THE EFFECT OF PATERNAL X-RAY EXPOSURE ON THE SECONDARY SEX RATIO IN MICE (F_1 GENERATION)[1]

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WHEN the mouse testis receives an X-ray dose greater than 350r, fertility ceases within four weeks and, after an interval of sterility which may last several months (depending on the dose), fertility is restored. During the presterile and poststerile periods, matings with unirradiated females yield significantly different litters. RUSSELL (1954) has reviewed the earlier work on this point, especially the important experiments of SNELL (1933), HERTWIG (1938), and STRANDSKOV (1932), as well as his own. Litter size in the presterile period decreases by 15 to 85 percent as the X-ray dose is increased from 200 to 1000r, but the sex ratio of the progeny remains constant. The reduction in litter size is the result of embryonic or fetal death, not of a failure in fertilization. These results are analogous to those in Drosophila, with the exception that in the latter the sex ratio tends to change in favor of the males (CATCHESIDE and LEA 1945).

In contrast, the change in litter size during the poststerile period is small or questionable, as is the change in sex ratio, even after doses up to 1200r. The difference is considered to be due to the type of germ cell irradiated: the spermatozoa of the presterile period were irradiated as such, or matured from irradiated spermatids, or perhaps spermatocytes; the spermatozoa of the poststerile period are derived from irradiated stem cells whose progeny matured successfully.

In the course of an experiment designed for another purpose, in which irradiated males were mated in the poststerile period with unirradiated females, we observed an increase in the sex ratio of the progeny from 42 to 52 percent males, which was significant at the five percent level by the χ^2 test and obviously differed from results reported in the literature. In view of the exceptionally low sex ratio in our unirradiated stock (42 percent males), we entertained the possibility that this stock might be peculiarly suitable for the demonstration of such an effect. In order to investigate the matter further, the original experiment was enlarged and a second independent one was set up in which the regression of sex ratio on X-ray dose was determined. The data from both experiments are reported here.

MATERIALS AND METHODS

The mice used in these experiments were obtained from the Cancer Research Genetics Laboratory, University of California, Berkeley. The CAF_1 males were

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produced by mating BALB/cCrgl females with A/Crgl males. The original BALB/c stock, in its 50th generation, was obtained from Andervont (National Cancer Institute) and was bred through 11 more generations; the original A/He stock, in its 84th generation, was obtained from Heston (National Cancer Institute) and was bred through 15 more generations.

The animals used in our experiments were fed Purina Laboratory Chow Checkers and water *ad lib.*

The CAF_1 males were irradiated to the entire body (Experiment 1) or to the posterior 2.5 cm of the body (Experiment 2), as described previously (KOHN and KALLMAN 1956). The radiation factors were: 250 kvcp X-rays, HVL 1.5 mm Cu; exposure dose rate in the testes, 40–44r/min. The absorbed dose (rads) in the testes was taken to be five percent less than the exposure dose (r) in the testes.

The matings were carried out in both of the experiments by placing one male in a cage with three or five females for four or five weeks. When a female became pregnant, she was placed in a separate cage in which she raised her litter until weaning (at about age 35 days). In the first experiment the cages were examined only five days a week so that the data at birth were less accurate than those of the second experiment, in which the cages were examined at least once each day, and the progeny were sexed both at birth and at weaning. The data at weaning were used to correct errors in sexing at birth; about one percent of the diagnoses at birth required this correction.

RESULTS

Experiment 1: The CAF_1 males that had been irradiated (whole body) at age 385 days (±30 days) were mated at age 700 days (±30 days) with virgin unirradiated BALB/c females (ages 71–113 days).

It was considered important that the general health of the samples of males selected for breeding should be uniform. Therefore, the 18 males mated from each dose group were selected at random from the members of the group weighing at least 26 gm and having lost less than 2 gm during the preceding three months.

In brief, for the three dose groups, the sex ratio (fraction of males) at weaning was as follows:

(1) 0 rads: 216 progeny, sex ratio, 0.416;
(2) 525 rads: 182 progeny, sex ratio, 0.522;
(3) 2 × 262 rads: 239 progeny, sex ratio, 0.397.

