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THE GENETIC AND HEMATOPOIETIC EFFECTS OF LONG-TERM TRITIATED WATER (HTO) INGESTION IN MICE*

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With the increased use of nuclear reactors for power generation, it becomes increasingly important to evaluate the possible health hazards associated with their operation. Of prime interest is the possible effect of introducing large amounts of tritiated water (HTO) from reactors into the environment. To examine this problem, randomly inbred mice of the Hale-Stoner-Brookhaven strain have been maintained on HTO (3 μCi/ml) for extended periods. First generation animals on HTO from weaning (four weeks of age) have been evaluated for changes in growth pattern. Second generation animals on HTO have been evaluated for breeding efficiency, dominant lethal mutation rate and bone marrow integrity. A total of 18,831 embryos were examined. Statistical analysis of these results using either Student's "t" test or

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Kruskal and Wallis rank test indicates that there was a significant ($p < .01$) reduction in viable embryos and an increase in early deaths in matings involving animals drinking HTO. Beginning at 8 weeks of age and monthly thereafter, the hematopoietic stem cell content of the bone marrow was determined using the exogenous spleen colony technique. Results indicate that although the total cellularity of the bone marrow remains comparable in the control and treated groups, the total number of stem cells (CFU) was reduced beginning after approximately 12-20 weeks on the tritium regimen. This general reduction in CFU content continued with some fluctuation throughout the lifetime of the animal. These findings indicate a reduction in the total number of pluripotent stem cells in the marrow together with the ability of this reduced number of cells to maintain normal levels of total cellularity in the bone marrow.

The results of these studies indicate that continuous ingestion of HTO at a concentration of 3 μCi/ml by mice results in: (1) A reduction in the number of viable embryos present in the female at late pregnancy from matings when either the female or both parents have been on HTO. (2) An increase in the number of early post implantation deaths when both parents are on HTO. (3) Reduction in bone marrow stem cell content after 12 weeks or longer on HTO. (4) No apparent effect on breeding efficiency (% females pregnant) or body weight. These results will be discussed in relationship to the accumulated radiation dose.

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The present and projected needs for additional electrical power in established and developing nations makes it apparent that the world will by necessity become more dependent upon nuclear power reactors.

With the acceptance of nuclear power as a fact of life comes concern over the possible long-term deleterious effects arising from the radioactive byproducts generated in these reactors. Of concern is the possible effects from tritium in the form of tritiated water (HTO), a major byproduct of both fission and fusion reactions. A program has been instituted in this laboratory to investigate the possible genetic (dominant lethal mutations) and somatic (hematopoietic stem cell alterations) effects in mice maintained on HTO.

Materials and Methods:

All animals were randomly inbred albino mice of the Hale-Stoner-Brookhaven strain. The HTO test animals were first litter animals resulting from breedings of 8-week-old animals that had been maintained on HTO (3 μCi/ml) since weaning at 4 weeks of age. The control animals were first litter animals taken from the mouse colony and maintained on tap water. From the second generation animals 4 experimental groups were established for dominant lethal testing (1). Group 1 consisted of animals where both the male and female were on HTO. Group 2 consisted of females on HTO and males on tap water. Group 3 consisted of males on HTO and females on tap water. Group 4 consisted of males and females on tap water (controls). When these animals reached 8 weeks of age, breeding was established by placing one male with 5 females for a 5-day period. Fifteen days after the midpoint of this breeding period,

the females were killed and the ovaries and uterine contents examined. The corpora lutea (CL) on each ovary were counted and the uterine contents classified as to viable embryos (VIA), early embryonic deaths (ED) and late embryonic deaths (LD). The early and late deaths refer to death of the embryo after implantation. ED's occurring between approximately 4 and 10 days of pregnancy were evidenced by a small, black body sometimes referred to as a "mole". The LD's occurring between approximately the 10th day and sacrifice were evidenced by a formed but dead embryo. The data from each female was placed on a computer card for subsequent analysis. New breeding groups were started each week so a continuing program of data accumulation took place in all 4 experimental groups.

The somatic effects evaluation was done by examining the size and competence of the hematopoietic stem cell pool from second generation animals maintained on either the HTO or tap water for 80 weeks. At approximately 4-week intervals, animals were sacrificed, and the bone marrow removed from the hind legs (femora and tibia) using the quantitative technique of Stoner and Bone (2). Determinations were made on at least 4 animals at each point and the average total nucleated cell content determined. The number of stem cells/leg was then determined using the spleen colony technique of Till and McCulloch (3). This method involves injecting a known number of bone marrow cells into a recipient mouse which has received a single whole-body 250 kVp x-ray exposure of 750 rads within 24 hrs previous to the bone marrow injection. After 7 days the recipient animals were killed, their spleens removed, fixed in Bouin's solution and the number of surface colonies determined. The colony number is directly related to the number of pluripotent stem cells in the injected sample. In addition to the genetic and hematopoietic determinations, weight records were kept on previously selected groups of mice to determine any effect on body growth.

