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MASTER

GENETIC AND SOMATIC EFFECTS IN ANIMALS MAINTAINED ON TRITIATED WATER

[SPECIAL CONSIDERATION OF TRITIUM DISTRIBUTION, TURNOVER AND MICRODOSIMETRY]*

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Introduction

The increasing worldwide need for additional electric power makes it apparent that there must be an increased reliance upon nuclear power reactors. A major byproduct of nuclear reactors is tritium which will be released to some extent into the environment either from the reactors or from nuclear fuel reprocessing plants. The projected tritium release is of great concern to health physicists, environmentalists, and those who must provide for the public welfare through the controlled licensing of nuclear reactors and reprocessing plants. In order to assess the magnitude of the health impact of tritium release it is necessary to obtain detailed information concerning the somatic and genetic effects of chronic exposure to tritium. Also, inherent in answering the questions concerning biological effects, additional information is required concerning the metabolism, distribution and dosimetry of tritium in mammalian systems. To provide this necessary information a program was instituted in the Medical Department, Brookhaven National Laboratory to investigate the possible genetic (dominant lethal mutations (DLM's) and cytogenetic changes in the regenerating liver) and somatic (hematopoietic stem cell changes, growth and nonspecific life time shortening) effects in mice maintained on tritiated water (HTO) throughout two generations. This program, which spans several years and involves a number of individuals, is summarized in Figure 1. Some results from this comprehensive study have already been published (1-5). Other aspects are continuing and some new studies have just begun. This report will summarize results to date and serve as a progress report on continuing research.

Materials and Methods

1. Mice breeding and maintenance

Mice of the Hale-Stoner-Brookhaven strain were used. This is an albino strain, maintained in a single colony for more than 20 years in the Medical Department at Brookhaven National Laboratory. Animals are normally maintained on Purina Laboratory Chow, Ralston Purina Company, St. Louis, Missouri, ad lib. and tap water acidified to pH 2.4. Breeding partners are established by random selection from animals born within the same week without attention to litter mate selection. Breeding partners remain together throughout their reproductive lifetime. In these studies only first litter animals were used. At four weeks of age the animals were removed from the mouse colony and divided into two experimental groups. The first of these was maintained on tritiated water (3.0 μCi/ml) and the second group on tap water throughout the experiment. For long term studies, half of the animals on tap water were maintained in the tritium room whereas half were removed to a similar room which contained only mice on tap water. When the animals reached 8 weeks of age breedings were done within the two experimental groups resulting in second generation animals whose parents had either been maintained on HTO or tap water. Groups of male and female animals from both treatment groups were then maintained for long term observation. From these larger groups, 20 male and 20 female animals were randomly selected and put aside for monthly weighing. Weight measurements were made until too few animals remained in each group to give significant mean values. At the time of weighing, each animal was evaluated for general appearance.

2. Breeding efficiency and dominant lethal mutation (DLM) testing

When the second generation animals reached 8 weeks of age they were divided into four experimental groups for DLM testing (6). Group 1: males and

females maintained on HTO. Group 2; females on HTO, males on tap water. Group 3; males on HTO, females on tap water. Group 4; males and females on tap water (controls). In each group one male was placed with five females for a 5 days breeding period. Fifteen days after the midpoint of the breeding period the females were sacrificed, the number of pregnant females noted and the ovaries and uterine contents examined. The corpora lutea (CL) were counted and the uterine contents classified as to viable embryos (VIA), early embryonic deaths (ED), as evidenced by a dark "mole", and late embryonic deaths (LD) as evidenced by a formed but dead embryo. The data from each pregnant female were entered on a separate computer card for subsequent analysis.

New breeding groups were started each week so that a continuing program of data accumulation took place in all four experimental groups.

From the 4 parameters measured CL, ED, LD and VIA the preimplantation loss (PRE) for each mating was calculated:

$$PRE = CL - (VIA + ED + LD).$$

The results of the DLM evaluation were compared using three statistical tests; first, Students 't' test (7) a parametric test which assumes normal distribution of the data. In our analysis this test makes use of the pooled errors of all groups. The second test was the rank test developed by Kruskal and Wallis (8). This is a non-parametric test described as being a test for a complete random design with any number of populations in which the final analysis is made using a $(chi)^2$ test. The third test is an arcsine transformation which normalizes the data and computes the mutation index for each treatment (9). The mutation indices may then be compared using a $(chi)^2$ test. Evaluations were made on animals maintained on 3.0, 1.0 and 0.3 μCi/ml HTO.

3. Liver cytogenetics studies

The measurement of chromosomal aberrations in dividing cells is a sensitive measure of radiation damage. Such measurements can only be made in a mitotically active tissue where individual chromsomes can be examined in cells undergoing division. In the adult mouse the liver is relatively mitotically inactive. Therefore, individual liver cells will tend to accumulate injury resulting from continuous exposure to ionizing radiation. This damage becomes visible as chromosome aberrations when the cells are stimulated to division. Following partial hepatectomy the remaining liver cells become very mitotically active and may be examined for chromosomal aberrations resulting from the ^{3}H exposure.

In the initial determination experimental animals were maintained on HTO (3.0 μCi/ml) from weaning until sacrifice while matched controls were maintained on tap water. After 90, 330, 500, 560 and 700 days animals underwent partial hepatectomy followed at 54 hours by chromosome analysis, using previously published methods (10).

In a second series of determinations now underway the HTO animals being tested were maintained on 0.3 μCi/ml HTO.

4. Bone marrow evaluation

The leg bone marrow (femur and tibia) of animals on HTO and tap water were analyzed for total cellularity, relative number of hematopoietic stem cells (CFU-S) and total number of CFU-S per leg. The spleen colony assay (11) was used to make this analysis. In this assay bone marrow cells are removed from the test animals and injected into recipient mice which have received a lethal dose (750 rads, 250 KVP X-rays, 120 rads/min) whole body radiation. After 7 days the recipient animal is killed, the spleen removed, placed in Bouin's

solution and after 24 h the number of surface colonies on the spleen counted. The number of colonies bears a direct relationship to the number of viable pluripotent stem cells injected into the recipient mouse. The marrow from the donor mice was harvested by a quantitative method (12), which allows determination of the total cellular content of the femur and tibia. After dilution, 60,000 nucleated cells were injected into each recipient. From the measured values for total cells per leg and colonies obtained from 60,000 cells it is possible to calculate the total CFU-S or stem cells per leg. Lifetime CFU-S measurements were made on animals maintained on 3.0, 1.0 and 0.3 μCi/ml HTO.

