranking and the interdependence of the various subdivisions will be made clear in section 5.

Since the purpose of the present report is to analyse the possibility of extrapolation to man of results obtained experimentally, this study will be restricted to experiments on mammals. Data referring to other living systems are mainly relevant for radioecology and their significance for human protection is only of an indirect nature.

4.2 — Toxicity of tritiated water

The intake of tritium as tritiated water by the absorption pathways illustrated above (see para. 3.1.2) is considered to be the most common source of contamination for man, and particular attention will therefore be paid to experiments conducted with tritiated water.

4.2.1 — *Toxicity at the cellular level*

For obvious reasons of experimental convenience, the cells in culture are a favoured biological system for these studies. From the qualitative point of view, the effects of tritium on the cells are identical to those encountered when the culture receives external irradiation and consist in a loss of the reproductive capacity of the cells (followed by various other effects at the population level), in an increase of the cell cycle time and a decrease of the population doubling time and in the appearance of chromosome aberrations. For what concerns the mechanisms of action, the targets of the radiobiological effects, the dependence of these effects on physical, chemical and biological factors, the results of tritium irradiation are not significantly different from those brought about by external X or gamma irradiation. The quantitative aspects of these phenomena are, on the contrary, of paramount importance and they relate to the magnitude of the effects as a function of the absorbed dose, of the specific ionization of the tritium beta radiation, of the effectiveness relative to other standard radiations. The reaction mechanisms, the site of radiant action, the interdependence of the various effects and the action of tritium on the cultures do not differ significantly from those encountered in X or gamma radiation. The important factor is, however, the quantitative aspect of these phenomena (absorbed dose, difference in specific ionization of the tritium beta radiation relative to other radiations and, consequently, the difference in its relative biological effectiveness.

At a concentration of tritiated water of 1 mCi/ml of culture medium (therefore a dose rate of the order of 12 rad/hr, see para. 2.1), the proliferation of HeLa cells is considerably reduced (Nias and Lajtha, 1964) and the growth of the population stops on the fourth day of treatment; if the concentration of the isotope is reduced to one-tenth, however, the same effect is observed on the ninth day. In similar experiments conducted on cell cultures of a heteroploid strain of human origin (Fraccaro *et al.*, 1966) it was possible to reduce the rate of growth with dose rates in excess of 30 rad/day (which would correspond, according to formula 5.1, to a concentration of about 0.1 mCi/ml of culture liquid) on the sixth day from the beginning of treatment. The growth of cells treated with 300 rad/day, on the other hand, ceased on the third day of treatment. In all these cases, the overall effects were observed on cell populations under artificial conditions of continuous growth, and they included a large number of primary effects (chromosome aberrations, delayed mitosis, inhibition of the reproductive capacity). These results may nevertheless be of some use for the purposes of radiation protection; for example, they indicate that the doses necessary to produce serious and reproducible effects of this type are about 10 orders of magnitude greater than the tritium concentrations now present in the biosphere (Radiation Data and Reports, 1972).

Some experiments conducted by Colvin and Everts (1971), in which two luminous compounds activated with tritium were administered via the percutaneous route to Chinese hamsters, are of particular interest to environmental protection. Although the results do not allow precise quantitative conclusions in the form in which they were presented, they show nevertheless that tritium is taken in by this route and, within 30 days after administration, may produce a significant increase (3-6 times) in the number of cells carrying various types of chromosome aberrations in primary lung and kidney cultures.

Not surprisingly, in view of our general radiobiological knowledge, the literature contains no data on possible metabolic alterations in mammalian cells as a result of irradiation with tritiated water.

4.2.2 — *Toxicity at the organ and whole-body level*

The toxicity of tritium administered I.P. or I.V. as tritriated water is most evident in the tissues which renew themselves rapidly (haematopoietic organs, intestine, testes) self-reversing, fast-cycling tissues. Myelolymphoid aplasia develops for doses between 0.2 and 1.4 mCi/g (Furchner, 1957). There is no clear distinction between bone-marrow and intestinal death, probably because the radiation dose due to tritium is never delivered acutely.

Lesions of the bone marrow shown by a depression of incorporation of ^{59}Fe into the circulating blood have been observed in rats (Furchner *et al.*, 1953; Storer *et al.*, 1957) after injection of tritiated water.

Atrophy of the spleen and thymus in the mouse (Worman, 1954; Storer *et al.*, 1957) and in the rat (Moskalev *et al.*, 1970) following the administration of tritiated water have also been used quantitatively in studies of relative biological effectiveness. These studies have demonstrated that tritium has a higher biological effectiveness than gamma radiation in producing such lesions (see para. 5.2.3) by factors of the order of 1.3-1.6. The tritium concentrations at which very harmful or lethal effects are encountered *in vivo* are of the same order of magnitude as those necessary to obtain comparable effects *in vitro*.

Clinical and pathological diagnoses carried out on two subjects whose work involved exposure to luminous compounds activated with tritium (Seelentag, 1971) are particularly significant. In the first case, a man who had been exposed to levels of activity estimated at approx. 2500 Ci/year in the last six years before his death, the diagnosis was panmyelophthisis. In the second case, also involving a man who was exposed for three years before retiring from work (followed, one year later, by death) to a level of activity estimated at several thousand Ci per year, the diagnosis was chronic aplastic anaemia.

As regards testicular lesions, Lambert (1969) studied the loss of reproductive capacity of intermediate spermatogonia into primary spermatocytes in mice treated with tritiated water in doses up to 40 μCi/g, and concluded that cellular death occurred within 4 days after treatment even with doses lower than 2 μCi/g. The tritiated water and the X-ray doses necessary to obtain an identical effect are 20 μCi/g and 30 rad, respectively. The fact that lower doses also produce this effect is probably due to the high sensitivity of the spermatogonia, even compared with external irradiation, to interphase death mechanisms.

The criterion most often used to study effects on the entire organism has been the lethal dose at 30 days. After injecting scalar doses of tritiated water Brues *et al.* (1952) estimated an $LD_{50/30}$ in the mouse of 1 mCi/g of body weight. This does not differ substantially from the $LD_{50/30}$ of approx. 1 mCi/g estimated for the same animal by Trujillo *et al.* (1955) after endoperitoneal injection and is close to the estimate of 1.45 mCi/ml of body water which was the LD_{50} obtained for pulmonary administration of tritium in gaseous form. In these latter experiments no acute histological lesions of the lungs were detected at a dose to the lungs calculated at approx. 135 000 rep although, in the absence of direct observations, one cannot exclude the appearance of delayed effects in these organs following such high doses of tritium. A higher effectiveness compared with gamma radiation was also found at the whole-body level of tritium (Brues *et al.*, 1952; Furchner, 1957) (see para. 5.2.3).

A comparison of the sensitivity of various animal species of animals was given by Zhuravlev (1971) and it was found that dogs have a high sensitivity to tritium oxide, probably due to differences in the repair capacity of haematopoiesis.

