RADIATION-INDUCED MUTATION RATES IN FEMALE MICE

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Information on radiation-induced mutations in female mice has remained far below the level attained for males as early as 1951.[1] The reason has been that adult female mice given acute X-ray doses comparable to those used in our experiments with males become permanently sterile after bearing only one or two litters. Thus, in experiments with females, in order to obtain a total number of offspring equivalent to that from experiments with males, a much larger number of animals has to be irradiated.

In spite of this difficulty, it was feasible, and seemed important, to conduct an experiment that would at least go far enough to determine whether females showed any indication of a much higher sensitivity to mutagenesis than males. Such an experiment, using acute X-irradiation, was consequently carried out at an early date, 1949–1951. More recently, a much larger investigation has been started with chronic gamma irradiation, following the demonstration that the sterilizing effect on females can be minimized by administering the dose at a low intensity.[2, 3] The present paper will discuss mutation-rate data from both the acute X-ray and the chronic gamma-ray experiments, the latter of which is still in progress.

The data from the acute X-ray experiment are presented in Table 1. This in-

TABLE 1

Mutations at Specific Loci in Female Mice Exposed to Acute X-Irradiation

Experiment	Dose (r)	No. of Offspring	No. of Mutations at Locus						
			a	*b*	*c*	*d*	*p*	*s**	*se*
1949	300	498	...	...	...	...	...	...	...
	400	760	...	...	...	...	...	...	...
	500	48	...	...	...	...	...	...	...
1950–1951	0	5845	...	...	...	...	...	...	...
	400	1729	...	...	...	...	...	1	...

*See text for qualification concerning 1949 experiment.

vestigation was carried out in two parts. In the experiment proper (1950, 1951), adult wild-type F_1 hybrid females from a cross of 101 × C3H strains were exposed to 400 r of X-radiation (250 kvp.; 15 ma.; inherent filtration 3 mm. Al; H.V.L. 0.4 mm. Cu; dose rate 96 r/min) and mated to males of our T stock, which is homozygous for seven recessive marker genes. Only one mutation at any of the specific loci was obtained in 1,729 offspring of irradiated females. No mutations were found in 5,845 young born to control, unirradiated females (Table 1, 1950–51 experiment). In a still earlier pilot study, no specific locus mutations had been found (1949 experiment). At this earlier time the test stock had not been fully developed, and some of the test males used in this experiment, although homozygous for the marker genes at six of the loci, may not have been homozygous at the seventh (*s*) locus. The mutation rate of 12×10^{-8} per roentgen, per locus, obtained by combining the data of the 1950–1951 and the 1949 series, and its 95 per cent con-

fidence limits of 0.3×10^{-8} and 68×10^{-8} may, therefore, be slightly biased, but, since this bias is small compared with the sampling error, it will be ignored in the following discussion.

Although the estimate of the mutation rate is based on only one mutation and therefore has the wide confidence interval given above, the results obtained were nevertheless adequate to answer the question posed: they ruled out the possibility of females having a much greater sensitivity to radiation-induced mutagenesis than that found in males. Thus the upper 95 per cent confidence limit of the female mutation rate is less than three times the acute X-ray-induced mutation rate of 25×10^{-8} per roentgen, per locus, originally reported in males,[1] or the rate of 28×10^{-5} later found in males exposed to 300 r.[4] Therefore, it could be concluded that calculation of the average risk of equal exposure of both sexes on the basis of the far more reliable mutation rate obtained in males would almost certainly not underestimate the hazard from acute X-rays by more than a factor of 2. Of course, such a calculation is actually more likely to overestimate the hazard, since the point estimate for females is below that for males, and the lower 95 per cent confidence limit of the female rate is very much below that. However, it is obvious that a calculation based on the correct male rate can never overestimate by more than a factor of 2, no matter how low the true rate in females is.

The limited experiment on females was, then, sufficient to show that if the more reliable mutation rate in males is used to calculate risks, any error introduced by not allowing for a difference between the acute X-ray-induced rates in males and females is likely to be well within the limits of other uncertainties in the estimation of genetic hazards in man. Consequently, the obtaining of more accurate information on specific locus mutation rates in females was postponed until recently, and the use of the specific locus method for the exploration of the many other factors that enter into the estimation of genetic hazards[4, 5] was pursued in males.