5. Relative biologically effectiveness (RBE) determination

At the time these studies were begun, there was considerable debate concerning the assignment of a correct RBE or Quality Factor ("Q") for tritium as HTO. We therefore chose to compare the effects of continuous (22 hours/day) exposure to ^{137}Cs gamma rays and the chronic ingestion of HTO. The geometry of the gamma irradiation facility was arranged such that the depth dose within the peritoneal cavity of exposed mice, as measured by implanted thermoluminescence dosimeters, was equal in dose rate to the exposure resulting from the average soft tissue dose in animals maintained on HTO for extended periods.

The measured values for tritium content of soft tissue varied somewhat dependent upon their water content. On the basis of several determinations it was established that a dose rate of approximately 0.67 rads per day would be a reasonable value for the dose in soft tissue. The gamma irradiation facility was thus constructed so that the initial dose rate was 0.69 rads/22 hour day. Over the period of the experiment, this was slightly reduced due to decay of the source.

Lifetime shortening, induction of DLM's, growth and effects on the bone marrow cellularity and CFU-S content were compared between the two experimental groups. For the initial study, the Cesium gamma source was of sufficient activity to equal the dose resulting from the ingestion of 3.0 μCi/ml HTO. In the second study, now underway, a Cesium source was prepared to give a dose equivalent to the continuous ingestion of 0.3 μCi/ml of HTO.

6. Tritium incorporation and radiation dosimetry calculations

A number of determinations have been made to determine the radiation dose delivered to several tissues of interest on an activity/gram basis, and also on the basis of tritium incorporated into specific subcellular fractions. In addition the tritium turnover has been measured in the cell nuclei, chromatin, DNA and histone of these tissues.

The pattern of ^{3}H buildup and equilbirium levels were determined by measuring the amount of tritium in blood plasma and soft tissues (liver, muscle, and gonads) at various times after animals began drinking the HTO. The pattern of tritium incorporation was determined by analysis of fresh tissue. Animals were killed by cervical dislocation and tissues were removed and immediately placed in a weighed counting bottle containing a tissue solvent-scintillator fluid. The bottle plus the tissue was then weighed to obtain the tissue weight. After dissolution of the tissue the sample was counted using a well type scintillation counter. The total tritium content was calculated on the basis of the wet tissue weight. Plasma activity was similarly determined on blood obtained from heart puncture immediately before sacrifice. Determinations of tritium content in specific subcellular constituents were carried out as follows.

Chromatin was isolated from the tissues of mice under conditions designed to minimize degradation. Tissues were homogenized in a Potter-Elvejhem type

apparatus, nuclei were freed of cytoplasm by pelleting through 2.2 M sucrose, and chromatin was extracted from washed nuclear fragments with 10^{-4} M EDTA, pH 8, lyophilized and redissolved in 2 M NaCl. This material represents a complex of DNA, RNA, histones and residual protein which respectively constitute roughly 30%, 3%, 30%, and 37% of the total by weight. These components were extracted in sequence: First histones on the basis of their solubility in 0.2 M HCl then RNA which becomes acid soluble when heated 18 h at 37°C in 0.3 N KOH (13) and finally DNA, which becomes acid soluble when heated 30 min at 90° in 1 M $HClO_4$. The acid insoluble material which remains represents residual protein.

The tritium content of these fractions was determined by liquid scintillation. Counts obtained were corrected for background and chemiluminescence by comparison with tissue fractions from mice not exposed to tritium. These counts were converted to dpm by means of the counter efficiency determined for each fraction by subsequent addition of a tritiated toluene standard. The total number of ^{3}H atoms present in each fraction can be calculated by multiplying dpm by the half-life of tritium [12.35 years (14)] in minutes and dividing by the natural logarithm of 2.

The DNA content of whole chromatin can be determined from UV absorption measurements of the DNA fraction since this fraction contains 96% of the total chromosomal DNA and negligible amounts of RNA. The number of diploid nuclei which this amount of DNA represents is calculated from the UV absorption (after correcting for the hyperchromic effects of fractionation) by dividing by the molar extinction coefficient, taken as 6418 (15), and the weight of DNA per diploid nucleus, taken as 6×10^{-12} g (16) and multiplying by the average molecular weight of polymerized mouse deoxyribonucleotides (308.9).

The above procedure allows the determination of tritium exposure during continous ingestion of HTO. The situation after a transient exposure to tritiated water, such as might happen accidentally to a human population, is more complex. Water turnover is rapid in comparison with the turnover of organic components of the nucleus, particularly DNA which is metabolically inert in viable non-dividing cells except for possible repair replication (17). Even relatively small amounts of tritium in these organic components in the nuclei can contribute significantly to the radiation dose after transient exposure to HTO if they persist for long periods of time. This is particularly true for cells with a long lifespan such as primary oocytes (18). In order to evaluate the significance of radiation from tritium remaining in the organic components of the nucleus after transient exposure to tritiated water, the rate of disappearance of tritium from the nuclei of testes, bone marrow, liver and brains of mice was measured after discontinuing exposure to tritiated water. In addition, we have measured the distribution of tritium among various components of nuclei from the liver and brain, searching in particular for nuclear components other than DNA and histone which retain comparable amounts of tritium and are long lived. Animals used for these determinations were obtained from litters born to parents maintained on 3.0 μCi/ml HTO for 4 weeks before mating and were themselves maintained on this until 8 months old. After this time they were given only tap water to drink. At various times after discontinuing exposure to the HTO, tissues were removed and nuclei isolated as previously described (19) except that nuclei from the brain were pelleted through one additional layer of 2.3 M sucrose to remove contaminating cytoplasmic material. Chromatin was isolated and fractionated as before. Tritium activity in DNA concentrations were also measured as before.