Information on delayed effects is very scanty and, under these circumstances, negative results may also be of some interest. Mice and rats fed for about six months on a diet containing tritiated water with a specific activity of 5 μCi/ml (Thompson and Ballou, 1956) tolerated the treatment; similarly, no somatic damage was detected in man about two years after acute exposure to concentrations of tritiated water of the order of 1 mCi/kg of body weight (Snyder *et al.*, 1964;

Sanders and Reinig, 1967). No macroscopic evidence of harmful radiation effects was observed in a population of *Dipodomys merriami* after several generations in an environment contaminated with tritium such that the activity of the body water and tissues was 0.1-0.4 μCi/g (Hatch *et al.*, 1970). No increase in the death rate or in the induction of tumours up to the age of 27 months was found in mice which had received 15 μCi/g of tritiated water at birth (Baserga *et al.*, 1966). It must therefore be concluded that single doses of tritiated water of the order of 10^{-2} or 10^{-3} of the lethal acute dose have given little or no evidence of somatic damage.

As regards the study of the effects on the foetus, Moskalev *et al.* (1969) reported that tritiated water administered to pregnant rats in doses of the order of 0.08-3 mCi/g led to typical vascular lesions (œdema, hematoma) in the mother, the placenta and the foetus. At higher dosage levels a prenatal mortality rate of 80% is reached, due mainly to intrauterine implantation defects.

In recent experiments (Cahill and Yuille, 1970) rats were kept during pregnancy at constant levels of tritiated water ranging between 1 and 100 μCi/ml of body water corresponding to dose rates in the embryo and the foetus of 0.3-30 rad/day. The effects observed were extremely heterogeneous, based on biometric measurements at birth, sterility and the induction of tumours in the adult up to a maximum of 270 days of life. The authors' conclusions indicate that significant effects are not produced by continuous exposure to tritiated water during pregnancy at doses of 1 μCi/ml, whereas higher concentrations produce distinct effects on the gonads and the brain which appear in the form of atrophy and sterility in the first case, and microcephaly in the second.

A particular problem connected with the effects on the foetus is that of the incorporation of tritium in the oöcytes of female foetuses, which do not divide until the time of sexual maturity. Under these conditions the tritium incorporated during foetal life remains in the nuclei of these cells for very long periods, of the order of the entire reproductive life of the animal. This gives rise to radiation protection problems and difficulties in dose calculations which will be dealt with in more detail in para. 5.2.3.

No data whatever are available concerning the genetic effects on the germinal cells of mammals due to tritium in the water or food (Russel and Cumming, 1971), although there is reason to presume that damage of this type may occur since a small proportion of the tritium ingested in this form is stably incorporated into the genetic structures (Hatch and Mazrimas, 1972).

4.3 — Toxicity of tritiated thymidine

The radiation damage caused by tritium present in the thymidine molecule, and as such preferentially incorporated into DNA, is essentially a damage to the genetic structures. At the sub-cellular level this consists in mutations and chromosomal aberrations, which are well established. At higher levels or organization various lesions may occur; although none of these seems to be typical of this isotope, the degree of such lesions as a function of dose is, in our opinion, of considerable interest. Since this compound is widely used for labelling DNA, a great deal of data is available on its radiotoxicity. For a review of this literature and a more detailed discussion, the reader is referred to specialized texts (Feinendegen, 1967), since the present report will only be limited to those aspects which most closely affect radiation protection.

4.3.1 — *Mutagenic effects*

The beta radiation due to tritium incorporated into the genetic structures has a distinct mutagenic effect (Feinendegen, 1967). In the mammal, these effects have been studied on the mouse after the administration of tritiated thymidine in drinking water (Greulich, 1961). Over periods of time varying between 24 and 32 days (therefore in practice during the whole period of

gestation) mice undergoing this treatment received a total activity of 245-300 μCi. The progeny of the first mating, with males who had received 8-167 μCi over periods of 1-18 days, was phenotypically normal, whereas in subsequent matings, either between the same treated animals or between treated and normal animals, a significant decline was observed in the reproductive capacity owing to an increase in neonatal mortality rate, foetal resorption and male sterility. Subsequent crosses between apparently normal first-generation animals also showed a decrease in the reproductive capacity due to a high neonatal mortality rate.

In an attempt to establish a relationship between the frequency of lethal mutations and tritium content of the sperms (Bateman and Chandley, 1962), tritiated thymidine was administered by injection to male mice which were then mated when the number of labelled sperms reached the maximum. Based on the frequency of intrauterine death and autoradiographic data, it was calculated that 25 grains/sperm (after an exposure period of three weeks) were necessary to produce a dominant lethal mutation.

Apart from the literature referred to above, which relates essentially to dominant lethal mutations, no other data are available on specific point mutations in the mammal or other information on genetic mutations caused by tritium in any form.

4.3.2 — *Chromosomal aberrations*

The first information on the induction of chromosomal aberrations in human leukocytes treated with 1 μCi/ml of thymidine for 30 minutes was published by Bender *et al.* (1962). The value of this information today is more of a qualitative nature, since some aspects of a technical nature have since been recognized as invalid. Marin and Prescott (1964) studied chromatid exchanges in Chinese hamster cells in culture exposed to tritiated thymidine at concentrations of 0.05-2.7 μCi/ml for 1 or 2 hours, to test the hypothesis that such exchanges are produced by the beta radiation of the isotope, and concluded that these aberrations do not depend on the radiation dose but are in most cases spontaneous events.

Other data gathered by Dewey *et al.* (1965) are reported in para. 4.4.

Chromosomal aberrations without any character of specificity have been described in the caryotype of human fibroblasts in culture following the incorporation of tritiated thymidine at a concentration of 1 μCi/ml of medium for 30 minutes (Ockey, 1967).

It can be concluded that exposure to concentrations of tritiated thymidine of the order of 1 μCi/ml is capable of inducing chromosomal aberrations in mammal cells, as has been shown after exposure to external sources. However, the significance of these aberrations, particularly *in vivo*, is not well-understood and we are faced with all the uncertainties involved in the interpretation of aberrations caused by external irradiation.

4.3.3 — *Cellular toxicity*

Concentrations of tritiated thymidine in cultures of mammal cells exceeding 0.02 μCi/ml of culture medium for 24 hours reduce the cloning ability of the cell (Drew and Painter, 1959): under these conditions the D_{37} is approx. 0.07-0.08 μCi/ml when the duration of exposure does not exceed 30 minutes, much higher doses (2 μCi/ml) are apparently incapable of producing the same effect.

In lymphocyte cultures (Osgood, 1959), the toxic effect of tritiated thymidine (at a dose of 1 μCi) becomes manifest through a reduction in the number of cells and changes of their morphology. Similarly, the addition of tritiated thymidine to cultures of HeLa–S_3 cells alters the growth kinetics (Marin and Bender, 1963 a): in this case the D_{37} is 0.21 μCi/ml for a treatment period of 15-16 hours.

The considerable variations in the reported concentration levels can be explained by the fact that the incorporation of tritiated thymidine into the DNA of the cells depends on the concen-

tration in the culture medium and on the exposure time but also, and perhaps critically, on the parameters of the mitotic cycle of the population under study (for example, total cycle length, relative length of S phase, etc.).

During *in vivo* tests to investigate the inhibition of cell proliferation, ascitic tumour cells were transplanted into the peritoneum of mice (Lisco *et al.*, 1961 b): 48 hours after inoculation the animals received a total dose of tritiated thymidine of 0.1, 1.0 and 10.0 μCi/g of body weight over a period of 20 hours, the total dose being administered into 5 fractions. The growth of the tumour was inhibited in proportion to the dose and it was found that the effects of 10 μCi of tritiated thymidine per gram of body weight were the same as those obtained with an X-ray dose of 600-1000 rad.