Dosimetry:

At selected times after being placed on the tritium regimen, animals were sacrificed and the amount of tritium in the blood plasma and various soft tissues determined by scintillation counting. Calculations of accumulated dose were based on these determinations.

Results:

Genetic Effects: A total of more than 3450 animals were bred in the four treatment groups. There was no significant difference in the per cent pregnant females in any of the four groups. The mean values for the number of viable, early deaths, late deaths, and preimplantation deaths are given in Table I.

The significance of the differences between various experimental groups was then tested using three statistical tests. These were: 1) Students' "t" test (4), a parametric test which assumes normal distribution of the data. In our analysis this test makes use of the pooled data of all groups. 2) The rank test of Kruskal and Wallis (5), a nonparametric test for a complete random design with any number of populations. The final analysis in this test was made using a Chi^2 test. The third test is the arcsine transformation of Salzberg (6). This test normalizes the data and computes the mutation index for each treatment which may then be compared using a Chi^2 test.

The results of all three analyses were consistent, indicating a significant difference in the number of viable embryos between the control group when compared with either group 1 (male and female on HTO), or group 2 (female only on HTO). Similarly, a significant difference was seen in the number of early deaths when comparing the control group with group 1.

Stem Cell Determinations: No significant difference in total cellularity of the leg bone marrow was noted between the HTO and control animals. However, when the relative number of pluripotent stem cells (CFU) and the total number of CFU/leg were determined ; marked differences were noted. The first

depression in total CFU's/leg was evident as early as 12 weeks of age in the second generation animals on tritium. With some variation this depression continued throughout the remainder of the 80-week observation period.

Dosimetry: After the initial increase in tritium concentration in blood plasma and soft tissue, a relative equilibrium was maintained throughout an observation period of 570 days (Figure 1). Dosimetry calculations were made on the basis of the average tritium content over the period from 17-265 days. These values in μCi/gm were: blood plasma = 2.36, soft tissues (liver, spleen and gonads) = 1.61. If these values are converted to rads/day absorbed dose, average soft tissues = 0.47 rads/day. The activity in other tissues varied directly with the water content of the tissue. In ovaries the rad dose/day was 0.36 and in testes 0.48.

Using the blood plasma value for calculating the dose to the bone marrow, the accumulated dose for the first 25 weeks (onset of significant reduction in CFU content) was approximately 120 rads. If a similar calculation is made for the second generation animals' ovaries and the resulting embryos, the value is 31.3 rads. This calculation assumes; the first appearance of the ovaries at the 8th day of gestation in the first generation animal, 56 days of aging after the birth of the second generation female, a 5-day breeding period and 15 days until sacrifice. This is a most conservative estimate (overestimation of dose) since the radiation dose delivered during the final days in the pregnancy of the second generation animals could not contribute to the early death findings. Measurements of body weight indicated no difference between HTO and control animals.

Discussion:

The possible genetic and somatic effects of chronic ingestion of HTO (3 μCi/ml) has been investigated in mice. A significant reduction in the number of viable embryos resulting from matings of animals with both partners

on tritium and in matings where only the female was on tritium have been observed. Similarly, an increase in the number of early deaths was noted in matings where both partners were on tritium. Since the reduction in viable embryos is due to a number of factors (early death, late death and pre-implantation loss), it is not surprising that an effect on the number of viables would be seen in both groups 1 and 2; whereas, for the early deaths, an effect was seen only when both mating partners were on tritium. Continuing replacement of mature sperm in the male limits the total accumulated exposure to these cells whereas in the female, the dose accumulation persisted in the second generation animals beginning in utero and continuing throughout pregnancy.

The positive effects seen using the somewhat insensitive dominant lethal test system and the effects seen on the blood-forming cells indicates that at least in the mouse there is a hazard in the continuous ingestion of HTO at a concentration of 3 μCi/ml. A direct comparison of these results to the human drinking an equivalent amount of HTO is impossible due to the obvious differences in water metabolism between the two species. Until further experimentation at lower levels of ingestion are completed, it is difficult to comment concerning the significance of these results as related to current concepts of maximum permissible concentration. Studies are now underway examining the possible effects of lower concentrations of chronic HTO ingestion.

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TABLE I

Dominant Lethal Analysis Parameters.

FIGURE 1.

Tritium incorporation into blood plasma and soft tissues.