7. Deuterium-tritium micromapping

In order to measure the amount of tritium incorporated into primary oocytes by standard counting or autoradiography techniques, it would be necessary to maintain the mother on such high levels of tritiated water that radiation-induced inhibition of oogenesis would take place. To avoid this, deuterium was used as a stable isotope tracer for tritium. Technical trials have been performed to investigate the feasibility of obtaining clean preparations of single oocytes from ageing, unbred, nonsuperovulated mice grown to maternity from the litters of deuterated and nondeuterated mothers. Female mice were maintained on 7½% and 15% deuterated drinking water before and during pregnancy. The yield and appearance of oocytes from one-year-old offspring of these animals were evaluated. The single oocyte preparations were then prepared for triton bombardment and alpha-track analysis as previously described (20).

Results

1. Growth and appearance

Physically the animals on all concentrations of HTO and tap water appeared the same. By visual examination no difference was apparent throughout the observation period of 560 days. The weight curves were also identical, and there was no evidence of lifetime shortening.

2. Breeding efficiency and DLM findings

Measurements have been completed on animals maintained on 3.0 and 1.0 µCi/ml HTO. Determinations are still in progress on animals maintained on the lowest concentration (0.3 µCi/ml). Similarly, results are now complete on animals exposed to ^{137}Cs at a dose rate equivalent to 3.0 µCi/ml of tritium. No gamma exposure comparable to 1.0 µCi/ml group were made, however, determinations are being made on gamma exposed mice equivalent to the 0.3 µCi/ml HTO animals.

A summary of typical data for 3.0 μCi/ml group has been previously published (21). A summary of the computer analysis of parameters measured in DLM testing appears in Table 1. Note that this includes the preliminary findings on animals exposed 0.3 μCi/ml. Lack of evidence for an effect in the 0.3 μCi/ml animals should not be considered as a final result since at this time only a limited number of animals have been examined.

3. Liver cytogenetic studies

Evaluation of animals maintained on 3.0 μCi/ml HTO for 100, 330, and 500-560 days indicated a significant increase in the number of abnormal cells as compared to animals maintained on tap water (Figure 2). Details of this study have been previously published (1). Preliminary results on animals maintained on 0.3 μCi/ml HTO indicate that there is no evidence for a detectable effect in these animals using the system described. However it should be noted that analysis of all animals in this study are not complete and the lack of a detectable effect should be noted as preliminary.

4. Bone marrow toxicity evaluation

There was no effect on total marrow cellularity in any of the animals maintained on HTO, at any of the concentrations. However, reductions in number of bone marrow CFU-S were noted as early as 8 to 12 weeks in the 3.0 μCi/ml mice, and by 24 weeks in the 1.0 μCi/ml animals. In both of these groups the depression continued with some variability throughout the observation period. In the 0.3 μCi/ml animals there was no obvious pattern of response, other than a somewhat greater than normal variability in number of CFU-S per leg (Figure 3).

5. RBE determinations

As shown in Table 1, in no instance was there a significant difference in any DLM parameters between animals maintained on 3.0 μCi/ml of HTO and animals

exposed to the equivalent ^{137}Cs gamma dose (22 hours/day exposure). As shown in Figure 4 the hematopoietic stem cell response was also equivalent for the two types of exposure. Similar comparisons between animals maintained on 0.3 μCi/ml HTO and receiving an equivalent Cesium gamma exposure are now underway to see if at the lower dose and dose rate a difference in response is evident.

6. Tritium incorporation and turnover studies

When animals were placed on the 3.0 μCi/ml HTO regimen ^{3}H concentrations in body water and soft tissues rapidly approached equilibrium levels (Figure 5). When removed from the HTO regimen, the ^{3}H level in tissue water dropped rapidly from 2.02 μCi/ml before withdrawal to 0.07, 0.01 and 0.001 μCi/ml 7, 14 and 28 days later. The rate at which nonexchangeable tritium disappears from brain and liver histones indicates a half-life of 117 days for liver histone and 159 days for brain histone with 95% confidence intervals of 85 to 188 days for liver and 129 to 208 days for brain (Figure 6).

Tritium activity in liver and brain DNA is plotted in Figure 7. The brain data points fit a straight line with a slope indicating a half-life of 593 days with 95% confidence limits of 376 to 1406 days. The curve for the liver shows a pronounced curvature indicating the presence in the liver of two cell populations with distinctly different turnover times. The least squares fit drawn indicates the two liver cell populations with half-lives of 12 and 318 days representing 23 and 77% of the total DNA respectively. The 95% confidence interval for the half-life of the cells with the longer life span is 241 to 466 days. The initial specific activity in liver DNA was 0.90 dpm per microgram, which is in excellent agreement with the value of 0.89 expected on the basis of previous studies (22). The initial specific activity in brain DNA was 0.99 dpm/microgram, somewhat higher than expected.

7. Micromapping determinations

Clean single cell oocyte preparations were obtained and exposed to triton beams in the manner previously described (18). Good track detection has been obtained; however, it is felt that better preparations might be obtained with higher detection efficiency using slight modifications of the existing techniques. Final determinations of the atom amounts of deuterium present in oocytes in progress.

Summary

Some somatic and genetic effects of the continuous ingestion of HTO at concentrations of 0.3, 1.0 and 3.0 μCi/ml have been investigated in mice. At these levels there is no measurable effect on growth, general appearance or nonspecific lifetime shortening. Genetic effects as measured by the DLM assay indicate a significant effect ($P > 0.01$) on the number of viable embryos and early deaths depending upon the treatment of either or both of the mating partners in the 3.0 μCi/ml HTO group. Similarly, a significant effect on the number of viable embryos was noted when both breeding partners were maintained on 1.0 μCi/ml of HTO. Preliminary results on animals maintained on 0.3 μCi/ml HTO indicate no significant effects on any of the parameters measured.

Cytogenetic evaluation of the regenerating liver in animals maintained on 310 μCi/ml of HTO for extended periods of time indicated a significant increase in the number of abnormal cells. Preliminary results indicate no similar effects in animals maintained on 0.3 μCi/ml HTO.