The lethal effect of tritiated thymidine at high specific activity (6 Ci/mmol) and high concentrations (2 μCi/ml) has been used to obtain synchronization of cells in culture (Whitmore *et al.*, 1965), but the data are not relevant to radiation protection, although they do confirm the lethal action of the compound. This lethal effect has also been demonstrated on bone marrow cells and tumour cells *in vivo* without particular specificity characteristics at doses of the order of 1-10 mCi/mouse administered in 6 injections over a period of 24 hours (Bruce and Meeker, 1965) and on bone marrow *in vitro* at doses of 100-600 μCi/ml for 20 minutes (Becker *et al.*, 1965).

4.3.4 — *Organ and organism toxicity*

In vivo experiments on the radiotoxicity of tritiated thymidine (Johnson and Cronkite, 1959) have shown that the survival of mouse spermatogonia is reduced at a dose of 1 μCi/g of body weight and that a dose higher than 5 μCi/g in the rat causes the appearance of pycnosis in the lymphocytes (Cronkite *et al.*, 1962). Histological damage to the testes has also been reported in mice 145 days after they had received 167 μCi of tritiated thymidine over 18 days (Greulich, 1961).

Hepatic regeneration after partial hepatectomy has been used to study the radiotoxicity of this compound on organs (Grisham, 1960): by the analysis of three parameters (mitotic index, percentage of chromosome aberrations, increase in organ weight) it was found that tritiated thymidine at a dose of 1 μCi/g of body weight inhibits hepatic regeneration in the rat. At the same dose levels it interferes with the normal division kinetics of the hepatocytes (Post and Hoffman, 1965, 1967) and of rat intestinal cells (Post and Hoffman, 1968) by lengthening the S and G_2 phases. Changes in the mitotic cycle have also been observed in spleen lymphocytes after the administration of 1-10 μCi/g (Post and Hoffman, 1961); the evolution of these changes was followed as a function of time, after various doses of tritiated thymidine.

Mouse bone marrow undergoes a partial loss of its capacity to repopulate the hematopoietic system of irradiated animals after doses above 20 μCi/g of body weight (Smith *et al.*, 1962): it is nevertheless possible to cancel this effect by transplanting a greater number of cells, as is to be expected from our knowledge of the lethal effect of external irradiation on stem cells.

The continuous perfusion, over periods up to 18 days, of 864 μCi of tritiated thymidine per day per animal brings about morphological changes in rat marrow and gives a picture of medullar hypoplasia-aplasia (Calvo *et al.*, 1971).

Haas *et al.* (1971) recently studied the effects on rats of the perfusion of tritiated thymidine starting on the ninth day of pregnancy and continued until birth; the doses varied between a minimum of 72 μCi/day and a maximum of 1440 μCi/day per animal, with a wide range of intermediate doses. Only chromosome aberrations and a reduction in the number of oöcytes in newborn animals were found with doses up to 288 μCi. With progressively larger doses this treatment injured the bone marrow and caused serious malformations of the progeny; at the maximum dose the subject did not survive after the 12th day from the start of the treatment. All the effects described were in proportion to dose.

4.3.5 — *Delayed effects*

In the case of tritiated thymidine, as with tritiated water, the available data are very poor. Post and Hoffman (1961) showed changes in the percentage of polyploid cells in rat liver following the endoperitoneal administration of 2 μCi/g of thymidine in 3-week old rats. With these animals, in which the percentage of polyploid cells increases with age, the percentage of polyploidy two weeks after treatment with thymidine reaches a level in the liver equal to that of untreated 2-year-old animals.

Treatment with tritiated thymidine (0.1-10 μCi/g of body weight) increases the incidence of spontaneous tumours in the mouse (Lisco *et al.*, 1961 a; Baserga *et al.*, 1966): at higher doses the life span is shortened and there is a higher incidence of tumours compared with lower doses. The more common tumours appeared to be less easily induced than the less common types in the particular strain of animal used. These effects are more readily produced when the animals are young and the dose is administered in several fractions.

Induction of lymphatic leukaemia in C57 BL mice following the administration of 0.3 μCi/g has been reported by Mewissen (1965). In animals of the same strain the incidence of tumours was also increased significantly by doses of 0.3-1.5 μCi/g in the newborn (Mewissen, 1969; Mewissen and Rust, 1971). It should also be noted that the strain of mice used in this research shows a particularly high incidence of spontaneous tumors. In contrast, Johnson and Cronkite (1966, 1967) were not able to produce variations in mortality rate or in the incidence of neoplasia in adult mice with doses between 1 and 5 μCi/g.

In conclusion, there appears to be sufficient experimental evidence that doses of tritiated thymidine, corresponding to about 0.1-1.0 μCi/g of body weight administered to young mice may produce an increase in the incidence of some types of tumours which can be experimentally observed. It is possible that these same doses are insufficient to produce this effect when administered to adult animals.

4.4 — Comparison between the effectiveness of tritiated water and tritiated thymidine

In order to make the existing data more useful in practice and for a better understanding of the many problems discussed in section 5, a comparison between the effects produced by tritiated water and tritiated thymidine may be of value. The available data is, however, limited and difficult to correlate because different effects have been studied and these have been obtained under different experimental conditions, especially with respect to the time of exposure.

By adding increasing doses to HeLa cells in culture, Painter *et al.* (1958) found that equal indices of growth were obtained at concentrations of 5 μCi/ml of thymidine in the medium and 5 mCi/ml of tritiated water. A comparison between these two treatments in inhibiting the division of intermediate spermatogonia in the mouse revealed a higher effectiveness for thymidine compared with water by a factor of approx. 4 (Lambert, 1969). The inhibition of antibody synthesis was used as an *in vivo* test for a comparative study of thymidine and water (Dutton, 1961): it was found that the dose of tritiated water required to obtain comparable effects was about 100 times greater than that of tritiated thymidine. Further confirmation of the higher toxicity of thymidine was found by Sanders *et al.* (1961), who put forward the hypothesis that the higher effectiveness of thymidine was due to mechanisms which were different from those of beta irradiation: it is known that non-radioactive thymidine causes cells to block at the beginning of the S phase. In a comparison carried out by Haas *et al.* (1971), based on various effects on organs and entire animals (cf. para. 4.3.4), the equal effect ratio of thymidine to water is about 10:1.

Completely different results were reported, however, by Dewey *et al.* (1965) in a comparison between various types of irradiation (tritiated water, tritiated thymidine, ^{60}Co gamma) in producing chromosomal aberrations *in vitro* in hamster cells. In this case tritium

radiation from thymidine was found to be less effective than tritium from water. According to Feinendegen (1967) the most likely explanation of this anomalous behaviour should be sought in non-uniform distribution of the tritium bound to water.

It can be concluded that, according to the end-points studied, the effectiveness of tritium administered thymidine is greater by factors varying between 10 or less and 1000.

5 — DOSIMETRIC CONSIDERATIONS

The purpose of biological dosimetry is to measure with the greatest possible precision the amount of energy deposited by radiation in biological materials, so that the observed effects may be quantitatively related to this energy. The dosimetric problems can be very different depending both on the type and energy of radiation, and on the effects which are to be measured. Tritium is a rather special case, because of the low energy associated with its radiation and because of its complex distribution within the biological structures of higher organisms, including man. Some of the problems posed by tritium do not yet appear to have been satisfactorily settled, and any attempt to evaluate the current state of our knowledge is therefore difficult. The high rate at which new research reports are being published (some of them, however, in the form of abstracts or pre-prints) makes it unlikely that any such attempt may result in a complete and exhaustive review.

