UCRL-88376
PREPRINT
Conf-821167--1

# TRITIUM EFFECTS ON GERM CELLS AND FERTILITY

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This paper was prepared for submittal to
Proceedings of
European Seminar on the Risks from Tritium Exposure
Mole, Belgium
November 22-24, 1982

November 19, 1982

Lawrence Livermore National Laboratory

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# TRITIUM EFFECTS ON GERM CELLS AND FERTILITY

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UCRL--88376

DE83 007349

ABSTRACT

Extreme radiosensitivity is manifest by certain mammalian germ cells. Primordial oocytes in juvenile mice, for example, even more sensitive than spermatogonia, show acute gamma-ray $LD_{50}$ as low as 6 rad. This provides opportunities for determining dose-response relations at low doses and chronic exposure in the intact animal -- conditions of particular interest for hazard evaluation. Examined in this way, $^{3}HOH$ in body water is found to kill murine oocytes exponentially with dose, the $LD_{50}$ level for chronic exposure being only 2 μCi/ml (delivering 0.4 rad/day). At very low doses and dose rates, where comparisons between tritium and other radiations are of special significance for radiological protection, the RBE of tritium compared with $^{60}Co$ gamma radiation reaches approximately 3. Effects on murine fertility from tritium-induced oocyte loss have been quantified by reproductive capacity measurements.

Chronic low-level exposure has been examined also in three primate species -- squirrel, rhesus, and bonnet monkeys. In squirrel monkeys the ovarian germ-cell supply is 99% destroyed by the time of birth from prenatal exposure to body-water levels of $^{3}HOH$ (administered in maternal drinking water) of only 3 μCi/ml, the $LD_{50}$ level being 0.5 μCi/ml (giving 0.1 rad/day), one fourth that in mice. Though not completely ruled out, similar high sensitivity of female germ cells has not been found in macaques; and it probably does not occur in man.

The exquisite radiosensitivity of primordial oocytes in mice is apparently due to vulnerability of the plasma membrane (or something of similar geometry and location), not DNA. Evidence for this comes from tritium data as well as neutron studies. Tritium administered as $^{3}HOH$, and therefore generally distributed, is much more effective in killing murine oocytes than is tritium administered as $^{3}H$-TdR, localized in the nucleus. This situation in the mouse may have implications for estimating radiation genetic risk in the human female.

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## INTRODUCTION

The unusually great radiosensitivity of germ cells in certain mammals provides rare opportunities for obtaining quantitative dose-response data in the critically important low-exposure range (e.g., see ref. 12). Here, we consider the killing of germ cells by tritium. Focus is on low-level exposure, because of its importance for hazard evaluation, and on effects in mammals, including monkeys, because of their relevance to questions of human health. Primary attention is on $^3H$ as water ($^3HOH$), because this is the tritium exposure of most practical concern to radiation protection.

Germ-cell loss induced by tritium (or by other radiations) can affect fertility; and measurement of this is also considered here. Moreover, murine oocyte killing by radiation is an example of induced interphase death, a phenomenon that occurs in other cell populations as well (20,23), where it is more difficult to quantify; oocyte destruction is readily measured. Considered also is a finding of special interest: that radiation-induced oocyte killing in the mouse apparently involves the plasma membrane, not the nucleus, as the radiosensitive target (24,18); and this may have implications for the estimation of genetic risks in women.

## TRITIUM-INDUCED GERM-CELL KILLING

### Effects in Mice

Germ-cell killing by tritium has been studied in mice in both males (21) and females (e.g., see ref. 12). In Fig. 1, dose-response curves for spermatogonia in the male and for oocytes in the female are compared. Following single i.p. injections of $^3HOH$, oocytes are more readily destroyed ($LD_{50}$ about 6 rad) than are spermatogonia ($LD_{50}$ about 33 rad). This reflects the generally higher radiosensitivity of murine oocytes, which is also seen in Fig. 1 by comparing the dose-response curves for acute gamma irradiation (where dose rates were more than 100-fold higher than for the $^3HOH$ exposures). Here again, the $LD_{50}$ for oocytes is about 6 rad; that for spermatogonia is approximately 7-fold higher. From comparisons between spermatogonial killing by injected $^3HOH$ and by 200-kVp x irradiation given at the same exponentially decreasing dose rate (also shown in Fig. 1), Lambert found an RBE value of approximately 2 (21).