Mice maintained on both 3.0 and 1.0 μCi/ml HTO exhibited a reduction in bone marrow stem cells without an attendant reduction in total marrow cellularity. These results are of interest since they demonstrate both the presence of an effect as well as the animals' ability to compensate for this effect

by recruiting stem cells from the G_o resting state. The evidence for damage leads one to contemplate the possible importance of radiation exposures at these levels in the induction of leukemia or other blood dyscrasias. However, at present there is no evidence for such effects. Studies aimed at such determinations are in progress.

Results of determinations made at 0.3 μCi/ml HTO indicate that the spleen colony forming system is insufficiently sensitive to measure an effect if one does exist.

Using the DLM assay and the CFU-S assay, it is impossible to detect a significant difference in effectiveness when comparing animals maintained on HTO and those receiving a chronic ^{137}Cs gamma exposure at an equivalent dose and dose rate.

Comparison of the tritium content of nuclei with the tritium content of DNA and histone at various times after discontinuing exposure to tritiated water indicates that after two months essentially all remaining tritium is in these two components. Because of the short range of tritium radiation, nearly all of the significant biological damage is produced by tritium atoms located within the nucleus. Consideration of the relative amounts and biological half lives of tritium present in the nucleus as water, DNA as histone suggests that after transient exposure to tritiated water, nearly all significant radiation damage can be attributed to tritium present in the nucleus as water. The exceptions are cells with a life span of the order of years which happen to be proliferating at the time of exposure. Such cells are rare in adults but much more common in juveniles and embryos. In these cells tritium in DNA, and, to a lesser extent, tritium in histone will also contribute to radiation damage.

Techniques involving the use of deuterium as a tritium tracer together with track detection methodology looks promising as a method for measurement of atom amounts of tritium present in nuclei where standard counting or autoradiographic methods are not applicable.

The detection of measurable effects at the levels tested in these studies should not be interpreted as being representative or indicative of hazards to which the population might be exposed in a nuclear energy based economy. The tritium levels investigated are many times greater than the highest estimates predicted for tritium release from either fission or fusion reactors or nuclear fuel reprocessing plants. It appears that the effects noted could be predicted on the basis of standard beta radiation-dose effect curves, with RBE values assigned dependent upon the reference radiation used. (This may range from values of 1 for low energy x-rays to as high as 3 when cobalt gamma rays are used as the reference source.) A source of confusion in the assignment of a correct RBE has been in the use of improper reference radiations. The ICRP value of 1.0 (23) is appropriate when the proper reference radiation is used. Special attention should still be focused on problems relating to the passage of tritium through the biosphere and its differential degree of incorporation into subcellular components with varying half-lives. The importance of radiation exposure to cells and/or subcellular components of interest, most specifically DNA, should be noted on the basis of a single, short-term or chronic tritium exposure. The hazards from tritium attendant with normal reactor operation should not at this time be considered as a deterrent to the further development of fission and/or fusion reactor technology.

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TABLES

1. The First Three Columns Show the Statistical Comparison for Various Dominant Lethal Mutation Parameters for Animals Maintained on 3.0, 1.0 and 0.3 µCi/ml of HTO and Their Matched Controls. Column Four Compares the Dominant Lethal Mutation Parameters for Animals Maintained on 3.0 µCi/ml HTO to Animals Receiving an Equivalent External Cesium-137 Gamma Exposure.

FIGURES

TABLE I

COMPARISON OF DOMINANT LETHAL PARAMETERS (STATISTICAL EVALUATION OF GENETIC RESULTS)

PARAMETER		3.0μ Ci/ml vs CONTROLS		1.0m Ci/ml vs CONTROLS		0.3μ Ci/ml vs CONTROLS		3.0μ Ci/ml HTO vs ^{137}CESIUM	
		(p)	pCIII	(p)	pCIII	(p)	pCHI	(p)	pCHI
VIA	1/C	.0000**	.0000**	.0014**	.0040**	.4932	.5000	.0696	.1773
	2/C	.0019**	.0030**	.0894	.0491	.4143	.5000	.7641	.5000
	3/C	.0013**	.0129	.3810	.5000	.2260	.5000	.6536	.5000
ED	1/C	.0062**	.0059**	.4340	.5000	.9132	.5000	.3896	.5000
	2/C	.2489	.1117	.2795	.5000	.8242	.5000	.2703	.5000
	3/C	.1579	.0798	.6621	.5000	.7644	.5000	.5156	.5000
LD	1/C	.4649	.5000	.2107	.5000	.9370	.5000	.9484	.5000
	2/C	.9934	.5000	.2698	.5000	.8587	.5000	.5000	.3349
	3/C	.3251	.5000	.1490	.5000	.1310	.2029	.4858	.5000
PRE	1/C	.0363	.0170	.2281	.5000	.5605	.5000	.0107	.1245
	2/C	.0436	.0200	.2528	.5000	.5718	.5000	.9762	.5000
	3/C	.1967	.0503	.9408	.5000	.5841	.5000	.3381	.1529
# BRED		3454		3232		2647		1902	

** $p < .01$

TREATMENT:

GROUP 1 MALE + FEMALE	HTO
GROUP 2 FEMALE	HTO
GROUP 3 MALE	HTO
GROUP C	CONTROL

BROOKHAVEN TRITIUM TOXICITY PROGRAM

I. GENETIC AND REPRODUCTIVE EFFICIENCY
- A. DOMINANT LETHAL MUTATION RATE
- B. CYTOGENETIC STUDIES

II. SOMATIC EFFECTS
- A. GROWTH (BODY WEIGHT)
- B. NONSPECIFIC LIFETIME SHORTENING
- C. BONE MARROW CELLULARITY AND CFU-S CONTENT

III. RELATIVE BIOLOGICAL EFFECTIVENESS (RBE)
- A. COMPARISON OF HTO AND ^{137}Cs EFFECTS

IV. BIOCHEMISTRY AND MICRODOSIMETRY STUDIES
- A. RATE OF TRITIUM INCORPORATION
- B. SITE OF TRITIUM INCORPORATION
- C. RATE OF TRITIUM DISAPPEARANCE

