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RADIATION DOSIMETRY IN EXPERIMENTAL ANIMALS EXPOSED TO TRITIATED WATER UNDER DIFFERENT CONDITIONS*

R. B. Cumming, G. A. Sega, and M. F. Walton

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

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R. B. CUMMING, G. A. SEGA, and M. F. WALTON
Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee
United States of America

Abstract

RADIATION DOSIMETRY IN EXPERIMENTAL ANIMALS EXPOSED TO TRITIATED WATER UNDER DIFFERENT CONDITIONS.

The radiation dose to the germ cells of male mice, which sired the offspring scored in a specific-locus mutation test of injected tritiated water, has been calculated. The weighted mean dose for germ cells which received all of the radiation at postspermatogonial stages was 430 rads, while that for germ cells irradiated almost entirely as spermatogonia was 615 rads. Most of the animals received a single intraperitoneal injection of HTO of 0.50 mCi/g body weight, but a few of them received 0.75 mCi/g. These weighted mean doses reflect the contribution of both groups. The percentage of the total dose delivered from ^{3}H incorporated into macromolecules is small — less than 0.5%. The percentage of the total radioactivity in dry material from the testis on day 1 postinjection is 0.6%, on day 7 it is 3.2%, and on day 605 it is 95%. Tritium is incorporated into testicular DNA from tritiated water, and peak levels of radioactivity in this macromolecule are reached from 3 to 9 days following injection. The stable incorporation of tritium into trichloroacetic acid insoluble materials is about 75% in protein and 25% in nucleic acids at all time periods following injection. Doses from single injections of tritiated water are inherently more variable than for protracted low-level exposures. This is because small differences in water balance near the time of injection can make a very large difference in total radiation dose.

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INTRODUCTION

Human exposure to environmental tritium would be expected to largely be from tritiated water at low levels for protracted periods. In obtaining quantitative biological data for use in the calculation of potential risk to human populations, it is frequently necessary to choose other exposure conditions for the treatment of the experimental animal models. It is the purpose of this report to explore one such set of conditions, the single intraperitoneal injection of relatively high levels of HTO, and to examine the nature and magnitude of errors which could result from departing from chronic low level exposure in experiments using this technique.

The specific-locus mutation test in mice [1] provides the best currently available method of obtaining quantitative transmitted gene mutation data in an intact mammal. However, this method, or any method designed to obtain such data, requires the examination of relatively large numbers of offspring of individuals exposed to doses which are high compared to the expected environmental exposure. In addition, it is useful to perform the experiment in such a way that effects due to exposure of different germ-cell stages can be distinguished. The only type of exposure with which one can practically achieve these objectives for a specific-locus test in mice is that of the single injection of HTO.

For any type of exposure conditions the total dose is a function of the product of the average radiation intensity (dose rate) and time. For tritiated water the time-intensity curves are very different in shape for single exposure and chronic exposure conditions. In principle the areas under both types of curves could be integrated and total dose to any germ-cell stage calculated. These total dose figures would be strictly comparable with each other only if there is complete reciprocity for time and intensity, i.e., that biological effect per unit of dose is independent of dose rate over the range of dose rates tested. The highest dose rates obtained by injecting 0.50 mCi/g body weight of tritiated water are below the levels where dose rate effects are seen in males from other types of radiation exposure in the specific-locus test. Thus dose rate, *per se*, is not likely to produce major differences in results between the two types of experimental protocol.

There are, however, other complicating factors in comparing chronic *vs.* acute exposures to tritiated water. One such factor is the possibility that for the two sets of exposure conditions, different proprotions of the total dose may be from tritium in different molecular sites. Another is that the precision of dose calculations for acute exposures is inherently less precise than that for chronic exposure conditions.

Differences of this type between chronic and acute exposure conditions must be effectively dealt with to achieve a realistic assessment of the environmental impact of man-made tritium release. The dosimetry of biologically significant radiation from exposure to tritiated water is extremely complex. However, it is hoped that useful values can be obtained for the purposes of risk calculations.

