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Gonadal cell kinetics in male mice treated with sulphur-35 during prenatal development

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Abstract. Investigations on the possible hazards of the use of internally administered radioisotopes in human medicine either as therapeutic or diagnostic agents before or during child bearing age are of late gaining importance. The present investigation has been taken up to screen the effects of sulphur-35 on spermatogonia.

CBA pregnant mice were injected (ip) with a dose of 20 μ Ci of sulphur-35 on 3·5, 10·5 or 15·5 days of gestation. At the similar intervals pregnant mice injected with physiological saline were kept for control data. All the animals were allowed to litter and F_1 male progeny were killed at maturity at the age of 10 weeks and the testes collected. Sections of both the testes were prepared and stained by PAS-haematoxylin technique and the survival of spermatogonia types A, Int and B and preleptotene spermatocytes was evaluated. There was a significant reduction in all the cell types in the sulphur-35 treated animals. Thus the results indicate the cell-killing effect of radionuclide.

Keywords. Sulphur-35; gonadal cell kinetics; prenatal development; mice.

1. Introduction

^{35}S is one of the important radionuclides used in human medicine. It is used to treat chandrosarcoma (Andrews and Holland 1965; Gottschalk 1960; Alpert and Albert 1959), mycosis fungoides (Lund and Davis 1962), skin lesions (Mc Laren 1965) and to estimate extracellular water in animals (Walser *et al* 1953). Its half-life is 87·1 days and it decays to $^{35}Cl_{17}$ by emission of β-rays with energy of 0·167 MeV.

The effects of internally administered radioisotopes such as ^{32}P and ^{131}I on gonadal cells of mammals and their possible hazards of using in medicine before or during child bearing age were shown earlier (Lunning *et al* 1963; Reddi *et al* 1968; Reddi *et al* 1971; Reddi 1971). Hence in the present investigation the effects on spermatogonia of mouse treated with ^{35}S during prenatal development were studied.

2. Materials and methods

CBA pregnant mice were injected intraperitoneally with a dose of 20 μCi of ^{35}S in the form of carrier-free $Na_2\,^{35}SO_4$ (supplied by Bhabha Atomic Research Centre, Bombay) in 0·5 ml of saline on 3·5, 10·5 or 15·5 days of gestation. Three more batches of pregnant mice injected with normal physiological saline at the same intervals were kept for control values. All the pregnant animals allowed to litter. The F_1 male progeny were killed at maturity. Immediately after killing the testes were collected and fixed in Zenker's formalin solution for 3½ hr. They were washed in running water overnight and transferred to lugol's iodine solution for 45 min to remove excess of mercuric chloride. The excess of iodine was removed by giving 20 min of wash in 5% sodium thiosulphate solution. After washing in running water and a rinse with distilled water the testes were dehydrated in ascending grades of alcohol and cleared in cederwood oil and embedded in paraffin wax. Five micron thick serial sections of both the testes were prepared. The slides were stained with periodic acid—Schiff-haematoxylin technique (Oakberg and Clark 1961).

Type 'A' spermatogonia from all tubular stages, intermediate and type 'B' spermatogonia from tubules of II to VI stages and preleptotene spermatocytes from VII stage tubules were scored. All the cell types were counted in 100 randomly selected tubule cross-sections per mouse both in control and ^{35}S treated groups. The cell counts thus obtained were then converted to experimental controls ratios as described by Oakberg and Clark (1961).

3. Results and discussion

In the present investigation the F_1 males were treated during different periods of prenatal development with sulphur-35 and the survival of various germ cells was assessed at maturity and the results are presented in table 1.

A drastic reduction in all the cell types was recorded in males treated with ^{35}S at all the three intervals indicating the cell killing effect of sulphur-35. In animals treated on 3·5 day of gestation the loss of type 'A' spermatogonia was 43·40% while the losses were 38·70% and 26·00% in animals treated on 10·5 and 15·5 days of gestation respectively. A similar reduction was also noticed in other cell types (Int, B and PLS). In animals treated on 3·5 days the loss of all the cells put together was about 40·60% while the losses were 32·70% and 23·00% in animals treated on 10·5 and 15·5 days of gestation respectively. The results agree with earlier studies of Reddi *et al* (1971); Henricson and Nilsson (1967); Henricson *et al* (1962); Reddi *et al* (1968) who have reported gonadal cell mortality in adult male mice after treatment with different radionuclides. The results are also in accordance with x-ray, neutrons and gamma ray studies (Oakberg 1959; Oakberg and Clark 1961). The acute depletion of the cell types scored can be explained on the basis of direct cell killing by transmutation effects of ^{35}S after its incorporation into spermatogonia.

From the results it is evident that the maximum damage to spermatogonia was caused to the animals treated during pre-implantation period when compared to the damage observed in animals treated during major organogenesis and fetal

Table 1. Numbers of type 'A' spermatogonia (A); intermediate and type 'B' spermatogonia (int and B); and preleptotene spermatocytes (PLS) in male mouse treated with saline (control) and 20 μCi of sulphur-35 on 3·5, 10·5 or 15·5 days of gestation.

Period	Treatment	Cells scored			
		A	Int and B	PLS	Total cells (A, Int and B PLS Pooled)
3·5 day of gestation	Control	954	873	1044	2871
	^{35}S	540	552	612	1704
		(0·566)	(0·632)	(0·586)	(0·594)
10·5 day of gestation	Control	1008	912	1071	2991
	^{35}S	618	603	792	2013
		(0·613)	(0·661)	(0·739)	(0·673)
15·5 day of gestation	Control	888	855	1014	2757
	^{35}S	657	651	816	2124
		(0·740)	(0·761)	(0·805)	(0·770)

$P < 0·05$.
Experimental control ratios are given in parentheses.

periods. This might be due to the continuous irradiation of embryos by ^{35}S during subsequent stages since it has a long half-life.

The present study indicates that the treatment of males as embryos during any stage of prenatal development may result in the incorporation of ^{35}S into the testes of embryos and might lead to physiological upset, genetic death and inherited low fertility.

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