FIGURE 1

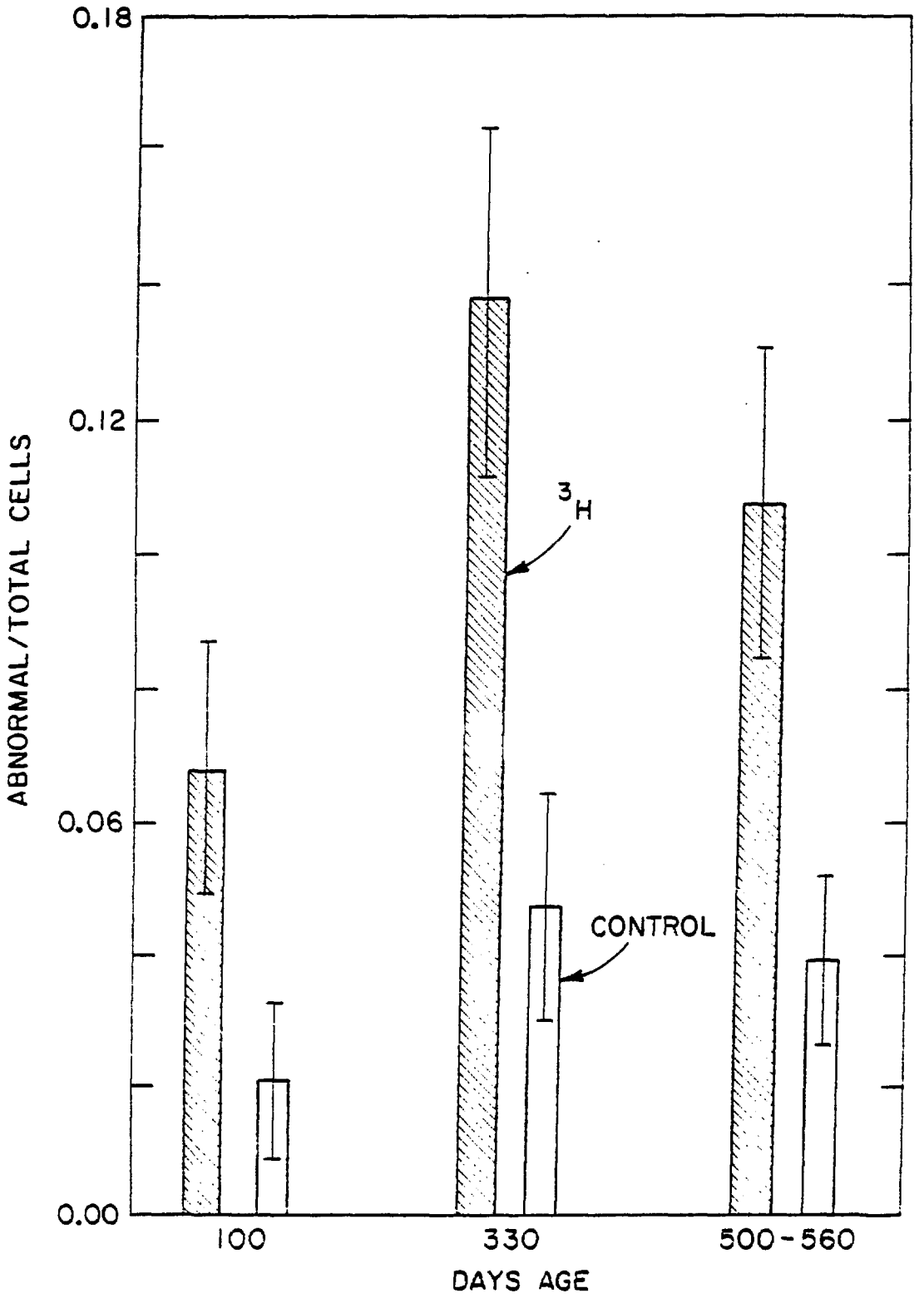
0.18
0.12
0.06
0.00
ABNORMAL/TOTAL CELLS
^{3}H
CONTROL
100
330
500-560
DAYS AGE

FIGURE 2

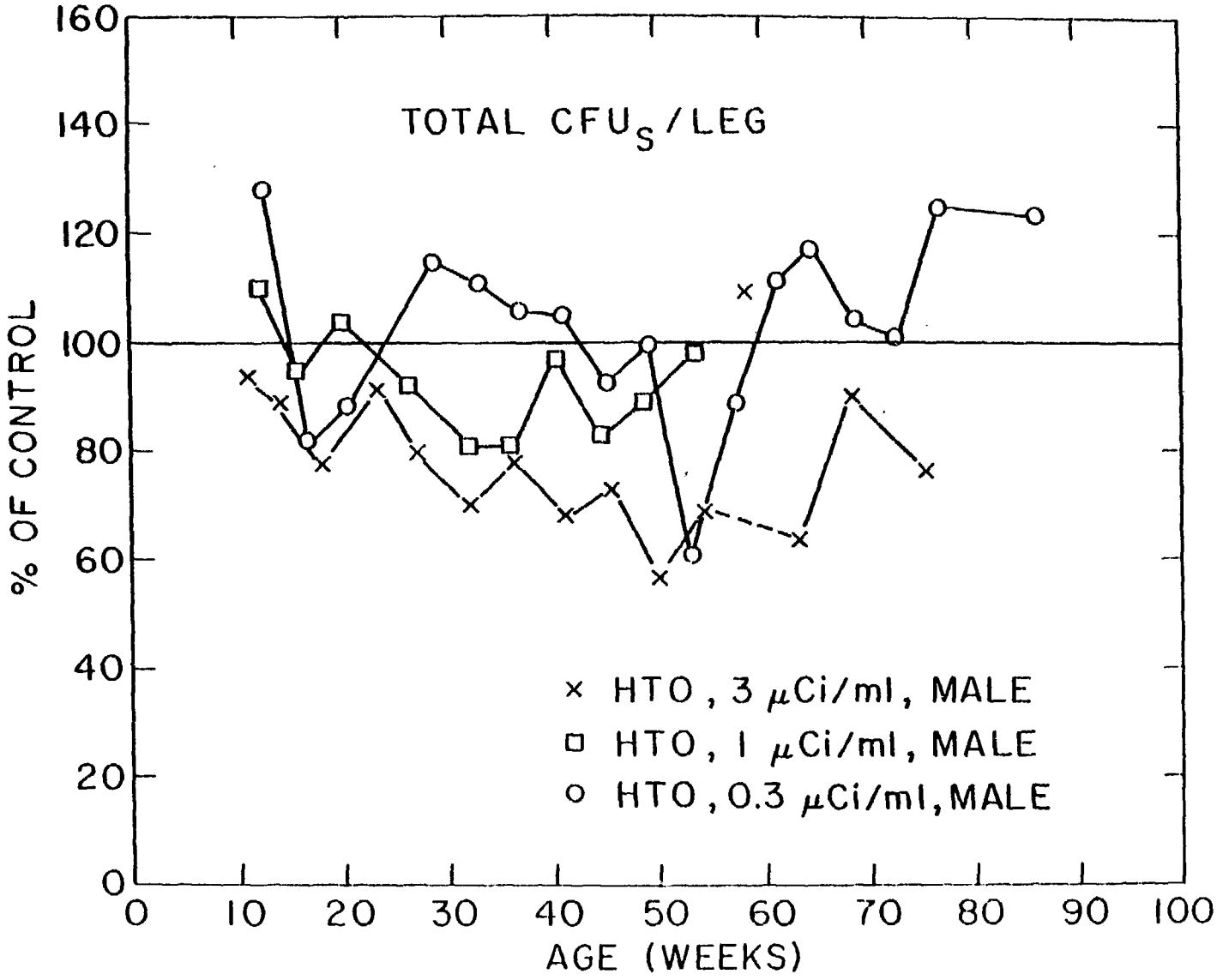
TOTAL CFU$_S$ / LEG
% OF CONTROL
AGE (WEEKS)
× HTO, 3 μCi/ml, MALE
□ HTO, 1 μCi/ml, MALE
○ HTO, 0.3 μCi/ml, MALE
0
20
40
60
80
100
120
140
160
0
10
20
30
40
50
60
70
80
90
100