Of particular interest is chronic exposure. This, however, is more difficult to study in the male than in the female. The germ-cell population in males is a cell renewal system, allowing insufficient time for accumulating significant dose in the

sensitive intermediate and type-B spermatogonia at low dose rates, whereas the oocyte population is set by the time of birth, lost cells being irreplaceable, so that chronic exposure can be examined experimentally.

Our most extensive studies have been on mice (10-12,15), with animals chronically exposed to low levels of $^3$HOH in body water during early life, and with oocyte loss being quantified by microscopic counting in serial ovarian sections. Dose-dependent germ-cell destruction was found at all tritium levels examined in a more than 100-fold range of concentrations from 12 μCi/ml down to 0.085 μCi/ml. Results from several investigations (10-12) are shown in the left-hand panel of Fig. 2, where it is seen that oocyte survival decreases exponentially with exposure level, that there is no evidence of a threshold, and that the $LD_{50}$ level is only 2 μCi/ml which, under these chronic exposure conditions, gives about 0.44 rad/day.

This remarkable effectiveness of tritium, with measureable cell destruction at exposure levels below 0.1 μCi/ml (10), in a range comparable with ICRP's maximum permissible concentrations for occupational exposure, raised the question of what might happen in man.

## Effects in Primates

Studies were therefore done in primates. In squirrel monkeys (Saimirai sciureus) exposed continuously (via maternal drinking water) from conception to birth, ovaries in the newborn showed massive germ-cell loss (17). Results from oocyte enumerations are shown in the right-hand panel of Fig. 2, in comparison with the results from mice. Response in this primate was even more dramatic than in the mouse (murine oocytes were previously considered perhaps the most radiosensitive of mammalian cells). The primate germ cells showed an $LD_{50}$ level of 0.5 μCi/ml (giving 0.1 rad/day), one fourth that found in mice. (The dashed curve in Fig. 2 is discussed in ref. 17). The data shown in Fig. 2 demonstrate that the female germ-cell supply in a squirrel monkey would be devastated (99.9% destroyed) before birth by only 1 rad/day from $^3$HOH.

These startling results prompted studies in phylogenetically more advanced primates. We have examined two species of macaque, rhesus (Macaca mulatta) and bonnet monkey (Macaca radiata), using continuous $^3$HOH exposure (via maternal drinking water) during the last two trimesters of gestation. While results are still under analysis, it is now clear that the striking effects seen at birth in squirrel monkeys are not found in the macaques (16). Aspects of this remain under study, but

results so far, as well as data from other radiation studies in rhesus monkeys (5,2,6), tend to match clinical observations in man (e.g., see refs. 7,4) and strongly suggest that the extreme radiosensitivity of female germ cells found in squirrel monkeys (and mice) probably does not occur in humans.

Tritium and Gamma Radiation Compared

It was important to compare $^{3}$HOH exposure with a more "standard" radiation. Mice were therefore chronically exposed to $^{60}$Co gamma rays in a manner rigorously comparable with the $^{3}$HOH exposure. Oocyte survival results (11,12) are shown for both gamma rays and $^{3}$HOH in the upper portion of Fig. 3, and the RBE obtained from these results is shown, as a function of dose (and dose rate), in the lower portion of Fig. 3. At very low doses (and dose rates) the RBE reaches a value of about 3. Its increase with decreasing dose is clearly not due to any increased effectiveness of tritium, but to decreased effectiveness of gamma rays at low doses. Relationships of these findings to radiation protection recommendations have been discussed elsewhere (13).