5.1 — Applications of the general principles to tritium

In principle, tritium does not differ from all other radionuclides with respect to the general mechanisms of energy deposition in matter, and particularly in biological materials, following radioactive decay: the energy liberated by disintegration of the nucleus is transferred by the beta particle to the surrounding medium through the well known processes of ionization and excitation, resulting in free radicals formation, the breakage of chemical bonds, etc. It should also be expected that some of the overall observable effects might be attributable to atomic transmutation, that is to the fact that the helium atom produced by radioactive decay has still some recoil energy and its physico-chemical properties are completely different from those of tritium from which it had originated. In general terms, this phenomenon cannot be without consequences on the molecular structure of which tritium was a component.*

As a first approximation it is therefore possible to apply to tritium all the principles and methods of calculation developed for radioactive emitters deposited within biological materials, about which a large body of literature is available (Attix and Tochilin, 1969). It is not within the scope of this report to present a detailed discussion, because some recent reviews are dealing specifically with tritium (Feinendegen, 1967), and because attention will be focussed to those problems that appear to be of particular interest from the viewpoint of radiation protection.

5.1.1 — *Fundamental dosimetric relationships*

If we take, for example, the simplest case of a uniformly labelled isotropic substance, and assuming total absorption of the disintegration energy within the substance, the dose deposited by a beta emitter such as tritium is a function only of specific activity and mean disintegration

* The problem of transmutation falls outside the scope of the present report, which is mainly concerned with aspects related to radiation protection. In fact, the relevant experiments have been carried out only in lower organisms, from which extrapolation cannot be justified at present. The reader is then referred to more detailed discussions of this particular problem (e.g., Feinendegen, 1967).

energy. Making suitable adjustments to the dimensions and units of measurement of the parameters, it is possible to use an equation of this type (Feinendegen, 1967):

$$D = \frac{3.7 \times 10^7 \times 3600 \times 1.6 \times 10^{-6} \times \bar{E}}{100 \times g} \tag{5.1}$$

where D = the absorbed dose in rad $\times$ mCi^{-1} $\times$ h^{-1}
3.7×10^7 = the number of disintegrations $\times$ mCi^{-1} $\times$ sec^{-1}
3600 = the number of sec $\times$ h^{-1}
1.6×10^{-6} = the number of erg $\times$ MeV^{-1}
$\bar{E}$ = mean energy of the beta particle spectrum of tritium, in MeV
g = mass of the substance in g
100 = conversion factor from erg $\times$ g^{-1} to rad.

The numerical value of $\bar{E}$ is usually taken as 5.6-5.7 keV, and therefore the dose rate delivered by tritium at a concentration of 1 mCi $\times$ g^{-1} is approx. 12.14 rad $\times$ h^{-1} (see para. 2.1). Using other units it is simple to obtain the well-known value of 106 mrad $\times$ year^{-1} $\times$ μCi^{-1} $\times$ kg^{-1}, or to calculate that for a dose of 1 mrad $\times$ year^{-1} the concentration required in soft tissues is of 9.4 nCi $\times$ kg^{-1}, which is also equivalent in current environmental protection jargon to 2940 TU, where one TU (tritium unit) corresponds to one atom of tritium in 10^{18} atoms of hydrogen (Osborne, 1972).

5.1.2 — *Criteria for calculation of cumulative doses*

Due to the high rate with which tritium is uniformly distributed throughout the free water in the organism, the concentration in urine at any given time is practically equal to the concentration of the radionuclide in the free water (see para. 3.2.1); by making a number of simple assumptions, it is therefore possible to estimate the dose rate delivered at the time of sampling from the urine concentration. It is sufficient to know the relative proportion of free water to the mass of the entire organism (Feinendegen, 1967) and to use a conversion factor between specific activity and dose of the type indicated above (see equation (5.1)). It is also possible to estimate the cumulative dose within a certain period, provided that the measurement of urine concentration relates to the beginning of the period under study and a reasonable estimate is made of the biological half-life of tritium for that period. This last point is very important because biological half-life is strangely dependent on the time elapsed after a single intake and, in the case of chronic administration, on the length of the period of contamination (see para. 2.3). For cumulative doses deposited over relatively long times more complex calculation models are required which make use of all currently available data on the metabolism of tritium in mammals, and particularly in man. These models are based on further assumptions of numerical values to be given to some physiological parameters which at present are not completely known (for example, the relative dimensions of compartments with widely different binding capacities for tritium). One of these models was developed by Woodard and Harley (Woodard, 1970), dealing mainly with the problem of estimating the contribution to the total cumulated dose due to the tritium which is 'combined' with the tissues, i.e. the tritium which has the longest biological half-life (of the order of hundreds of days in man; see para. 3.3.2). The model is valid only for acute intake of tritium in the form of tritiated water (regardless of the intake route) and in all cases assumes a uniform distribution of the tritium, excluding concentration factors in particular organs and tissues, even over long periods. In applying such a model to an actual case, however, the authors made assumptions regarding the amount of tritium present in the various tissues and fluids on the basis of data contained in the literature, and these assumptions were incorrect. This was printed out by Osborne (1972, and personal communication), and the conclusions reached in the Woodard report must therefore be rejected. Bennett (1972) recently published a complete

revision of the model, together with calculations based on more accurate estimates. His report states that, assuming biological half-lives 9, 30 and 450 days (in a three-compartment model), the resulting total tissue dose is 84 mrad/mCi of intake. 84% of this dose is due to tritium which remains in the form of tritiated water in the free body water, the remaining 16% being represented by tritium 'combined' with the tissues. It is very important to note how this total dose appears to be distributed in time: 50% is deposited within 11 days after intake and 90% within 200 days; the remainder (10%) may be regarded as an 'infinite dose'. It can be seen that the relative contribution to the total dose due to 'combined' tritium is not large, but it is not insignificant and is highly diluted in time.

5.1.3 — *Criteria for calculation of permissible maximum concentrations*

Complex models based on similar concepts have been used to determine maximum permissible levels for exposure of the whole body and maximum permissible concentrations of tritium in air and water for exposed workers (ICRP, 1959, 1968). In these cases, however, the basic methodology is more complicated since ICRP (and also the other national and international agencies) introduces further correction factors into the calculations. These factors must make allowance for at least: (1) considerations relating to individual organs, e.g. in cases in which it is necessary to apply the concept of 'critical organ', or individual parts of the body specifically related to internal contamination; (2) considerations of relative biological effectiveness, where doses are expressed in units of equivalent dose, i.e. in rem and not in rad; (3) considerations which make allowance for the non-uniform distribution of the radionuclide. In the case of tritium, the following numerical values have been used since the original calculations made by Pinson and Langham (1957), which have been adopted in principle by the ICRP: about 43 kg for the mass of the compartment of interest (corresponding to about 62% of the weight of an average man weighing 70 kg; this fraction represents the 'total free water' in which the tritium is considered to be uniformly distributed); an RBE factor of 1.7; a non-uniform distribution factor equal to unity, i.e. the distribution of the radionuclide is considered to be uniform both at the macroscopic (organ) and at the microscopic level. Using these criteria and these numerical assumptions, it can be estimated that 1 mCi of tritium delivers in man a dose of the order of 5 rem/year or 0.1 rem/week, which corresponds to the present maximum permissible dose for exposed workers (ICRP, 1966).

