Bull. Environ. Contam. Toxicol. (1985) 34:803–808

Bulletin of Environmental Contamination and Toxicology

Tritium Toxicity: Age-dependent Radiosensitivity of Mouse Testes

A. L. Bhatia

Radiation Biology Laboratory, Department of Zoology, University of Rajasthan, Jaipur - 302 004, India

Tritium, a heavy and unstable isotope of hydrogen (half-life-12.36 yrs, atomic mass 3), finds its way to the environment from two sources, natural and man made. In nature it is formed by interactions of cosmic rays with the gases of upper atmosphere. Large quantities are also released from nuclear energy operations as a significant by-product, thereby proving hazardous to human population due to a bound gradual increase. Thus tritium has emerged of primary concern due to a persistence and ubiquitous distribution in the environment and in biological system. However, as a radiation hazard of biological significance it has largely been ignored because of its rapid turnover from the body, its low average beta energy(5.7 KeV), and short range in the tissue (6.0×10^{-4}cm.). In all the available reports of the investigations, little research effort has been noticed on the age-specific consequences of HTO-irradiation of mammals in the perinatal to pubertal stages of life as compared to the adult stages. The present study is an attempt to evaluate qualitative and quantitatively the age dependent radio-sensitivity of mice testesto tritium toxicity.

MATERIALS AND METHODS

Swiss albino mice, procured from Cancer Research Institute, Bombay, were maintained and bred in air-conditioned laboratory and were fed with balanced food manufactured by Hindustan Lever. Water was given ad libitum. Tritiated water was received from Bhabha Atomic Research Centre, Trombay, Bombay, (sp. activity 10 mCi/ml). The mice belonging to different six age groups were injected intraperitoneally, 20.00 μCi/ml of their body water. Autopsies were made 48 hours post-injection. The body water taken into account and the total doses injected is shown in the table 1. Other detail of materials and methods has already been described elsewhere (Bhatia, 1978, 1982). Qualitative and quantitative studies were made and compared with their respective age-matched controls. The dose 20 μCi/gm body weight in this experiment estimated to deliver approximately 4137.83 ± 815.0 mrads/day 'initial dose rate' to the body.

This work was presented in 70th Session of Indian Science Congress, held in Tirupati, India from 3rd to 8th Jan. 1983.

Table 1. Experimental Design

S. No.	Age of Animal	No.of Animals Control	Treated	Body Wt.	% b.wt. as body water	Injected doses μCi
1.	1 week old	10	8	4.5	80	72.00
2.	2 week old	10	8	5.5	75	82.40
3.	3 week old	10	7	9.0	70	130.00
4.	4 week old	10	7	12.0	60	135.00
5.	5 week old	10	6	13.0	62	140.00
6.	6 week old	15	5	14.5	60	160.00

RESULTS AND DISCUSSION

1 week old: Very mild cytoplasmic vacuolation with the pycnotic nuclei of some of the spermatogenic cells have been observed. A few tubules were containing desquamated cells in their lumen.

2 week old : Same degree of cytoplasmic vacuolationwith many dead and pycnotic cells has been observed. Mitotic figures were more in number than the normal, presumably due to mitotic arrest.

3 week old_ Some pycnotic nuclei and necrotic cells with mild cytoplasmic vacuolation and exfoliation were observed in the seminiferous epithelium. The tubules of the central region exhibited a marked reduction in their germs cells. Lymphocytic infiltration was noted at places. (Fig.1)

4 week old: This group of mice revealed a greater degree of damage as compared to other age groups. Almost all the tubules suffered from severe cytoplasmic vacuolation, greater reduction in the spermatogenic cells and distortion. Many cells nuclei appeared smaller or crenated in their shapes. Inter-tubular oedema of mild degree was also evident (Fig.2)

5 week old : A great degree of inter-tubular oedema spreading over all the testicular tissue wœ evident. Damaged tubules experienced cytoplasmic vacuolation of severe degree along with many pycnotic and necrotic cells. A few tubules had giant cells either formed from spermatocytes or from spermatids (Fig.3). Sperms were lacking in the lumen.

