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The genetic sensitivity to X-rays of mouse foetal gonads

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Estimates of the dose to human gonads of man-made radiation show that nearly all of it comes from radiation used in medicine, rather than from fall-out or atomic energy, and that of the medical dose approximately one-third is delivered to post-natal males, one-third to post-natal females and one-third to foetuses (Medical Research Council, 1956). Therefore in investigating the genetic hazard to man of ionizing radiation it is important to have some measure of the sensitivity of foetal germ-cells to radiation mutagenesis. Carter (1958) irradiated foetal male mice with 300 r. X-rays at 13½ days' gestation and tested them, as adults, for mutations at seven specific loci. He found only one mutant among 10,155 progeny tested and put forward the tentative conclusion that 'the yield of induced mutation, as recovered from spermatozoa of the adult, is lower for irradiation of foetal than of adult spermatogonia'.

The present paper describes further experiments on foetal germ-cells, designed to amplify this work.

Carter irradiated foetuses at 13½ days' gestation, when the gonad is still populated by primordial germ-cells and sex-cords are just developing. This corresponds to a stage of about 5 weeks' gestation in human embryos (Otis & Brent, 1954). But the greater part of the dose of medical radiation to human foetuses is delivered in late pregnancy. Therefore, in extending the work on mouse foetuses it was of interest to irradiate at a later rather than an earlier stage of gestation. The stage chosen was 17½ days, being the latest convenient stage, and the offspring of both sexes of foetuses were examined for mutations. At this stage female germ-cells are in the early stages of meiotic prophase (Brambell, 1927), and male germ-cells are still in the form of primordial germ-cells. This corresponds to a stage of about 5 months' gestation in human foetuses (Maximow & Bloom, 1952). Rugh & Jackson (1958) showed that irradiation of foetal mice may result in permanent sterility and that the dose which will cause sterility varies greatly between the sexes and among different foetal stages. The dose, 200 r. X-rays, used in the present experiment was the highest consistent with fertility of both sexes when given at 17½ days' gestation.

STOCKS AND METHODS

Female mice of the C3H/HeH strain were placed with 101/H males and examined daily for copulation plugs. Seventeen days after the finding of a plug they were given a dose of 200 r. X-rays (250 kV, 14 mA, HVL 1·2 mm. Cu, 72 r./min.) to the

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whole body from below. The young born to them, which were C3H × 101 hybrids, were mated when about 8 weeks old with animals homozygous for the seven recessive genes, *a*, *b*, c^{ch}, *d*, *p*, *s*, and *se*. The young born to these pairs were examined when $2\frac{1}{2}$ weeks old for mutations at these seven loci and for dominant visible mutations at any loci. A target of 30,000 progeny was set for each sex.

RESULTS

The animals irradiated as foetuses were smaller than unirradiated animals of the same genotype, but no congenital malformations were noticed among them, and their mortality in the nest was not unduly high, considering their small size. Altogether 621 males were mated, of which 47, or 7·6%, proved to be sterile and a few sired fewer young than normal, and 725 females of which only 4 were sterile.

Table 1 shows that among the progeny of irradiated females there were three specific locus mutants and one dominant visible mutant, and among the progeny of males there were nine specific locus mutants and five dominant visibles.

Table 1. *Mutant animals found*

Sex irradiated	Total progeny	Specific locus mutants							Total	Dominant visibles
		a	*b*	*c*	*d*	*se*	*p*	*s*		
♀	30,289	–	1	1	–	–	1	–	3	1
♂	31,253	–	1	2	1	(1)	1	4	9	5

All the specific locus mutants were genetically tested and found to carry mutant alleles at the loci shown. One, in the male series, carried mutant alleles at both of the very closely linked loci *d* and *se*. In the analysis of the results this has been counted as a single mutational event. Three of the four mutants at the *s*-locus occurred in two litters from the same irradiated male; these have been counted as three mutants.

The dominant visible mutation in the female series caused circling behaviour. Those in the male series were two in which heterozygotes resembled those of the *W*-series; one in which heterozygotes and homozygotes phenotypically resembled splotch, *Sp*; a sex-linked mutant resembling tabby, *Ta*; and a short-tailed mutant.

COMPARISON OF FOETAL AND ADULT SENSITIVITY TO MUTAGENESIS

Russell, Russell & Kelly (1958) showed that when adult male mice are given a dose of 300 r. or less of X-rays and young are raised from treated spermatogonia the average mutation rate at the same seven loci as were used in the present experiment is about 28×10^{-8}/r./locus. Russell, Russell & Cupp (1959) showed that the mutation rate after similar treatment of females did not differ from that obtained after irradiating males. Therefore, to test whether the sensitivity to mutagenesis of the foetuses in the present experiment differed from the sensitivity of adults, the numbers of mutants observed have been compared with the numbers that would have been expected had the induced mutation rate been 28×10^{-8}/r./locus (Table 2).

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Since the comparison is being restricted to the same seven loci throughout and there is no extrapolation to mouse loci in general, the confidence limits given by the Poisson distribution have been used (Fisher & Yates, 1953, Table VIIIi), rather than the wider confidence limits suggested by Kimball (1956) for use when arguing from species to species. In calculating the expected number for the male series allowance has been made for an expected 1·9 spontaneous mutants. This estimate is based on the combined figures for male spontaneous mutation of Russell, Russell & Kelly (1958) and Carter, Lyon & Phillips (1958), which were 25 mutants out of 406,343 animals, giving an average mutation rate of $0{\cdot}85 \times 10^{-5}$ per locus. The spontaneous mutation rate in the female mouse is at present unknown since Russell, Russell & Cupp (1959) found no spontaneous mutations among 46,763 offspring of untreated female mice. Therefore, since the number of mice raised in the female series here was less than 46,763 the expected number of spontaneous mutations has been taken as zero. If this should prove to be an error then the expected number of mutants here would be greater, and the significance of the results would be increased.