At this dose level, and by using reasonable numerical values for the various physiological parameters involved in the intake and excretion mechanisms (see section 3), it is possible to determine the maximum permissible concentrations in air and water for exposed workers (ICRP, 1959; Osborne, 1972).

The dose estimates referred to above have been calculated with sufficient accuracy for the purposes of radiation protection. Each of the numerical values used for all the parameters required in the calculations represents a reasonable average estimate, but it should be recalled that in practice these values are variable. The empirical approach is however still valid for these purposes, considering the continuing progress made by research in the refinement of the estimates. A typical example is the quality factor to be adopted for low-energy beta emitters, including tritium; the ICRP recently reduced this factor from 1.7 to 1.0, '... after having considered the physical and biological data relating to this problem, and having concluded that a unit value is justifiable within the limits of accuracy required for the purposes of radiological protection' (Sowby, 1969).

5.2 — Special problems posed by tritium

Mention has already been made of the physical characteristics of tritium radiation and the special role played by this radionuclide in the economy of living organisms as an isotope of

hydrogen (see sections 2 and 3). These characteristics make tritium a special case also in the matter of dosimetric problems. The general principles set out in para. 5.1, and the estimates derived from equations of the type given as (5.1) are based on simplified approximations. For example, equation (5.1) is applicable in all circumstances, provided that the mean range of the particles is small compared with the dimensions of the irradiated 'target', and that the proportion of energy dissipated as 'bremsstrahlung' is negligible. These two further assumptions are certainly valid on a macroscopic scale, but the first is no longer valid on a microscopic scale where the general concept of 'relevant organ or tissue' must be replaced by that of 'biological structure', damage to which is responsible for the final effect. At this level it is also necessary to take into account the following facts:

(1) the beta particles of tritium produce relatively dense ionization;

(2) the volume of biological material affected by the disintegration of one atom of tritium corresponds to a sphere with a diameter which, in 99% of cases, does not exceed 5 μm, since less than 1% of the beta particles of tritium have a mean range between 2.5 and 6 μm in material having a density approaching unity. In 80% of disintegrations this diameter reduces further to 2 μm. Dimensions of this order are comparable to or less than those of sub-cellular structures, particularly the nuclei of the majority of animal cells;

(3) the ionization density varies along the trajectory of the tritium beta particles, with consequent variations in the linear energy transfer. This parameter, which is measured in radiobiological dosimetry in keV/μm, clearly loses much of its usual significance in view of the short trajectories of tritium beta particles;

(4) the concept of relative biological effectiveness and the associated numerical value must therefore be accurately defined to be of any dosimetric or radiobiological significance;

(5) in addition to these factors, which can be defined as contributing to a 'microscopic *physical* heterogeneity' of the dose, there are also extremely important factors of 'microscopic *biological* heterogeneity' due to possible differences in tritium concentration in particular sub-cellular components (nucleus) or even molecular components (nucleic acids). These depend on the intake route, the chemical form in which the tritium is ingested and the subsequent chemical and metabolic processes within the organism, as referred to in section 3. This biological heterogeneity at the microscopic level will clearly cause substantial variations in the type of structure subjected to irradiation (and thus radiosensitivity), and is therefore of fundamental importance in defining the 'quality factor' or relative biological effectiveness;

(6) the possible effects of transmutation in relation to those due to irradiation, and particularly in relation to problems of non-uniform biological distribution of the dose.

In the following sections we shall attempt to give a general summary of the problems which have not yet been satisfactorily solved at present.

5.2.1 — *Linear energy transfer*

A tritium beta particle of mean energy loses its 5.6-5.7 keV over a trajectory of approx. 0.9 μm in biological materials. It can be seen from elementary notions about the structure of living material that these dimensions are within the range of sub-cellular organelles, where there are distinct variations in the density of the irradiated biological structures (Goodheart, 1961).

Apelgot and Duquesne (1963) calculated values of LET along the tritium beta trajectory and found that in the first 0.1 μm approx. 3.9 eV/nm are transferred, whereas at the end of the trajectory the LET rises to 5.5 eV/nm. In the case of particles of maximum energy, the initial value falls to about one-third. As stated above, the application of LET values at these submicronic scales is of doubtful significance; furthermore, the decision whether these variations

have dosimetric significance, in the sense that they are capable of influencing the quality factor, requires the solution of complex problems of biophysics and microdosimetry which have not yet been practically approached. This research becomes even more difficult when one considers the small number of measurable biological effects which could be used to detect such minute differences. Therefore it appears reasonable to assume that, at least for the present time, the variations in density of living material and the variations in LET can be disregarded, particularly for estimates of doses of interest in radiation protection. The fact remains that tritium produces radiation of greater ionization density in the immediate vicinity of the nuclide than other beta-decay isotopes; we do not know, however, whether this can affect radiotoxicity.

The Apelgot and Duquesne (1963) calculations are nonetheless interesting if they are considered in terms of the number of pairs of ions produced along the trajectory of a particle of mean energy; this number is within the range 90-200 in 0.9 μm of water (which can be reduced to approx. 0.7 μm in pure DNA, in view of the different electron density and the different energy level needed to produce one pair of ions). It is a basic statement in radiobiological literature (Lea, 1956) that the frequency of chromosome breakage is dependent, through complex mechanisms, on the ionization density, starting from a minimum of approximately ten pairs per μm. In spite of the considerable inaccuracy of these estimates, it is possible to conclude on a purely physical basis that chromosome breakage that is a well-defined biological damage, can result from a single disintegration of one atom of tritium by direct radiation action.

5.2.2 — *The concept of absorbed dose*

Para. 5.1 showed how general methods of estimating dose can be applied to tritium, provided that one assumes an effectively uniform deposition of the nuclide. Feinendegen (1967) discussed in some detail the question whether 'microscopic physical heterogeneity' could significantly affect dose estimates, by critically reviewing the data available at that time. The importance of such question for the purposes of dosimetry and health protection is clearly due to the need to use the rad as the unit of absorbed dose (and therefore the rem, once the RBE has been established).

Tritium does not in principle differ from many other similar internal emitters, particularly those undergoing alpha-decay (for reasons due to the short mean range of the particles), but it does have the advantage of being easier to study, at least in the case of tritiated water or tritium which freely exchanges with hydrogen.

If the energy deposited by individual beta particles of tritium in an animal cell nucleus or in a bacterial cell is expressed in rad, the numbers obtained have no precise value and, by the very definition of rad, which is on a macroscopic scale cannot be used in practice. For example, a single beta particle of mean energy would deposit approximately 170 rad within a sphere of 1 μm in diameter, the tritium disintegrating at the centre of this sphere (Oliver and Lajtha, 1960). In the case of a bacterium with a volume of 4.2 μm^3, uniformly tagged with tritium, each beta particle of mean energy would deliver a 'dose' of 14 rad (Apelgot and Duquesne, 1963), an estimate at least ten times lower than in the preceding case. Goodheart (1961) estimated that the 'mean' dose of radiation absorbed by cell nucleus 4 μm in diameter is of the order of 0.27 rad/disintegration; this value is even smaller and is approaching the dose estimate obtained for uniform distribution.