6 week old: Many tubules showed cytoplasmic vacuolation along with many pycnotic and necrotic cells. A few giant cells of both the types, hypertypic and multi-nucleated, were also seen. Sperms are not detected in the lumen (Fig.4).

In the group 1, 2, and 3 week old mice a slight decline in A type of spermatogonial population has been recorded as compared to those of later age groups (4, 5, 6 weeks). However, in all the age groups type B spermatogonial population was seen more affected. Conversely, in 1 week old mice

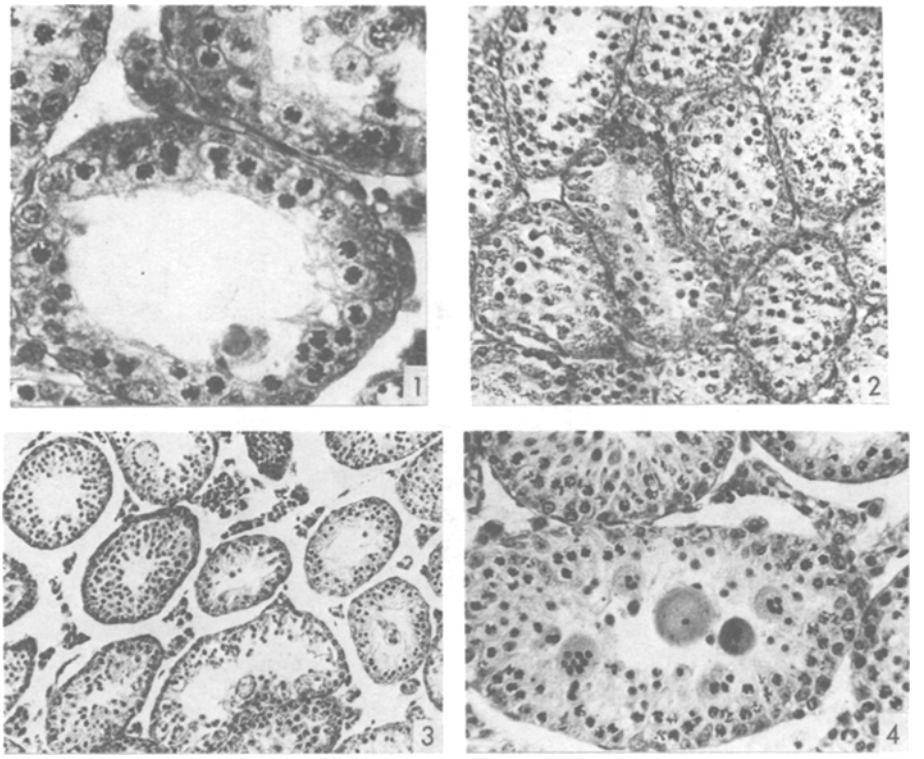

Figure 1 Photomicrograph of irradiated 3 week old testis showing central tubule with loss of germs cells and exfoliated cells and cytoplasmic vacuolation X 350.

Figure 2 Photomicrograph of irradiated 4 week old testis showing cytoplasmic vacuolation and pycnotic nuclei X 175.

Figure 3 Photomicrograph of irradiated 5 week old testis showing intertubular oedema and cytoplasmic vacuolation, pycnosis and necrosis of the germ cells in the tubules X 87.

Figure 4 Photomicrograph of irradiated 6 week old testis showing cytoplasmic vacuolation with prominent giant cellsX 175.

a slight increase in the A type of spematogonia was found, variations in cell which is statistically non-significant (P = N.S.). The maximum reduction in all, the type A, 1 and B of spematogonia was recorded in the 4 week old mice. The age-dependent radiosensitivity of the spematogonia population was found highest in the 4 week old mice testis (Fig.5). These variations in their crude counts have been found statistically highly significant.