EFFECTS ON FERTILITY

Depression of fertility by $^{3}$HOH exposure, and its relation to germ-cell loss, has been studied by reproductive capacity measurements and germ-cell enumeration in female mice (Dobson et al., unpublished). Following 33-day chronic $^{3}$HOH exposure (via the mother) during pre- and postnatal development up to 14 days of age, female Swiss-Webster mice were kept continuously with males (paired with proven breeder males that were regularly rotated among the cages) from 28 days of age until they ceased to litter. Wire grid cage floors assured that newborn offspring fell promptly out of reach of the adults. The young were counted and discarded. Thus, for female animals chronically exposed to $^{3}$HOH early in life, data were obtained for total numbers of litters and young produced. In identically exposed females at 14 days of age, oocytes were enumerated in serial sections of ovaries to provide data on oocyte survival as a function of $^{3}$HOH exposure.

Combined results show that reproductive capacity is less affected than oocyte number, as has been reported for other radiation exposure (22); the germ-cell-deficient mouse uses her remaining oocytes with increased efficiency (as measured by the ratio of offspring to available germ cells). Also, the distribution in time of litters and offspring becomes skewed to the left. The germ-cell-deficient

female shows peak litter production at a younger age, and she prematurely runs out of oocytes.

THE RADIOSENSITIVE TARGET IN MOUSE OOCYTES

The unusually great sensitivity of mouse oocytes was itself the first indication that the killing of these particular cells must involve a mechanism different from that in most radiobiologically studied systems, where the usual target is DNA. This was recognized by Davies and Evans (9). Interestingly, tritium data gave us the first clear signal that the target in these cells is not in the nucleus, but some distance from it. When survival data for oocytes in $^3$HOH-exposed mice were compared with radioautographic data for oocytes containing $^3$H-TdR in their DNA, it was obvious that tritium in chromosomes is less effective, not more, in killing these particular cells than is tritium in body water. Apparently the target is not easily reached by beta rays from $^3$H confined to nuclear DNA.

This phenomenon can be appreciated by referring to radioautographs and oocyte survival data provided by Baker and McLaren (3). In their Fig. 3, for example, seven to nine grains can be identified over the nucleus of a primordial oocyte in a 5-month-old mouse labeled with $^3$H-TdR during fetal development. If one considers 7 grains (and the 14-day radioautographic exposure used), the calculated (see ref. 19) dose received by this nucleus during five months is of the order of 300 rad or more. Now, as seen from Fig. 3 above, essentially no primordial oocytes can be expected to survive 300 rad from generally distributed $^3$HOH, yet the survival of Baker and McLaren's cells was about 60%! Clearly the discrepancy between $^3$HOH and $^3$H-TdR data requires resolution.

The realization that the target likely involves cytoplasmic structures, adjacent follicle cells, or the plasma membrane prompted neutron studies for information on target cross section. Results (24) indicated that the target area, assumed circular, had a diameter larger than the nucleus, but smaller than any combination of oocyte plus follicle cells. Together with a volume for the target (determined from gamma-ray data) of about 4 $\mu m^3$ (24), this suggests the plasma membrane as the sensitive structure. It is of course recognized that the target may not be the plasma membrane itself; but it is of similar geometry and location.

Assuming the membrane as target, data for tritium, neutrons, and gamma rays reconcile, and in each case $D_{37}$ corresponds to the deposition in the plasma membrane of about 500-1,000 eV (24). Now consider again the $^3$H-TdR situation. In

Fig. 4, $^{3}H$ beta-ray ranges are schematically compared with approximate mouse oocyte dimensions. These cells are characteristically spherical, with centrally positioned spherical nuclei, as pictured in A (Fig. 4); nuclear contact with the plasma membrane, as in B (Fig. 4), is rarely if ever observed, but is included here as a limiting case. In view of the energy spectrum and range distribution of $^{3}H$ beta rays, it is clear that only a small fraction of disintegrations occurring in nuclear DNA will deposit any energy at all in the plasma membrane. Monte Carlo calculations show that even for a typical oocyte experiencing 1,000 disintegrations in DNA (corresponding to a nuclear dose of approximately 300 rad), only about 860 eV will be deposited in the plasma membrane. This result is quite consistent with, and offers an explanation for, the 60% oocyte survival observed by Baker and McLaren, discussed above.