FIGURE 3

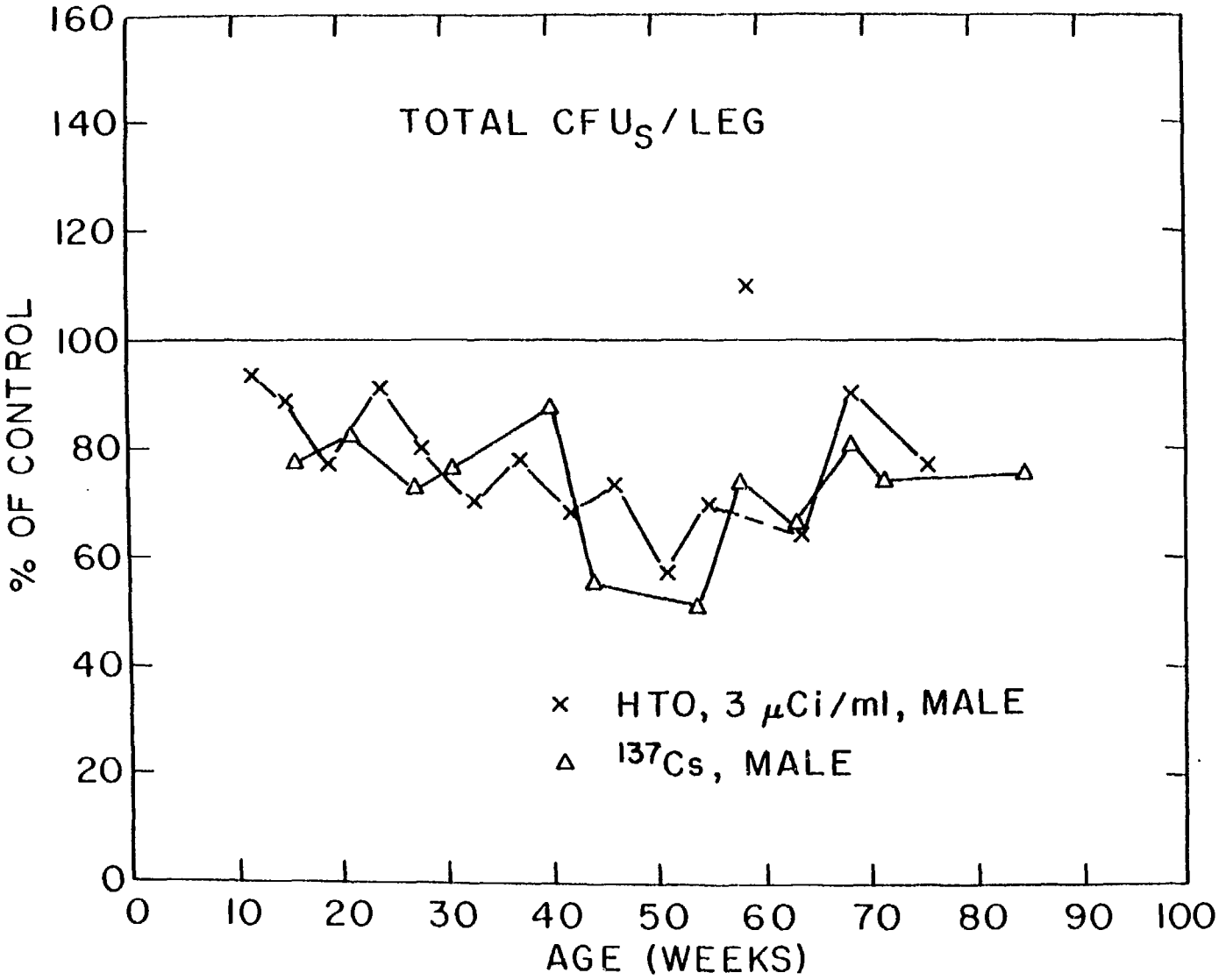
TOTAL CFUs / LEG
% OF CONTROL
AGE (WEEKS)
× HTO, 3 μCi/ml, MALE
△ 137Cs, MALE
0
20
40
60
80
100
120
140
160
0
10
20
30
40
50
60
70
80
90
100