Table 2. *Comparison of observed mutants with numbers expected on the basis of the adult mutation rate*

Sex irradiated	Total progeny	Mutants expected	Mutants observed	Confidence limits*		Observed induced mutation rate/r./locus $\times 10^8$
♀	30,289	11·9	3	0·619	8·77	7·1
♂	31,253	14·2	9	4·12	17·08	16·2

* Lower and upper 95% confidence limits of observed number. (Fisher and Yates, 1953, Table VIII.)

Table 2 shows that for both the male and female foetuses the observed number of mutants was below the expected number. For the females, but not for the males, the expected number was outside the 95% confidence limits of the observed number. However, the expected number was itself based on experimental data and therefore had its own confidence limits. The actual figures obtained by Russell, Russell & Cupp (1959) after giving 400 r. X-rays to adult females were 7 mutants out of 7,859 offspring examined, giving a mutation rate of $31{\cdot}8 \times 10^{-8}$/r./locus. The probability of obtaining by chance a difference as great or greater than that between the mutation rates per roentgen for these two groups of female mice was $P = 0{\cdot}023$ for a one-tailed test. Such a test has not been made for the males since the observed and expected figures differed so little.

In the case of the male foetuses we may also make another comparison: between the mutation rate obtained in this experiment using the $17\frac{1}{2}$-day stage and that found by Carter (1958) using the $13\frac{1}{2}$-day stage. Table 3 shows this comparison. The mutation rate per locus per roentgen at the $13\frac{1}{2}$-day stage was only about one-tenth of that at $17\frac{1}{2}$ days. However, the probability of finding a difference as great or greater by chance is as high as $P \sim 0{\cdot}1$ for a one-tailed test.

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Table 3. *Comparison of results from male foetuses of 13½ and 17½ days. The adult figures are those of Russell, Russell & Kelly* (1958)

Stage (days)	Dose (r.)	Total progeny	Mutants	Mutation rate $\times 10^5$	Induced mutation rate/r./locus $\times 10^8$
13½	300	10,155	1	9·85	1·76
17½	200	31,253	9	28·8	16·2
Adult	300	40,408	25	61·9	28

DISCUSSION

In discussing the results of this experiment one must first consider that it has been necessary to make comparisons between experiments carried out in different laboratories using different equipment and stocks of mice. The mouse stocks used at Harwell were branches of the stocks used at Oak Ridge, and Phillips (1960) found no evidence of any gross difference in sensitivity of adult males of the two branches to the mutagenic action of acute and chronic doses of 600 r. Therefore it may be concluded that differences in the stocks and experimental techniques do not account for the differences between the Harwell and Oak Ridge results that are discussed here.

The results of this experiment indicate that when female mice were irradiated as 17½-day-old foetuses the observed mutation rate among their offspring was lower by a factor of about four than after similar irradiation of adults. When the irradiated 17½-day-old foetuses were male mice the observed mutation rate was below but not statistically significantly below the comparable adult rate, but neither did it differ significantly from the rate for 13½-day-old male foetuses, which Carter found to give a lower mutation rate than adults.

There are two main possible explanations for the differences found: (1) that the rates of induction of mutation were different in the various life-stages irradiated, and (2) that the rates of induction were constant but that differing amounts of elimination of mutant cells by selection during gametogenesis affected the rate of discovery of mutants. Either of these explanations could be the real one. Russell, Bangham & Gower (1958) showed that in adult male mice the mutation rate varies with the germ-cell stage irradiated. In foetal gonads the germ-cell stage attained is different from that in adults. In 17½-day-old foetuses the female germ-cells are in early stages of meiotic prophase whereas in adults they are resting in the dictyate stage at the end of prophase; in the male gonad the primordial germ-cells are still present at 17½ days whereas the results from adults which have been quoted refer to spermatogonia. Thus it is possible that the rate of induction of mutation in foetuses differs from that in adults as a result of the difference in germ-cell stage irradiated. On the other hand, selective elimination of mutant cells might also occur, particularly in males. In this experiment about 7·6% of males were sterilized by the radiation dose they received. This suggests that the cell-killing effect of the radiation on the germ-cells was severe, and that the testes were repopulated from a

very small number of surviving cells. If there were any differential sensitivity the observed mutation rate among the surviving cells might be spuriously low.

Thus this experiment, though amplifying Carter's earlier work, still leaves much unknown about the genetic effects of foetal irradiation. Though female foetal mice of the stage used seem clearly less susceptible than adults, this is not certain for males. It seems reasonable to expect that different foetal stages will vary in their sensitivity to mutagenesis, but the data are not so far adequate to establish this. From the point of view of human hazards, however, it is reassuring that there is no indication so far that any age or sex of foetus is more susceptible than the adult male.

SUMMARY

The mutation rate at seven specific loci was measured among the offspring of male and female mice exposed as $17\frac{1}{2}$-day-old foetuses to 200 r. X-rays. In the female series the mutation rate was lower, by a factor of about four, than the comparable adult rate; in the male series the mutation rate was lower but not statistically significantly lower than in adults.

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