The low sex ratio of the control group, 0.42, was not unexpected, since ratios in the range 0.44–0.48 were being obtained in BALB/c and CAF_1 matings; it was therefore not considered primarily due to the advanced age of the fathers. The sex ratio of the group given two doses of 262 rads (eight days apart) did not differ significantly from that of the controls. The high sex ratio for the group given 525 rads was surprising. Statistical tests (χ^2) showed significant heterogeneity among the three groups and that the result at 525 rads was significant at the five percent level.

To examine the matter further, it was decided to mate the males again (the median life span of the CAF_1 male is 870 days). Of the 18 males originally in each group, the following survived for the second mating (at about 820 days of age): 16 in group 1; ten in group 2; 16 in group 3. Each male was caged for five weeks with five females (one of whom had previously had a litter). The combined results for both matings are shown in Table 1. The elevation in sex ratio of group 2 was no longer significant, and the result for the pooled data of groups 2 and 3 (the irradiated groups) was almost identical to that of the control group. It was concluded that the difference in sex ratio originally observed may have been the result of random variation and that any further decision would have to rest on the results of a second independent experiment. With respect to progeny per litter, irradiation appeared to have reduced this from 5.5 to 5.0.

Experiment 2: Nine-month-old CAF_1 males (ten per group) were irradiated to the pelvic region; the absorbed doses received by each group are detailed in Table 2. Seven months later a series of four breedings five weeks apart was begun, arranged so that the first two were with virgin females (age 75–150 days) and would yield first litters, and the third and fourth would yield principally second litters. From each dose group, seven males were used; the ratio was three females per male: a total of 672 litters for eight groups was possible in four breedings—the yield was 577, or 86 percent.

The first breeding employed 168 virgin BALB/cCrgl females. The second breeding employed another 168 virgin females. The third breeding employed 116 females from the first breeding (whose litters were weaned) plus 52 virgins. The fourth breeding employed 132 females from the second breeding plus 36 virgins. Each male participating in the four breedings, therefore, had been caged with a total of 12 females; in each breeding the distribution of females was strictly random. One breeding male died in each of groups 6, 7, and 8, and was replaced by a "spare". Also, in groups 4, 5, and 8, one breeding male from each whose fecundity seemed low was replaced by a spare.

The over-all experimental design allowed two subsidiary factors to be examined for their possible influence on sex ratio and litter size.

First, the results showed that at birth and at weaning the sex ratio is practically the same and that it is independent of the parity of the female (zero or one), although the size of the first litter is smaller than the second, as is well known.

TABLE 1

Experiment 1: Number and sex ratio of progeny at weaning from mating whole-body irradiated CAF_1 males with unirradiated BALB/cCrgl females

Group	Dose (rads)	Litters (at birth)	Males	Females	Total: ♀+♂	Sex ratio*	Progeny/ litter†
1	0	81	185	260	445	0.416	5.5
2	525	63	145	168	313	0.46	5.0
3	262 × 2	84	161	268	429	0.38	5.1
Pooled:							
2 + 3		147	306	436	742	0.413	5.0

* ♂/(♂ + ♀).

† (Total weaned)/(No. of litters).

TABLE 2

Experiment 2: Number and sex ratio of progeny at birth from mating partial-body irradiated CAF_1 *males with unirradiated BALB/cCrgl females*