FIGURE 4

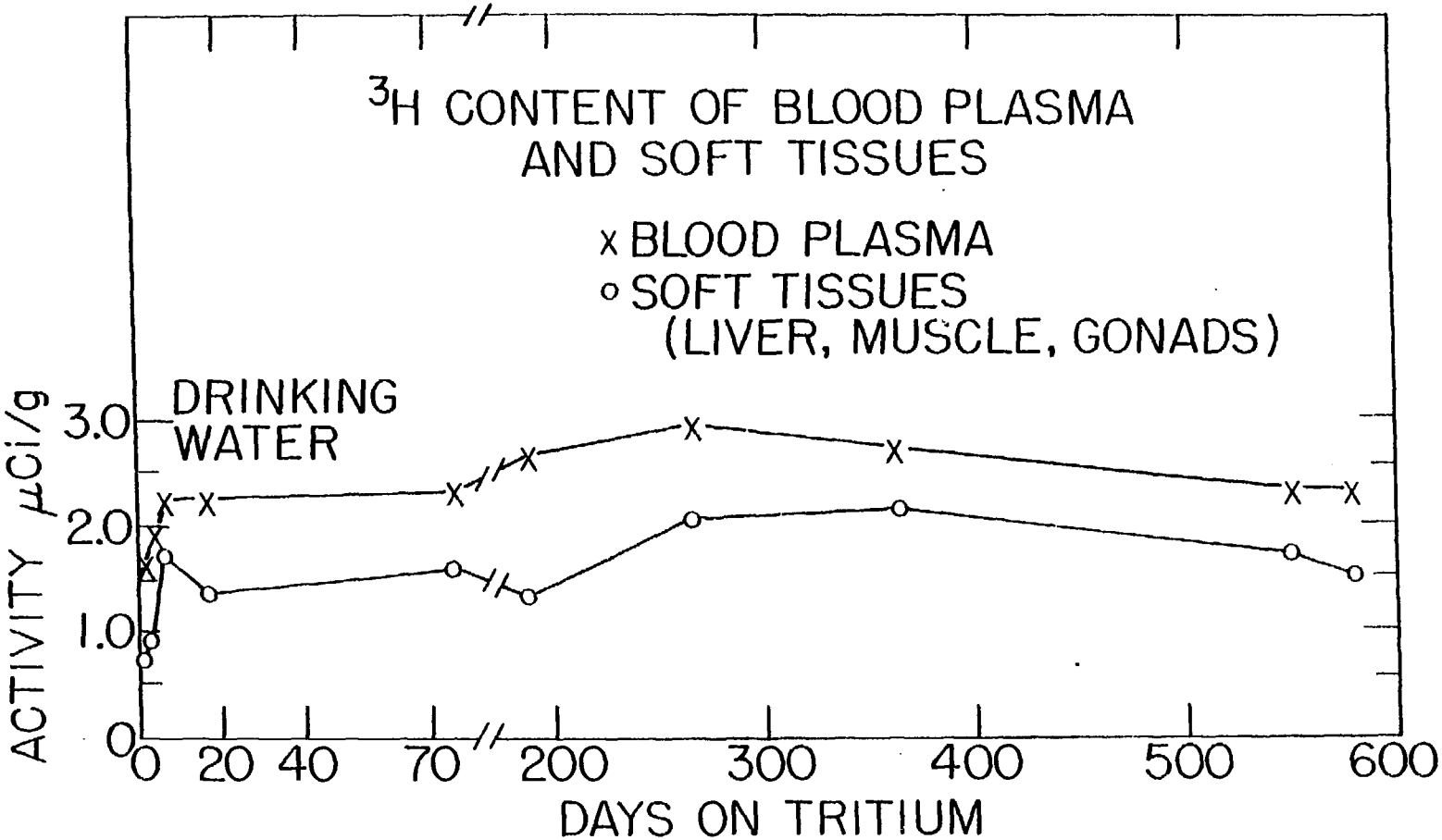
^{3}H CONTENT OF BLOOD PLASMA
AND SOFT TISSUES
x BLOOD PLASMA
o SOFT TISSUES
(LIVER, MUSCLE, GONADS)
DRINKING
WATER
ACTIVITY μCi/g
3.0
2.0
1.0
0
0 20 40 70 200 300 400 500 600
DAYS ON TRITIUM