In the interval between the early specific locus experiments in females and the present ones, to be described below, various other genetic effects in females were investigated in experiments that do not require large numbers and are not hampered by radiation-induced sterility. These studies[6-9] dealt with chromosome aberrations, particularly dominant lethals and translocations, which had been virtually unexplored in the female. They had been the subject of much work in the male, where it was shown that they are probably not a major hazard because the incidence, following irradiation of spermatogonia (the cell stage that is important in human hazards), was found not to be significantly different from that in controls. Dominant lethals could conceivably have turned out to be a hazard in the female, where oögonia are not present in the adult[10] and all germ cells are in primary oöcyte stage. However, it was shown that death due to dominant lethals occurs very early in embryonic development.[6] Translocations, which might also have proved to be a hazard in females, were shown to be induced with only low frequency.[9]

Concurrently with the work on chromosome aberrations, experiments were conducted to determine whether there were conditions of radiation that would minimize the sterilizing effect and so provide a more practical method for measuring specific locus mutation rates in females. Results from such investigations on fertility, taken together with the studies of dominant lethals and ovarian histology, aid not

only in the design of experiments but also in the interpretation of work on the genetic effects of radiation. Early experiments indicated that the radiation effect on female productivity was lessened by increasing fractionation of X-ray dose.[2, 11] More recent work shows that chronic gamma irradiation is even less sterilizing and that there is clearly an inverse relationship between dose rate and total young subsequently produced.[3] An example of the relative effectiveness of acute X-rays, fractionated X-rays, and chronic gamma rays is provided by the following data from the females used in our experiments: after receiving 300 r acute X-rays, a female produces, on the average, 7.6 young before sterility sets in; with the same total dose given in five 10-r fractions per week, she produces 13.7 young; if about the same weekly dose (47.2 r/wk) is, instead, administered continuously as chronic gamma radiation, females produce, on the average, 72.7 and 59.1 young for total doses of 256 and 342 r, respectively. A dose distribution that is compatible with long-continued fertility not only is of practical advantage in experiments but also tests the situation that is potentially most hazardous in humans, namely, that in which a high total dose is absorbed with minimal interference in transmission of genetic damage to descendants. On the basis of these results and considerations, the present specific locus experiment was started.

In this experiment (101 × C3H)F_1 young adult females were exposed to 258 r of gamma radiation from a cesium^{137} source at an intensity of 86 r/week. After removal from the radiation field, they were mated with T-stock males and the offspring examined for mutations at the seven specific loci. The results to date, including those from control females, are given in Table 2. Only two mutations have

TABLE 2

MUTATIONS AT SPECIFIC LOCI IN FEMALE MICE EXPOSED TO CHRONIC GAMMA IRRADIATION

Dose (r)	No. of Offspring	No. of Mutations at Locus							
		a	*b*	*c*	*d*	*p*	*s*	*se*	*d se*
0	29,739	...	...	...	...	...	...	...	...
258	22,234	...	1	...	...	...	...	...	1

occurred, one at the *b* locus and the other, presumably a deficiency, involving simultaneous loss of the phenotypic effects of the closely linked *d* and *se* loci.

The induced mutation rate of 5×10^{-8}/r/locus (95 per cent confidence interval: 0.6 to 18×10^{-8}) is significantly lower than the rate of 28×10^{-8}/r/locus already cited for irradiation of males at the comparable dose level of 300 r of X-rays delivered at 83 r/min.[4] In addition to sex, there are two other obvious differences between these experiments on males and females, namely, intensity and quality of radiation. The weight of genetic evidence from other organisms indicates that neither of these factors is likely to be of much importance, although the difference in quality between the X- and the gamma radiation used might be expected to have a slight effect. Thus, on the basis of the evidence considered so far, it would be tempting to conclude that the low mutation rate obtained in the experiment with females is due to sex. However, preliminary data from males exposed to chronic gamma radiation from the same cesium^{137} source also show a low mutation rate,[12, 4] which, as far as it goes, is not significantly different from the female mutation rate.