CONCLUSIONS

1. Studies of germ-cell killing by chronic $^{3}HOH$ exposure, particularly oocyte killing in mice, have revealed effects at exposure levels in a range comparable with those that have been considered acceptable for occupational exposure.
2. In such germ-cell killing, the RBE of tritium compared with $^{60}Co$ gamma radiation, at very low doses and dose rates, reaches approximately 3.
3. In at least one primate, the squirrel monkey, female germ cells are destroyed by $^{3}HOH$ exposure as readily as in mice, perhaps even more so. This is not true, however, in the two otner primate species that have been studied, rhesus and bonnet monkeys; and human oocytes are probably not highly sensitive to radiation killing.
4. Effects of $^{3}HOH$ exposure on fertility have been measured in mice. They are not unlike those found for other radiations.
5. The extraordinary radiosensitivity of mouse oocytes is apparently due to vulnerability of the plasma membrane (or something of similar geometry and location), not DNA. And since radiation genetic risk estimation in man is based primarily on mouse data (25,1), this special situation in the mouse, for which there is no evidence of occurrence in man, may have implications for genetic risk estimation in the human female.

ACKNOWLEDGMENT

This work was performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under contract number W-7405-ENG-48.

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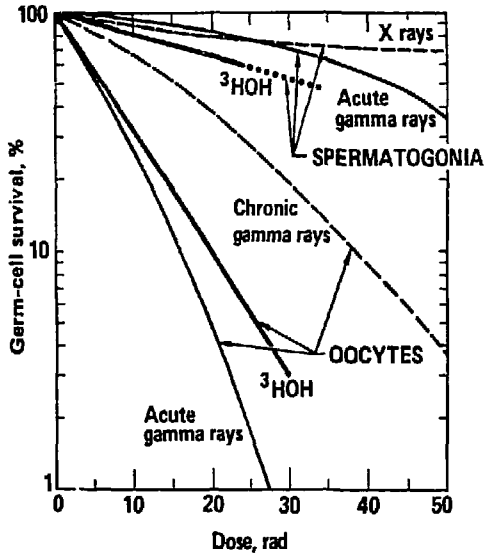


Fig. 1 Survival of intermediate and type-B spermatogonia and of primordial oocytes in mice following tritium and other radiation exposures. $^3$HOH: single i.p. injections; spermatids counted 72 hours later (data from (21)), oocytes counted 10 days later (data from Dobson et al., unpublished). Acute gamma rays ($^{60}$Co): spermatogonial data from (21), oocyte data from Dobson et al., unpublished. Chronic gamma rays ($^{60}$Co): data from (12). X rays (200 kVp): data from (21), dose rate exponentially decreasing to match $^3$HOH.