FIGURE 5

TRITIUM ACTIVITY IN HISTONES FROM THE BRAINS AND LIVERS OF MICE AFTER REMOVAL OF TRITIUM FROM THEIR DRINKING WATER

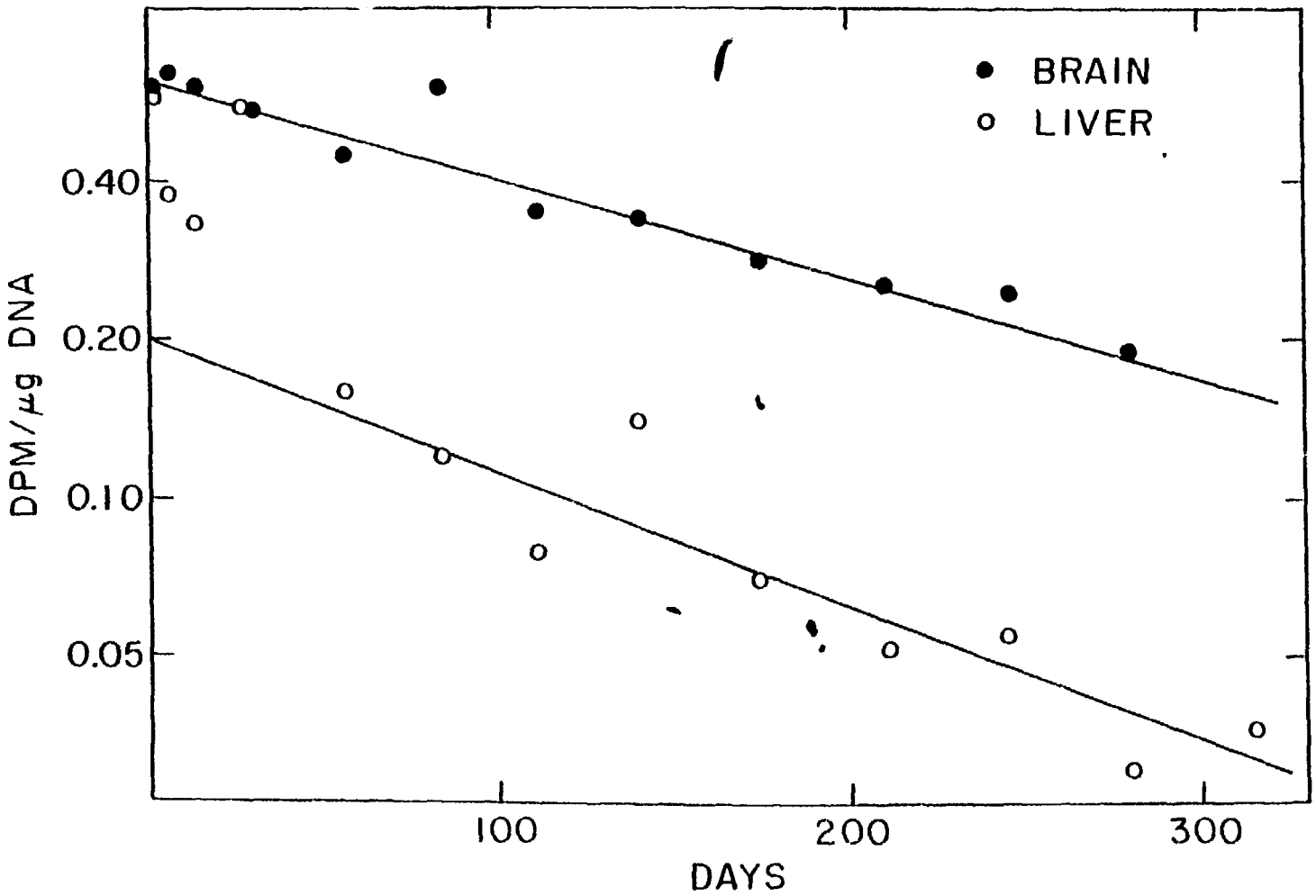

FIGURE 6

TRITIUM ACTIVITY IN THE LIVER AND BRAIN DNA OF MICE AFTER REMOVAL OF TRITIUM FROM THEIR DRINKING WATER

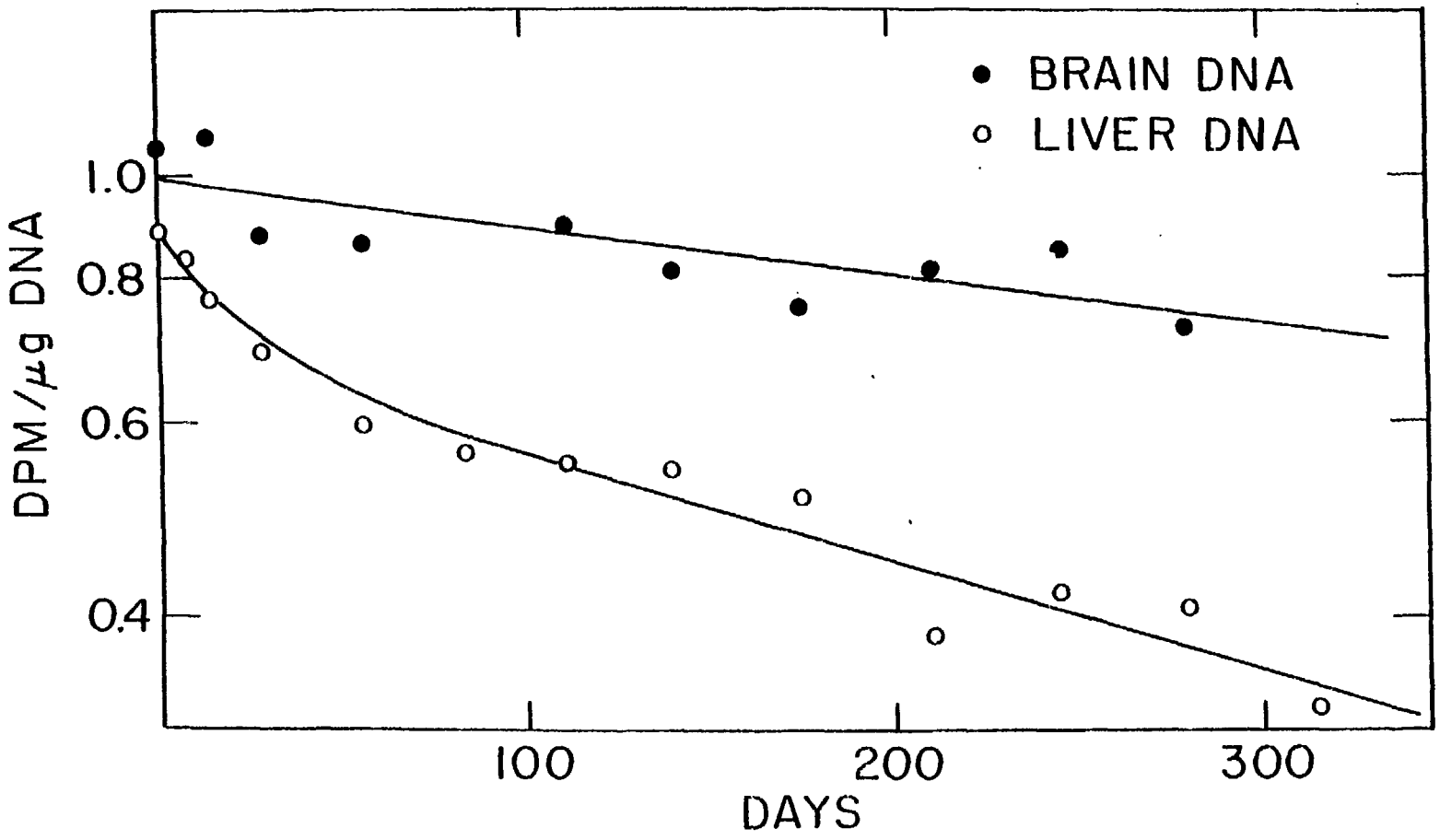

FIGURE 7