Tritium ingested as tritiated water is distributed uniformly throughout the organism and follows the same pathways as water, exchanging readily with hydrogen, and moves freely into the various fluids and tissues, so that microdosimetric considerations as mentioned above should not have any value in practice. Therefore, in conditions of uniform deposition, all cells, especially their nuclei, are not in a situation which is substantially different from that of cells bombarded with secondary electrons from electromagnetic radiation. Considering that the deposition of energy is at random and if we disregard, as a first approximation, the biophysical considerations

discussed in para. 5.2.1 and the problem of the irradiated volume on a sub-micronic scale, the rad retains its full value and significance as a unit of absorbed dose. With the exception of Barber (1969) all the researchers cited in this section, even the most recent ones, accept this fundamental principle in practice.

Serious complications arise, however, when we attempt to refine the calculation of absorbed dose at the sub-micronic level to obtain results having both biological and purely physical significance; the same complications are bound to arise when the tritium is localized exclusively in the nucleus, for example after incorporation of tritiated precursors of DNA. If the mean range of the beta particles is of the same order of magnitude as the biological structures under irradiation, only part of the beta spectrum energy will be entirely absorbed within the structures themselves. It is therefore incorrect to use a mean beta spectrum energy in equations such as (5.1). The significant physical parameter is the mean spectrum of the absorbed energy, or 'mean absorbed energy' (Tägder and Scheuermann, 1970), and this appears to be the core of the problem.

The literature contains many reports of attempts to achieve a theoretical solution by means of calculation (Goodheart, 1961; Bond and Feinendegen, 1966; Feinendegen, 1967; Lambert, 1969; Ertl, 1970), using increasingly complex models and the most recent estimates of physical constants (such as those relating to Mozumder, 1969). A useful survey of calculation methods is also given by Tägder and Scheuermann (1970), who conducted experiments on plants (root tips of *Vicia faba*) to test the validity of numerical assumptions associated with the biological parameters necessary for the calculation. The most important conclusion drawn by these authors is that the different calculation methods yield differing estimates of the dose in the nucleus and these are all highly dependent on the dimensions of the biological target in question. It is therefore reasonably clear that the problem which has been most satisfactorily solved so far is only that of estimating the proportion of the dose which escapes from the edge of the nucleus ('edge effect'; see Goodheart, 1961; Robertson, cited by Feinendegen, 1967; Ertl, 1970). Some autoradiographic evidence would suggest a possible non-homogeneous deposition of tritium within interphase nuclei and along metaphase chromosomes, but these observations do not seem to account for, nor contribute to the differences in the dose estimates. To the best of the present knowledge, no information are available on some very peculiar cases, such as the dose deposition from tritium incorporated mainly in nucleoli from tritiated precursors of RNA.

From the relatively large amount of data reported by all previously mentioned papers, mostly based on theoretical calculations, it would appear that the problem of choosing between the concept of 'mean spectrum energy' and that of 'mean absorbed energy' is still far from being solved. On the other hand it is a little surprising to see that such complex and difficult attempts at theoretical calculations have not yet stimulated similar attempts at any direct experimental verification. This does not imply that some sort of 'biological dosimetry' is suggested here, but merely that empirical approaches might be sometimes useful with the aid of suitable biological materials and end-points, and of conventional external radiation for which accurate dosimetry is available. Tägder and Scheuermann, for example, have calculated average dose estimates to the cell nuclei of root tips of *Vicia* under very well defined and reproducible conditions of labelling with tritiated thymidine; it should not be difficult to expose the same material under the same conditions to accurately measured concentrations of tritium as tritiated water and to external electromagnetic radiation, eventually comparing results of biological relevance, such as growth inhibition, chromosome aberrations, etc.

A completely different approach was adopted in recent calculations by Dugan and Ice (1971) and Dugan (personal communication, 1972) for the case of tritiated thymidine, in the attempt of considering the specific of incorporation of tritium into DNA and with the aim of obtaining dose estimates which could be practically used in the event of accidental contamination. This approach is perhaps too simple, because it attempts to extend the application of a general

formula of the (5.1) type, which is valid on the macroscopic level, from the entire organism to the organ (testis), the nucleus and eventually to the DNA molecule. The required numerical values were obtained experimentally in animals by measuring both the tritium concentration and the effective half-lives at each of four levels of biological organization, within 90 days after intake. This attempt would not appear to be completely acceptable without discussion. The model ignores, for example, all the considerations of microdosimetry mentioned in the preceding sections which are essential at the molecular level; in addition, an 'inhomogeneity factor' of 0.20 was introduced (for DNA only) based on widely divergent considerations derived from data in the literature, and not all of these are clearly justified. After the application of this factor, the dose in the DNA is expressed in units of equivalent dose (rem), so that this should be expected to include also an evaluation of Relative Biological Effectiveness. Nevertheless, it is interesting to note that the dose in the nucleus and the dose in the DNA expressed in rad are of the same order of magnitude (44-54 rad), whereas both are 20-80 times greater than those estimated for the entire organism and for the critical organ in question (testis). It is therefore clear that, if such great differences in the estimates reflect real differences in the absorbed doses, an empirical-experimental approach of the type used by Lambert and Clifton (1968) and Lambert (1969, 1971) is certainly feasible, provided that it is extended to cover times of exposure to the nuclide of the order of those considered by Dugan and Ice (1971).

5.2.3 — *Relative Biological Effectiveness*

Since the value of RBE is given by the ratio between the dose due to tritium and that from a standard radiation to obtain the same biological effect, all the difficulties discussed in the preceding paragraph are also encountered here. In order to place the problem of RBE of tritium in the correct perspective, it would be reasonable to keep the case of tritium ingested as tritiated water distinct from the case of tritium incorporated by tagged molecules or molecules which do not easily exchange with hydrogen.

In the first case (see para. 5.2.2) we have seen that the dose estimate calculated at the macroscopic level can be expressed in rad for effects which involve the entire organism and we do not therefore encounter any particular problems. In addition, it is possible to establish this RBE for tritiated water by experimental means and this was attempted in the 1950's when relatively low values of the order of 1.4–1.7 were found. Although these values were determined some time ago, they are still cited in the recent works on the subject (Bond and Feinendegen, 1966; Feinendegen, 1967; Vennart, 1968; Vaubert and Bittel, 1970; Bond, 1971; Osborne, 1972). It has already been seen that these values have recently been reduced to 1 on the basis of evaluations made by the ICRP (Sowby, 1969). More recent authors appear to be substantially in agreement with these evaluations and the problem is not considered to be of great relevance for health protection (Osborne, 1972). Problems of variation in linear energy transfer do not appear to be significant in this field (see para. 5.2.1), and the possible effect of inhomogeneity in the distribution of water at the endocellular level is not considered on the argument that water content and RBE would in any case be in inverse correlation (Osborne, 1972). Nevertheless, one must recognize that there is no recent experimental data which might be desirable at the present time. This is especially true because the research by the Moskalev group (Moskalev *et al.*, 1970 a and b), which give estimates of relative biological effectiveness of around 2 and show variations in sensitivity between species (Zhuravlev, 1971), are at present available only as summaries.