However, resting type of spematocytes were the most vulnerable type of primary spermatocytes to HTO in almost all the age groups of mice. A max-

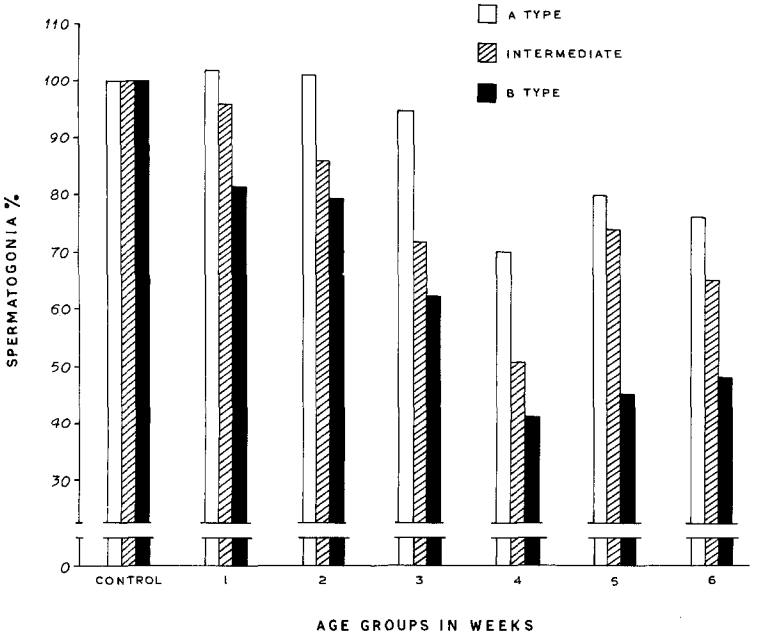

Figure 5 Histogram showing the variations in the percentage of spermatogonial populations in the testes of irradiated mice.

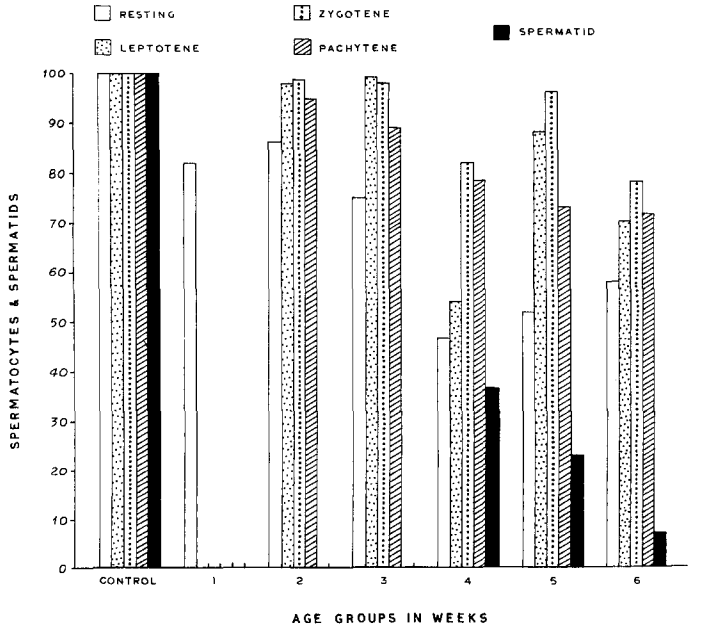

Figure 6 Histogram showing the variations in the percentage of spermatocytes and spermatid population in the testes of irradiated mice.

imum reduction inthe resting spermatocytes was noticed in 4 week old mice, followed by 5 week and 6 week old mice. The size of the zygotene type of spermatocytes population was found to be least affected in all the age groups. Pachytene and leptotene type of spermatocytes population were comparatively decreased in size. The spermatid population was reduced to a greater extent which may partly be due to their transformation into sperms and partly by a direct damage from high dose of β-radiations from HTO(Fig. 6).

The seminiferous epithelium is the tissue that changes its proliferative and functional characteristics as the animal matures. In this system, therefore, the regulation of cell reproduction is carefully maintained through the sequential development of spermatogenesis. Cell transit in the proliferative compartment is associated with progressive differentiation and maturation, and there is no evidence of a return to earlier forms of maturity.