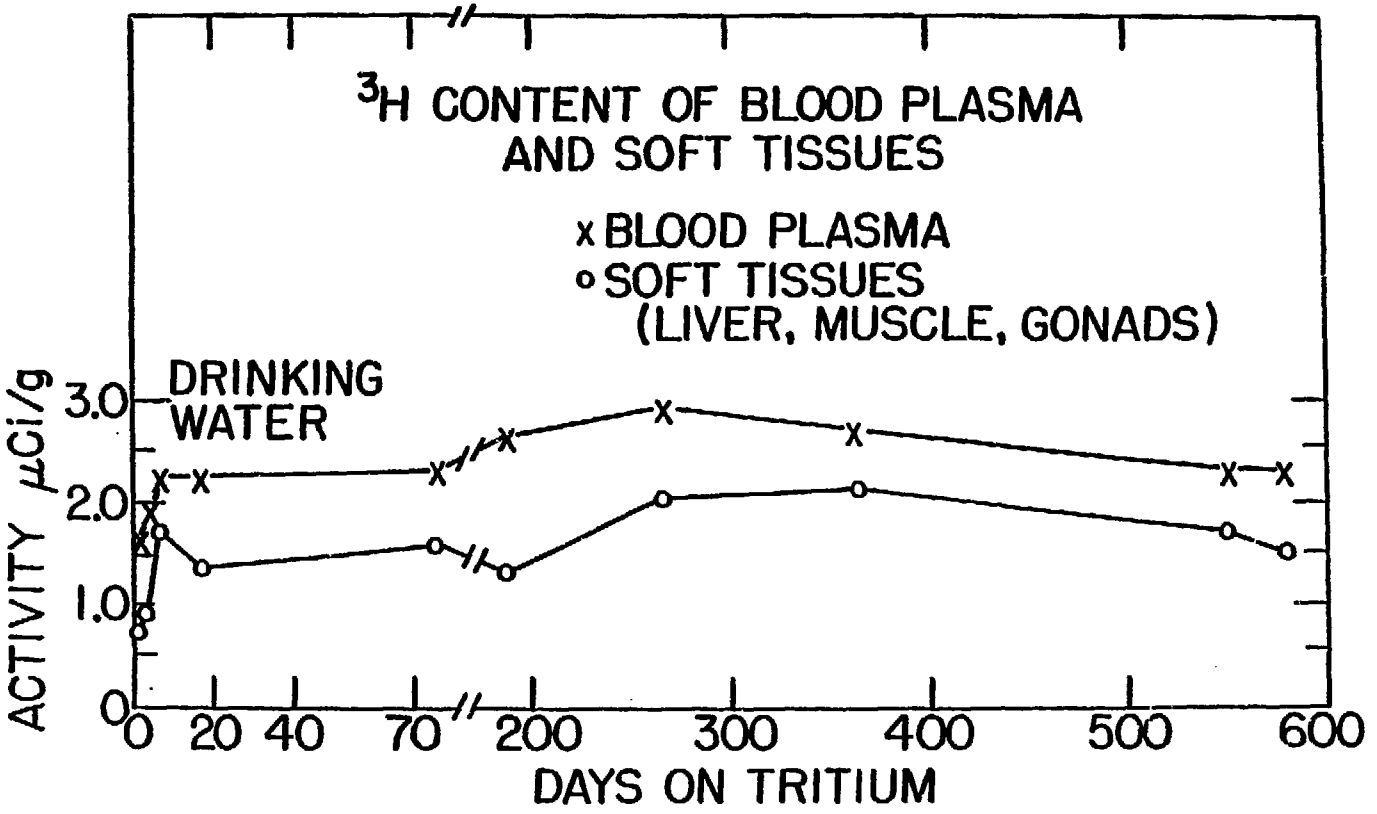
^{3}H CONTENT OF BLOOD PLASMA
AND SOFT TISSUES
x BLOOD PLASMA
o SOFT TISSUES
(LIVER, MUSCLE, GONADS)
DRINKING
WATER
ACTIVITY μCi/g
3.0
2.0
1.0
0
0 20 40 70 200 300 400 500 600
DAYS ON TRITIUM

TABLE I

DOMINANT LETHAL ANALYSIS PARAMETERS

	Group 1		Group 2		Group 3		Group 4	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
C1	10.564	0.052	10.666	0.084	10.718	0.079	10.967	0.071
Via	8.146	0.075	8.257	0.121	8.402	0.118	8.986	0.105
ED	0.623	0.033	0.546	0.046	0.563	0.044	0.470	0.036
LD	0.050	0.008	0.038	0.012	0.057	0.014	0.038	0.011
Pre	1.748	0.068	1.825	0.108	1.696	0.108	1.473	0.096

Group 1 = Male and Female HTO

Group 2 = Female only on HTO

Group 3 = Male only on HTO

Group 4 (control) = Male and female on tap water

R E F E R E N C E S

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