Preliminary accounts of this work have appeared in the Annual Reports of the Biology Division of the Oak Ridge National Laboratory [2, 3, 4].

METHODS

Exposure conditions

Male mice, strain (101 x C3H)F_1, were individually weighed and injected intraperitoneally with tritiated water which had been adjusted in specific activity to 25 mCi/ml. Each mouse received a single injection of 20 µl/g of body weight to give a total injected radioactivity of 0.50 mCi/g or 500 mCi/kg. Eighteen male mice were injected in each group at about 10:00 a.m., and each male was placed in a disposable plastic mouse cage with 5 uninjected T-stock female mice. The cage was immediately placed in an isolator (described below) designed to minimize atmospheric contamination in the animal facility. Exposure conditions for dosimetry were identical to those for the specific-locus mutation experiments reported elsewhere [5] and some of the animals in the dosimetry experiments produced offspring scored for specific-locus mutations and included in that report.

Isolators

For two weeks after injection the animals were housed in isolators designed to recover and measure HTO released into the atmosphere surrounding their cages. The isolators were made of a heavy flexible plastic with a rigid internal aluminum frame to maintain constant volume. These isolators were 61 x 61 x 152 cm in size and had an internal volume of 565 l. Each contained 18 disposable mouse cages and enough food and water to maintain the mice for one week. Each isolator was fitted with 2 pairs of heavy rubber gloves, which allowed the operator to reach any part of the internal space without opening the isolator. Isolators were operated at negative pressure relative to the room. Air was pulled from the isolator through a series of four cold traps, which removed >99% of the moisture it originally contained. It was then pulled through a large drying filter and released through a hood. Traps were removed at intervals and the volume and specific activity of the water they contained was measured. Air flow was maintained at about 1 isolator volume every 4 min. Animals were maintained in the isolators for two weeks after injection and then were removed into a special mouse room reserved for that purpose.

Experimental protocol

Animals were injected as described above and then serially sacrificed so that the quantity and distribution of radioactivity remaining in the testes could be measured. The postinjection serial sacrifice time points for this experiment were: 1 hr, 12 hrs, 1 to 10 days (1-day increments), 10 to 100 days (5-day increments), and 605 days. Nine animals were sacrificed for each time point to give an estimate of individual variability.

Tissue preparation

The testes of each treated animal were homogenized in distilled water and brought to a constant volume (10 ml). Aliquots of these homogenates were solubilized wet or dried under a vacuum on filter paper and counted for radioactivity

in a liquid scintillation spectrophotometer. Details of tissue handling procedures are as described previously [6]. Dried aliquots were oxidized by combustion prior to counting to obtain optimum samples for the determination of absolute activity. DNA and protein were extracted from pooled extracts at each time point and the specific activity determined.

Calculation of dose

Mean total tissue specific radioactivity was measured at the various time points from one hour to 605 days postinjection. These points were joined using an exponential extrapolation between points to generate a radioactivity time curve for the single exposure. Areas under this curve were determined by integration to give radiation dose estimates to germ cells at various injection-to-conception intervals. These doses were weighted for the number of offspring sired in each interval to give weighted mean doses in the specific-locus experiments for offspring resulting from germ cells irradiated only in postspermatogonial stages and offspring resulting from conceptions involving germ cells which were irradiated primarily as spermatogonia.

RESULTS AND DISCUSSION

An estimate of the radiation dose to the testes of mice involved in a specific-locus mutation experiment [5] has been calculated. This estimate was based on data obtained from mice injected with 0.50 mCi/g of HTO and handled and mated identically to those in the specific-locus experiment. The tissue specific activity of the testes was monitored through time by serial sacrifice and liquid scintillation counting of tissue homogenates. A summary of some of the data obtained is presented in Table 1. By exponential extrapolation between points a curve is generated and the area under this curve can be integrated to give the dose to any point in time after injection. The upper limit of the dose for these conditions and with this quantity of radioactivity is just under 600 rads. The animals injected with 0.75 mCi/g in the specific-locus experiment are assumed to receive 1.5 times the dose received by animals given 0.5 mCi/g at any equivalent time point. Using these values to calculate weighted mean doses to germ cells which produced the over 40,000 offspring scored for the specific-locus test [5] a value of 430 rads is obtained for germ cells irradiated in postspermatogonial stages and of 615 rads for spermatogonia.