Breeding*	Dose group (rads)	Litters	Male	Female	Sex ratio	♂/Litter†	♀/Litter†	Progeny/litter†,‡
1–4	All	577	1,709	2,181	0.439	2.96	3.78	6.81
1 + 2	All	279	752	955	0.441	2.70	3.42	6.17
3 + 4	All	298	957	1,226	0.438	3.21	4.11	7.41
1 + 3	All	297	832	1,113	0.428	2.80	3.75	6.59
2 + 4	All	280	877	1,068	0.451	3.13	3.81	7.04
1–4	0	76	234	287	0.449	3.08 ± 1.7	3.78 ± 1.8	6.95 ± 2.2
	248	75	205	304	0.403	2.73 ± 1.6	4.05 ± 1.8	6.80 ± 2.4
	360	78	234	274	0.461	3.00 ± 1.8	3.51 ± 1.9	6.68 ± 2.6
	495	72	232	266	0.466	3.22 ± 1.6	3.69 ± 1.9	6.96 ± 2.4
	248 × 2	74	246	288	0.461	3.32 ± 1.6	3.89 ± 1.8	7.32 ± 2.1
	610	70	211	263	0.445	3.01 ± 1.7	3.76 ± 1.8	6.77 ± 2.5
	720	66	182	264	0.408	2.76 ± 1.6	4.00 ± 2.0	6.85 ± 2.5
	248 × 3	66	165	235	0.412	2.50 ± 1.7	3.56 ± 1.6	6.08 ± 2.5

* In breedings 1 and 2, the females were virgin; in breedings 3 and 4, 75 percent had had one litter. In breedings 1 and 3 the females were from one pool, and in breedings 2 and 4, from another one.

† ± standard deviation.

‡ Thirty-nine partially eaten progeny that could not be sexed are included in progeny per litter.

This conclusion is based on comparison of the pooled data of breedings 1 and 2 (first litters only) with those of breedings 3 and 4 (over 70 percent second litters). The sex ratio was 0.44 in both groupings at birth, but the mean litter sizes were 6.2 and 7.4, respectively (Table 2). At weaning, the corresponding figures were 0.45, 4.8, and 5.4.

Secondly, although the sex ratio may have depended slightly on variations between different populations of females, the indicated effect was quite small and was not statistically significant. The sex ratio at birth for breedings 1 and 3 was 0.43, for breedings 2 and 4, 0.45; similar results were obtained at weaning (Table 2).

In Experiment 1, the preliminary result (but not the final one) had suggested that fractionating the X-ray dose might influence the ratio. In Experiment 2, fractionation of the dose did not affect the sex ratio at birth or weaning, nor did it have a consistent effect on litter size (dose groups 248 × 2 and 248 × 3 compared with 495 and 720, respectively (Table 2).

To estimate the effect of dose, unweighted linear regressions of the form $y = a + bx$ were calculated, in which y is the mean number of males, of females, or of males plus females per litter (or, the sex ratio), and x is the absorbed dose in rads. The values for the parameters a and b are given in Table 3. In no instance was the slope b significantly different from zero.

Since Experiments 1 and 2 were quite independent, the sex ratios at weaning may be compared. As noted above, the single-dose and fractionated dose groups of the first experiment did not differ from the control, nor did the corresponding groups in the second experiment (495 and 248 × 2). These corresponding groups

TABLE 3

*Values of parameters in regression lines**

y	a	b
At birth†		
Male/litter	3.123	—0.000372±0.000417
Female/litter	3.819	—0.000086±0.000314
(Male + female)/litter	7.040	—0.000520±0.000528
Male/(male + female)	0.4509	—0.000028±0.000041
At weaning		
Male/litter	2.489	—0.000376±0.000237
Female/litter	2.791	+0.000031±0.000309
(Male + female)/litter	5.281	—0.000351±0.000321
Male/(male + female)	0.473	—0.000045±0.000043

* $y = a + bx$, where x is the absorbed dose in rads.
† Based on data in Table 2.

within each of the two experiments were therefore pooled, with the following results:

Experiment 1: 491 males, 696 females; sex ratio 0.414
Experiment 2: 545 males, 602 females; sex ratio 0.475

The value of χ^2 is 8.94, and $P < 0.005$. The sex ratio was therefore significantly different in the two experiments.