The case of tritium which, regardless of the mechanism of intake, is subsequently incorporated preferentially in particular cells or molecules, is completely different. In this case the problems of evaluating the dose become critical and, since the problem of microdosimetry has not yet been solved, the same can be concluded for the relative biological effectiveness. It is surprising that the recent literature contains sophisticated work on dosimetry relating to tritium localized in the nucleus (see para. 5.2.2), whereas the problem of Relative Biological Effectiveness

has been practically ignored at the experimental level. A few works are available on the relative toxicity of the various tritiated precursors of nucleic acids (especially thymidine: Lambert, 1969, 1971), but the variability of results does not make it possible to draw definitive conclusions.

Unfortunately, the experimental conditions which make use of specific precursors of DNA, although they may be considered as fairly well controlled for research purposes (Feinendegen, 1967; Payne and Shaw, 1971), cannot be regarded as having direct relevance to public health at the population level, because in the latter instance the exposure conditions are so widely different. In any case, it appears to be far more important to determine the effectiveness of the tritium which is stably incorporated in the tissues, either long times after acute exposure or during chronic exposure. It is therefore not only necessary to refine the dose estimate, but also to confirm a biological effectiveness close to unity for the tritium which is taken in from the environment essentially in the form of tritiated water and characterized by half-lives of the order of hundreds of days (see para. 3.3). It should be noted that greater interest is now being taken in this fraction of the incorporated tritium in determining its endocellular and molecular localization (Hatch and Mazrimas, 1972) and in estimating the doses deposited by it (Bennett, 1972).

6 — CONCLUSIONS

Considerable difficulty is encountered in critically evaluating the data in the literature in order to draw conclusions which have some relevance to public health protection. In this chapter we shall first examine the applicability and the completeness of the data for the purposes of health protection and then indicate further points on which more data would be useful in formulating a final judgement in areas where we have little knowledge, if any.

6.1 — The risk of tritium contamination

There are clearly several angles from which this problem can be approached. There is, above all, a general problem regarding the tritium levels existing at present in the biosphere and the estimates of possible future levels, based on the various available assumptions. Secondly, it is clear that the various paths of tritium contamination are of different significance for public health. It is also necessary to discuss existing information on tritium metabolism in mammals and the associated doses. Finally, one should consider the toxic levels for various effects to see how these compare with existing and foreseeable future tritium levels, and how the currently accepted levels of contamination compare with toxic and actual levels.

6.1.1 — *Current tritium levels and future production*

The tritium measured at present in the biosphere at ground level in the northern hemisphere are of the order of 100 TU. Since it has been calculated that 1 TU is capable of giving a dose to the whole body equivalent to approx. 0.34 μrem/year (Osborne, 1972), the current dose is of the order of 34 μrem/year. If this dose is compared with an average limiting dose for the population at large of 170 mrem/year (NCRP, 1971)* it is simple to deduce that current tritium dose is lower by a factor of approx. 5×10^3. Estimates regarding the production of tritium in Western Europe are substantially in agreement (ENEA, 1971).

* Since the ICRP does not have any accurate information on the maximum permissible dose for the entire population, it is justifiable in individual cases to work on a dose of 5 rem over 30 years, which is valid for genetic damage.

As regards the future, it is thought that a controlled discharge of tritium from reactor systems such as to maintain the total activity of tritium at 200 MCi could lead to a dose of 3-15 μrem/year (Osborne, 1972). Other estimates (Jacobs, 1968) which calculate doses of the order of one μrem/year for 10^8 Ci of tritium are in fairly close agreement. It must, however, be taken into account that if some forecasts of the development of major sources of tritium (Cowser and Tadmor, 1968) are fulfilled, further increases by a factor of about 10 in terms of tritium dose could be hypothesized.

It is not unreasonable therefore to envisage some control of the tritium released by nuclear installations from now onwards, in order to prevent tritium contamination of such a degree that the dose delivered by it would amount to a disproportionately large fraction of the total maximum permissible dose. It does appear possible to exercise this type of control under even the most extreme conditions (real or hypothetical) foreseeable, on the basis of present technological knowledge and on the basis of the future expansion in the production of tritium from energy-producing plants using controlled fusion (Rose, 1970) which are, in any case, very much in the future.

It therefore appears justifiable to draw the following conclusions:

(1) Tritium contamination of the biosphere is significant for radiation protection in view of the ubiquity of the nuclide and of the number of persons exposed.

(2) Since the beginning of the nuclear age, the total amount of tritium present on the earth has increased by ten times without control, principally due to the production by nuclear weapons.

(3) At present the contribution made by tritium to the total dose can be estimated between one ten-thousandth and one-thousandth of the dose due to natural sources, and is therefore not particularly alarming.

(4) Since, however, it is unacceptable that a disproportionately high fraction of the maximum permissible dose of a 170 mrem/year should be taken up by tritium, controls and safeguard mechanisms should be envisaged in order to keep the total of tritium released by nuclear installations at reasonable levels. This control is technologically feasible.

6.1.2 — *Paths of contamination*

The available data, relating to both animals and man, on the intake of tritium under the form of tritiated water by the percutaneous, pulmonary and intestinal routes have now reached a fairly refined stage of development. The intake of gaseous tritium does not appear to be an insuperable problem or one significantly different from tritiated water. In addition, the intake of gaseous tritium is not a very common route of contamination, it is limited to a few accidental cases, and does not result in the ingestion of radioactivity in very large amounts.

In very general terms, none of the forms of contamination described above appears to pose serious problems at current contamination levels; at present, the risk is limited to groups of workers who are occupationally exposed and all of these together do not exceed some tens of thousands. In relative terms, it seems unlikely that the problem will become significantly more serious, in view of the foreseeable development of nuclear energy and its applications in the industrial and medical field and of the increase in the number of persons employed in these activities.

However, there is no sufficiently accurate or generally applicable information available on one form of contamination which may have much greater potential significance if the levels of water or soil contamination should greatly exceed the present levels: we refer to the tritium contamination by the ingestion of foods produced in contaminated environments or of drinking

water contaminated with tritium. Without considering a general increase in fall-out due to military events, which would obviously affect the whole world population, it is nevertheless conceivable that large groups of people could be exposed as a result of major accidental contamination or following an uncontrolled increase in intentional discharges into river basins, for example. It must be pointed out, however, that an increase over current contamination levels exceeding some tens of nCi/litre is not likely in the immediate future, and this level is about five orders of magnitude lower than those which have been experimentally shown to be toxic. In the almost complete absence of information on this point, however, it may be useful to undertake research to define the possibility of variations in the T/H ratio from a given environmental T/H ratio for particular food chains which are of interest to man. This research could at least indicate whether the current criteria for establishing environmental contamination levels and doses to the population on the whole are reasonable.

6.1.3 — *Tritium metabolism*

As regards the tritium pathways within the organism after intake, the basic physiological parameters necessary for the estimates of the dose received by man have been measured experimentally and are fairly well-established. This applies to the balance for the tritium intake by the various routes, the exchange and equilibrium times in the various tissues and fluids involved, the excretion kinetics and their temporal and quantitative parameters through the various routes. These data, together with basic dosimetric relationships discussed in para. 5.1.1, provide a sufficiently accurate estimate of the dose accumulated over short periods after acute exposure and also make it possible to derive estimates of maximum permissible tritium concentration for radiation protection purposes.