When aliquots of testis homogenates are counted both wet and after drying under a vacuum, one can obtain an estimate of the percentage of the radioactivity present in the form of water. The radioactivity which remains after such drying procedure is assumed to be incorporated into some nonvolatile molecule. Only 0.6% of the radioactivity remains after drying on samples taken 24 hours after injection, while 3.2% is stable to drying in samples taken 7 days after injection. In samples taken 605 days after injection of HTO 95% of the remaining activity is in nonvolatile molecules. This nonvolatile radioactivity forms the upper limit for tritium incorporated into macromolecules (proteins and nucleic acids). When nonvolatile radioactivity is integrated with time after injection, less than 0.5% of the total dose comes from tritium in this nonvolatile form. Thus a very small fraction of the total radiation dose comes from tritium incorporated into macromolecules.

In a procedure described earlier [6] it is possible to get an estimate of the ratio of ^{3}H incorporated into protein to that incorporated into nucleic acids. For all time periods following HTO injection this ratio is about 3 in testis homogenates. This indicates that about 75% of the total macromolecular incorporation is in protein and the other 25% in nucleic acids (both DNA and RNA).

If DNA is extracted from testis homogenates of animals sacrificed at various times following the injection of HTO, tritium incorporation into this macromolecule is readily measured. There is a broad peak of activity in DNA from 3 to 9 days postinjection. (See Fig. 1.) After this time the specific activity of DNA decreases, presumably due to dilution from new DNA synthesis incorporating less tritium. The fraction of the total radiation dose received from tritium incorporated into DNA is extremely small (<0.1% of the total).

We have a number of indications that total radiation dose from a single injection of HTO can be greatly influenced by rather minor changes in the experimental conditions. Water intake during the first few weeks following injection is clearly very important. It is also clear that the quantity and quality of the food consumed plays a role as does the number of animals housed in a single cage. Low-level chronic exposure conditions, such as those used by Carsten and Commerford [7] would be expected to be less subject to experimental variability. This is because the specific activity of the administered water remains constant and fluctuations in daily intake are relatively unimportant. The chronic low level exposures also provide a better model for the exposure conditions which humans are likely to encounter. However, the single injection protocol does make practical large genetic experiments such as the specific-locus test and thus yields vital information.