Fabrikant (1969) determined the median cell cycles times for three spermatogonia cell populations in 4 week old and 21 week old C57BL mice. He found that the cell generation times were somewhat longer in the young animals (in 4 weeks: 38, 35 and 39 hrs for type A, I and B spermatogonia, respectively) in comparison to the mature mice (29, 27 and 29 hrs for the same respectively). Four weeks old mice testes is found highly sensitive quantitatively which indicates possibility of the median cell cycle as one of the factors for the radiosensitivity of 4 week old mice testes observed in the present experiment. After continuous exposure from 16th day of gestation, a study during postnatal development showed that the 3 and 4 week old mice tests were more affected (Bhatia & Srivastava, 1982).

The difference in the radiosensitivity of seminiferous epithelium belonging to different age groups may be due to (i) elimination of tritium from organ i.e. effective half-life which may vary with the age. According to Ueno and Kawamura (1975), young mice have a longer half life of elimination of tritium than old mice. (ii) the dependence of spermiogenesis on the pituitary hormones which usually start their influence on the testes at 3 week may also play a role in the age specific radiosensitivity of 3 and 4 week old mice (Chadha, 1971). As the radiosensitivity of the whole animal body is concerned, Crosfill et al (1959) were of the opinion that mice are relatively radioresistant shortly after birth but they rapidly became more sensitive, reaching a time of maximum sensitivity at about 4 weeks of age. Thisis followed by a steady increase in resistance to adulthood. Abrams (1951) and Lindop and Rotblat (1962) found higher resistance at birth than at 30 days of age. Furth and Furth (1936) mentioned that among a series of mice exposed to 300-400, mortality was greatest in young animals aged about 5 weeks at the time of irradiation.

However, there are possibilities that this age-dependent radiosensitivity may extend to the tissue level which includes testes too. The directions for needed future research withTritium water in this field are many and multidisciplinary and essentially require an effort similar or parallel to that expended on research in other phase of mammalian development. The possibility of sign-

ificant hazard from an accumulated tritium from HTO, with relation to age can not be safely ruled out which warrants careful evaluation.

Acknowledgements _ The author wishes to thank Prof. P.N. Srivastava, Vice-Chancellor, Jawaharlal Nehru University, New Delhi for his valuable discussion in preparing the manuscript and Prof. A.S. Kapoor, Head, Department of Zoology, for providing necessary facilities.

REFERENCES

Abrams HL (1951) Influence of age, body weight, and sex on susceptibility of mice to the lethal effects of X-irradiation. Proc Soc Exptl Biol Med (N.Y.) 76: 729-732

Bhatia AL (1978) Effects of tri tiated water on mice liver in relation to age. J Radiat Res 19 : 153 - 162

Bhatia AL (1982) Tritium toxicity on postnatally developing mice testes: A qualitative and quantitative evaluation. Radiobiol Radiotherapia 23: 693-698

Bhatia AL, Srivastava PN (1982) Tritium toxicity on mouse testis: Effect of continuous exposure during pre- and postnatal development. Strahlentherapie 158: 752-755

Crosfill ML, Lindop PS, Rotblat J (1959) Variation of sensitivity to ionizing radiation with age. Nature 183 : 1729 - 1730

Chadha SR (1971) Qualitative and quantitative studies on the effect of radiophosphorus on gonads during prenatal and postnatal development of Swiss albino mice. Ph.D. Thesis submitted to Rajasthan University, India.

Fabrikant JI (1969) Cell proliferation studies in continuously irradiated mammals: Effect of age. In Radiation Biology of the Fetal and Juvenile Mammals pp 621-628 IAEA Vienna

Furth J, Furth OB (1936) Neoplastic disease produced in mice by general irradiation with Y-rays 1. Incidence and types of neoplasm. Am J Cancer 28 : 54 - 65

Lindop PS, Rotblat J (1962) The age factor in the susceptibility of man and animals to radiation. The age factor in radiation sensitivity in mice. Brit J Radiol 15 : 23 - 31

Ueno Y, Kawamura K (1975) Study on the elimination of Tritium in mice injected with tritiated water. Health Phys 29: 413 - 415

Received April 23, 1984; accepted July 10, 1984.