However, we do not have detailed information on the doses received in individual organs by tritium deposited in tissues, especially the slow-renewing ones, and it is therefore necessary in this case to extrapolate criteria from animals to man. The lack of information is due to two factors, the first being the absence, even in experimental animals, of accurate data to correlate the population kinetics of the various organs with the half-life of the incorporated tritium. A better knowledge of these phenomena is desirable because the dose due to tritium incorporated in the tissues may show up, eventually as the component of greatest radiobiological importance in the entire dose, whether received by acute or chronic exposure (see para. 5.2.2). Secondly, the scarcity of radiological data may be attributed to the dosimetric difficulties discussed in paragraph 5.2. These do not yet allow a clear picture of dose-effect relationships because there are no general criteria for calculating doses in the biological structures of importance for the most significant radiobiological effects. It seems unlikely that the formulation of refined mathematical calculation models will be able to resolve this problem unless their results are accompanied by sufficiently controlled biological tests having more relevance to radiological protection.

6.1.4 — *Radiotoxicity*

The harmful effects of tritium administered as tritiated water or tritiated thymidine have been adequately demonstrated at various levels of biological organization. However, the data available are of little use for public health protection because the effects measured are not of immediate practical application. For example, systematic studies of the lethal action of tritium *in vitro* and *in vivo* which are probably the most significant, even on a practical level, have only been conducted fragmentarily and without practical objectives.

As regards the toxicity of *tritiated water*, there is complete lack of information in the data examined in section 4 about the toxicity of this substance upon genetic structures, in causing either intergenic or intragenic mutations. Acute exposure to concentrations of tritiated water of the order of 0.1-1.0 mCi/ml or /g body weight inhibits the reproductive capacity of mammalian

cells *in vitro* and, mainly at higher dose levels, causes serious immediate somatic damage (bone-marrow failure). The existing data on delayed somatic damage are, of course, of an indirect nature and obtained from unsuitable experiments. It therefore appears reasonable to conclude that information on this subject is practically nonexistent. It would also appear that tritium concentrations capable of causing foetal lesions by treatment of pregnant animals are extremely low, of the order of 1-100 μCi/ml of body water.

Exposure of animals to *tritiated thymidine* in concentrations of approx. 10 μCi/g of body weight for periods of the order of a few weeks results in the induction of genetic damage, both dominant and recessive; *in vitro*, the acute exposure of cells to doses of the order of 1 μCi/ml results in chromosome aberrations. Inactivation of cells *in vitro* has been reported at concentrations of 0.1-10 μCi/ml or /g, whereas the same effects *in vivo* require concentrations of 5-500 μCi/g. Immediate somatic lesions occur at concentrations of approx. 1 μCi/g (chronic exposure over several weeks) and delayed lesions occur after acute exposure (especially in young animals) to concentrations of 0.1-10 μCi/g. Foetal damage occurs when pregnant animals are treated with doses of the order of one μCi/g.

It may be interesting to compare these concentrations with those measured at present in the biosphere and with the maximum permissible concentrations currently recommended by the ICRP (1959).

In the period July-September 1971, the mean tritium concentration in the drinking water in the United States was 0.2 nCi/l (Radiation Data and Reports, 1972). Using the equation suggested by Moghissi and Porter (1968) for the values recommended by the ICRU (1964), it is possible to estimate the radiation dose to man resulting from this tritium concentration. On the extreme assumption that the tritium concentration in all of the ingested water is equal to that found in drinking water and that the specific activity of body water is essentially the same as that of drinking water, one calculates a dose of 0.3 mrem/year, averaged over the whole population. This dose is approximately 0.2% of the proposed annual limit of 170 mrem. During the same period the maximum tritium concentration in surface waters in the United States was 3.9 nCi/l (Radiation Data and Reports, 1972). Measurements of tritium concentration in surface waters in various parts of Italy (Mastinu, 1972) gave mean values between 1 and 2 nCi/l, which can be compared with the maximum concentration of 30 μCi/l for individuals exposed at their places of work and 300 nCi/l in drinking water for the whole population, following the recommendations of the European Community (CNEN, 1967).

Therefore, the maximum permissible tritium concentrations for exposed workers are still four orders of magnitude greater than the concentrations generally observed, but this factor reduces to approximately two orders of magnitude or less when we consider the concentrations generally allowed for the population. Osborne (1972) made a detailed study of the problem of tritium intake by various paths, using the most recent estimates of the factors required for dose calculations. If one considers, for example, the case of chronic exposure from drinking water (which is a practical situation of considerable significance for public health), it can be concluded that the contamination limit proposed by the ICRP (1959) are more conservative by a factor of approx. 3 compared with the limits considered by Osborne.

At the present time, the problem is not so much one of further refining calculations of the type described above, but of establishing, on the basis of the available radiotoxicity data already discussed in detail but above all of the information not yet available, whether the currently proposed body burden and concentration levels should be regarded as in any way over-generous. In spite of the indications to the contrary made by some experts (Auxier, 1971), a more thorough discussion, possibly accompanied by reconsideration of these limits may be desirable in the particular case.

6.2 — The needs for future research

Although the points listed below have already been mentioned in the main body of this report, it may be useful to list separately the research topics which appear to be most interesting at the present time in order to fill in the gaps in our existing knowledge.

(1) It is important to study a number of food chains affecting man to determine whether tritium concentration exhibits any unusual behaviour in the various links of the chains.

(2) Because the tissue dose levels are dependent on the concentration of tritium bound to the various organic components, it is of vital importance for the correct estimation of these doses to have more information about that fraction of tritium associated with cellular components having a long half-life; more information on the proportion of tritium associated with those biomolecules, such as DNA, which are most probably the radiobiological targets for many effects seems particularly important. Within the framework of this research, greater standardization of techniques and a more accurate definition of terminology is also desirable.

(3) We are not yet in a position to quantitatively compare the genetic effects of tritium decay in germ cells with the effects produced by external radiation sources. It is essential to obtain these data for mammalian species in order to evaluate the genetic risk, because extrapolation from simpler forms of life could be unjustified and dangerous.

(4) It appears that delayed somatic effects have been largely neglected, especially in the case of tritiated water. No experimental work is available in which the problems of delayed damage have been examined by techniques which are acceptable for this type of work; on the contrary, owing to the inadequecy of methodologies and techniques, even the claims about the absence of effects appear often of little value.

(5) The lack of careful analyses of radiobiological phenomena based on extensive dose-effect relationships is often found in the literature. This increases the difficulty of extrapolating the effects at the low dose levels and therefore of making any judgement about the conditions which are most significant for public health. It is suggested that future experimental work should give special emphasis to the effect of the low doses.

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Appendix

COMMISSION OF THE EUROPEAN COMMUNITIES

RADIOTOXICITY OF TRITIUM IN MAMMALS

Critical analysis of the extrapolation to man of the results of tritium incorporation into animal tissues

by

G. SILINI, P. METALLI and G. VULPIS

1973

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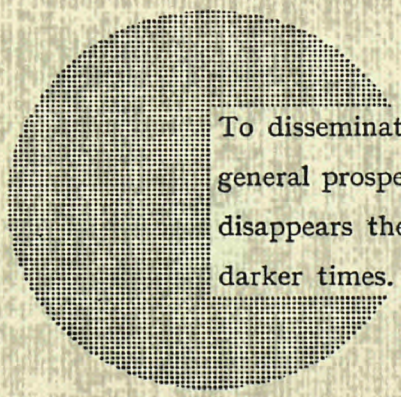

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