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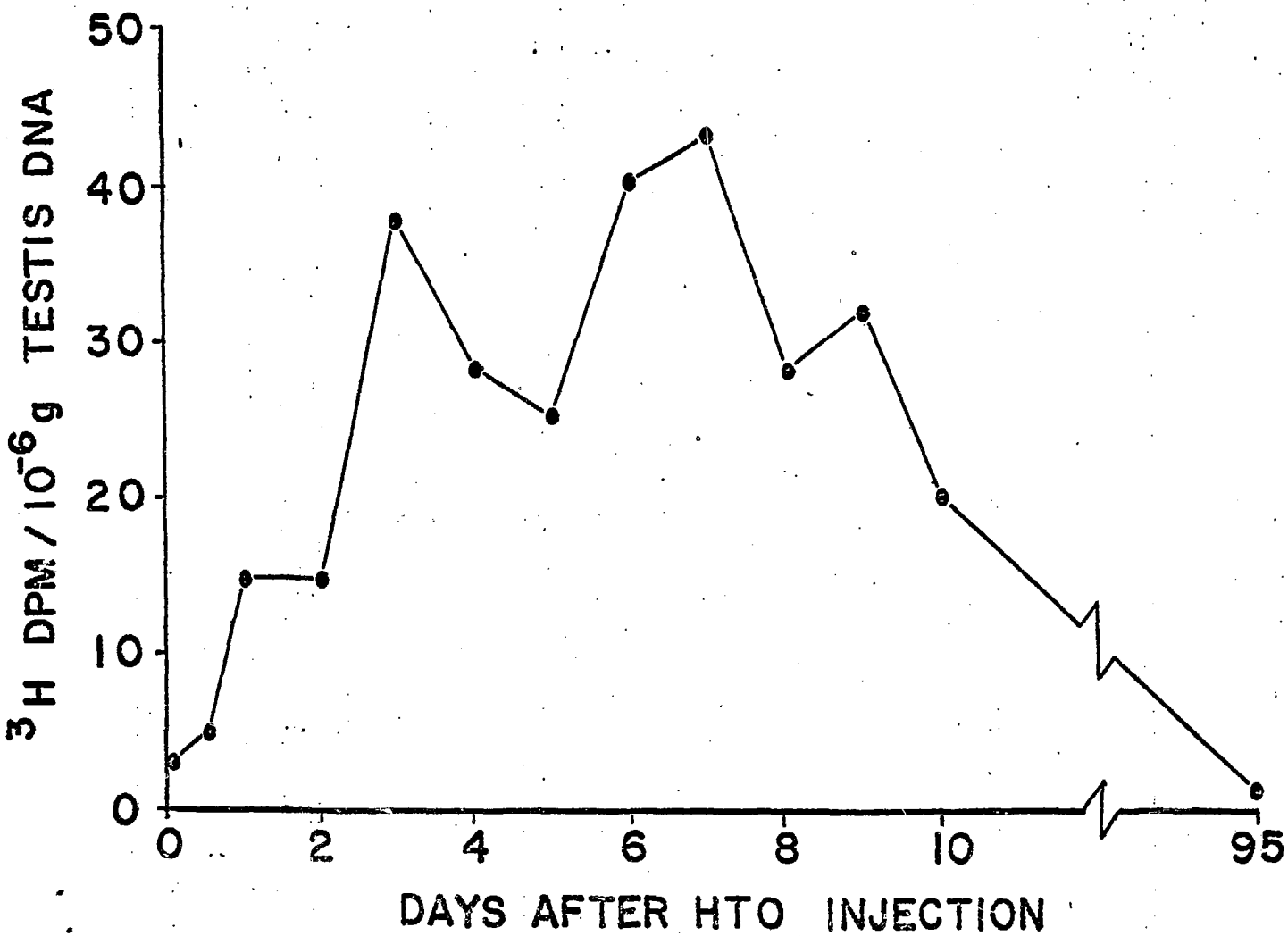
Table I. Summary of Data on Radiation Dose to the Testes of Male Mice Following a Single Injection of 0.50 mCi/g of HTO

Time postinjection (days)	Tissue radioactivity (DPM/mg)	Dose rate (rads/hr)	Cumulative dose (rads)
1 h	13.8×10^5	7.23	—
1	11.3×10^5	5.94	157.9
2	8.4×10^5	4.39	281.8
3	6.0×10^5	3.16	372.5
4	4.4×10^5	2.32	438.3
5	3.2×10^5	1.68	486.3
6	2.8×10^5	1.47	527.8
7	2.5×10^5	1.33	558.5
8	1.7×10^5	8.9×10^{-1}	564.1
15	1.9×10^4	1.0×10^{-1}	576.7
20	5.2×10^3	2.7×10^{-2}	583.4
30	1.2×10^3	6.3×10^{-3}	586.8
35	7.6×10^2	4.0×10^{-3}	587.4
40	4.0×10^2	2.1×10^{-3}	587.9
45	3.6×10^2	1.9×10^{-3}	588.2
50	3.0×10^2	1.6×10^{-3}	588.4
95	1.1×10^2	5.5×10^{-4}	590.0
605	4.4×10^1	2.3×10^{-4}	594.6

FIGURE LEGEND

FIG. 1. Specific activity of DNA extracted from the testes of mice as a function of time after a single injection of 0.50 mCi/g of tritiated water.


50
40
30
20
10
0
^{3}H DPM / 10^{-6}g TESTIS DNA
0
2
4
6
8
10
95
DAYS AFTER HTO INJECTION