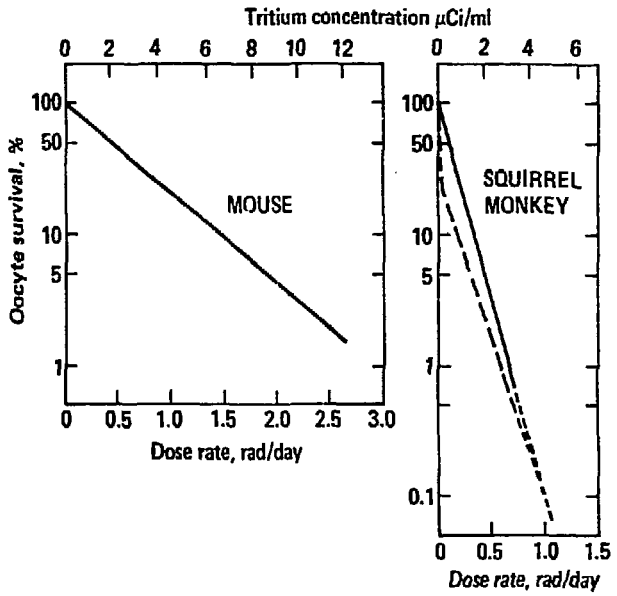


Fig. 2 Survival curves for primordial oocytes in developing mice chronically exposed to $^{3}$HOH in body water from conception to 14 days after birth (33 days) and for total oocytes in developing squirrel monkeys exposed from conception to birth (154 days). (Modified from ref. 14.)

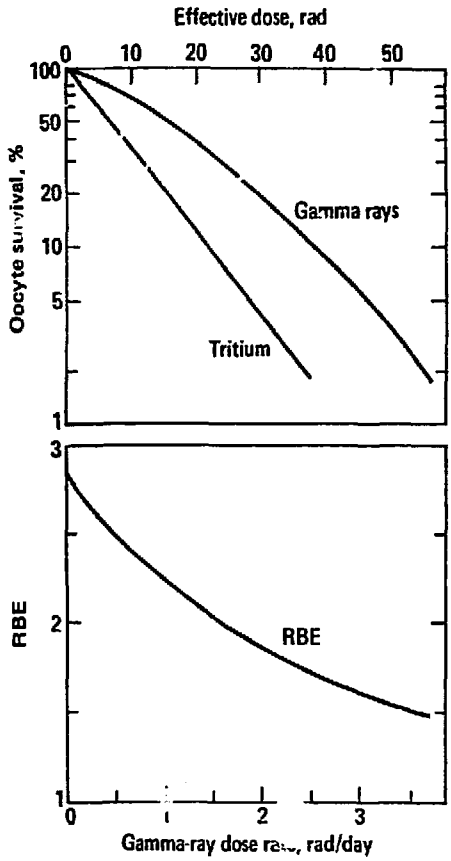


Fig. 3 Survival of primordial oocytes (as a function of dose and dose rate) in mice chronically exposed from conception to 14 days of age to $^{3}$HOH in body water or to $^{60}$Co gamma rays, and the RBE of tritium compared with gamma radiation. (From ref. 14.)

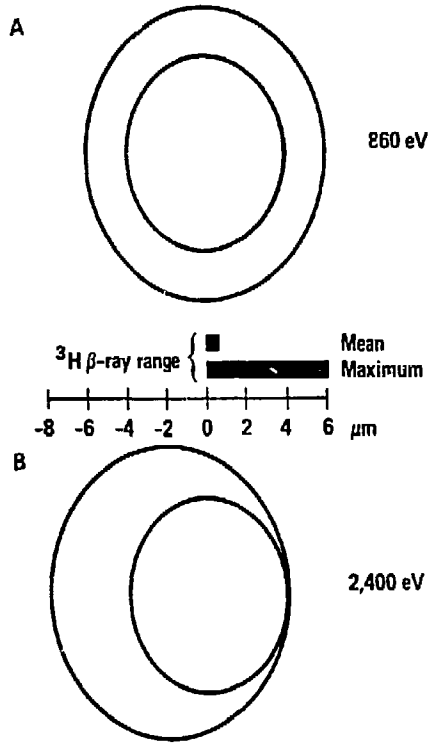


Fig. 4 Schema of mouse oocytes (typical diameter about 12 μm, nucleus 8 μm), with mean and maximum $^3$H beta-ray ranges for comparison, showing calculated energy (in eV) that would be deposited in the plasma membrane by 1,000 beta rays arising randomly in the nucleus, for two nuclear positions (see text).