Two alternative hypotheses may be formulated on the basis of these findings. According to the first hypothesis, the low mutation rate with chronic gamma irra-

diation is due to the same factor in males and in females. This could be either the lower intensity or the quality of radiation, although the weight of earlier genetic evidence is against both factors. According to the second hypothesis, the low rates in males and females are due to different causes. Thus the rate in males irradiated with chronic gamma rays may be low because of indirect factors, to be discussed below, while in females, where such indirect factors cannot be easily invoked, the low rate may be characteristic of the sex or of the oöcyte stage.

Possible indirect factors that may affect the mutation rate measured following irradiation of spermatogonia have been discussed elsewhere.[4, 5] They may be summarized briefly by saying that it is possible that the low mutation rate observed in chronic gamma experiments with males may have resulted from death of sensitive cells in the testis. The marked variation in sensitivity of spermatogonia to killing by irradiation[13, 14] and the observed actual reduction in mutation rate in an experiment with a high dose (1000 r) of acute radiation[15, 4] may both be taken as support for such a hypothesis. If this is the answer, then the low mutation rate observed in the chronic irradiation experiments with males would be a peculiarity of the levels of intensity and dose used. With males at lower doses or intensities, where the killing of spermatogonia or other interference with the dynamics of spermatogenesis was reduced to an unimportant level, one would expect, on this hypothesis, a mutation rate as high as, or even higher than, that found with acute irradiation. As has been pointed out elsewhere,[4,12] since this hypothesis is at least plausible, it would be incautious to conclude at the present time that chronic gamma irradiation of males will, at all doses and intensities, be less effective than acute X-irradiation.

However, it seems unlikely that an indirect factor of the type discussed for males would apply to the present data on females. Since oögonia do not occur in the adult ovary,[10] the chronic irradiation will be received by oöcytes, which, furthermore, for the bulk of the dose will be in the remarkably uniform dictyate stage. These oöcytes are, therefore, quite different from spermatogonia, which are going through rapid cycles of mitosis and consequently might be expected to show marked differential sensitivity to radiation. Thus it is reasonable to predict that the low mutation rate per roentgen observed in the present data may hold for other doses and intensities of chronic gamma irradiation in females. It also seems reasonable to assume that this information can be carried over to humans.

Summary.—Mutation rates in females have been measured by the specific locus method in two experiments.

A relatively small experiment with acute X-rays, at a dose which rapidly sterilizes females, was nevertheless sufficient to show that if the average risk of equal exposure of both sexes to acute X-rays is calculated on the basis of the far more reliable rate determined in males, the hazard will almost certainly not be underestimated by more than a factor of 2.

An experiment with chronic gamma rays, which allowed almost normal fertility of the females, has, to date, given a rate of 5×10^{-8}/r/locus with a 95 per cent confidence interval of 0.6 to 18×10^{-8}. This rate is significantly below that of 28×10^{-8}/r/locus found in males irradiated with a similar dose of acute X-irradiation. Possible reasons for the lower rate are discussed. Whatever the explanation turns out to be, the results indicate that the mutation rate in women exposed to chronic

gamma radiation may be less than that estimated from our earlier yardstick, namely, the mutation rate obtained from acute X-irradiation of male mice.

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FUNCTIONAL EQUATIONS IN THE THEORY OF DYNAMIC PROGRAMMING. IX

VARIATIONAL ANALYSIS, ANALYTIC CONTINUATION, AND IMBEDDING OF OPERATORS

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1. *Introduction.*—Consider the formal identity

$$\max_x [(x, Ax) - 2(x, y)] = (y, A^{-1}y), \tag{1.1}$$

where x is an element of a space S, and A is a symmetric operator defined over S with the property that (x, Ax) is negative definite. Since the Euler equation associated with this variational problem is $Ax = y$, we see that the element x furnishing the maximum is given in terms of the inverse operator. For the case of ordinary or partial differential operators; these operators are expressible in terms of Green's functions.

On the other hand, several classes of variational problems of this type can be treated by means of the functional equation technique of dynamic programming. Combining the two approaches, we can derive a number of properties of Green's functions. For the case of second-order linear differential operators, these techniques were applied in Bellman[1] and Bellman and Lehman,[2] while the classical Hadamard variational formula for Green's function associated with second-order