DISCUSSION

Two sets of experimental data in the literature for large numbers of mice from irradiated fathers are available (HERTWIG 1938, RUSSELL 1954). The chief conclusion from these and our own, drawn with due regard to the possibility of inter- as well as intraexperimental variation, is that for exposures up to 1000r a clear-cut change in sex ratio has not been demonstrated. If a change is indicated, it is probably in the direction of a decrease. The specific results are as follows:

HERTWIG pooled the data from various experiments in her laboratory, chiefly with the dilute blue strain (*dd bb aa CC*). She concluded that no significant change in sex ratio occurred after exposures of 400–1000r. We have fitted an unweighted regression line (sex ratio $= a + bx$) to these data based on 2595 control progeny and 2006 progeny from irradiated fathers; $a = 0.4931$, $b = + 0.0000177 \pm 0.0000204$. After 1200–1600r, the ratio rose abruptly to about 0.59. HERTWIG remarked that this last result, based on only 234 progeny, depended on the abnormally high ratios associated with several fathers, rather than a uniform increase in the ratio for all, and that further study was needed.

RUSSELL, for the stock used in his genetics experiments, found a sex ratio of 0.5100 (55,828 progeny) in the controls and 0.5035 (72,472 progeny) after 600r. Calculated by us, the slope *b*, based on these two points, is — 0.000011. The difference between the controls and experimental animals is barely significant: $\chi^2 = 5.3$; $P \backsim 0.025$.

In the second experiment of the present series, the regression analysis, based on 3890 progeny at birth, estimated that $a = 0.4509$ and $b = - 0.0000279 \pm$

0.0000412 (Table 3). In the first experiment, based on two points (zero and 525 rads) with 1187 progeny (at weaning), $a = 0.416$ and $b = -0.000006$.

The results with mice are at variance with the conclusions of SCHULL and NEEL (1958) for man, based on their study of Japanese data. For the comparable class of Japanese data (exposure of fathers only), there were the following estimated exposures and progeny: 0r, 46,166; 8r, 5463; 75–100r, 1309; 200r, 753. The value of b was $+ 0.000056$, but it was not significantly different from zero. SCHULL and NEEL, however, concluded that a significant increase had occurred, based on the following argument: they predicted the changes in the mammalian sex ratio after irradiation on the assumption that the Y chromosome is physiologically inert, whereas the X chromosome is susceptible to injury that can express itself as a dominant lethal; they found that the signs of the slopes of the regression lines fitted to several classes of data were in accord with the theory, even though the slopes of the individual regression lines were not significantly different from zero.

To the writer, such an argument seems insecure. SCHULL and NEEL were, of course, aware of the fact that small changes in the sex ratio might stem from causes other than radiation (Brit. Med. Journal Editorial 1953; MACMAHON and PUGH 1954). Furthermore, it is now known that the Y chromosome is physiologically active, exerting a positive masculinizing force in the mouse (WELSHONS and RUSSELL 1959) and in man (JACOBS and STRONG 1959; FORD, POLANI, BRIGGS and BISHOP 1959). The specifically stated theoretical basis of SCHULL and NEEL's argument has therefore been proved incorrect. The argument, however, might be advanced in a modified form, namely, that the smaller size of the Y chromosome renders it less likely to be the site of a dominant lethal hit than the X chromosome. Only further experimentation can provide the proof for this conjecture. Meanwhile, it is suggested that our view of the effect of radiation on the sex ratio in mammals be an empirical one.

In the present experiments, X-rays had no effect on the sex ratio as tested by linear regression (Table 3). The validity of the linear hypothesis, however, might be questioned. The sequence of ratios in Table 2 suggests but does not establish that the sex ratio fell after 248 rads, then returned to the normal range, and finally fell once more after 720 rads. It is of interest that the results of duplicate, independent determinations at 495 and 720 rads appeared to be in much closer agreement than would have been expected if the observed variation between doses had been a good estimate of random variation. An indication of a nonlinear response has been observed in the case of X-ray treated rooster sperm; after about 250r, the sex ratio fell, but it was normal after higher doses (DEMPSTER, LERNER and INOUYE 1959).

A significant change in sex ratio occurred between the first (0.414) and second (0.475) experiments of the present series; the cause is not known, but the difference in paternal ages may have been a contributing factor. The difference in radiation exposure (partial- and whole-body) is considered to be an extremely unlikely factor. In this connection, it may be noted that although genetic factors are known to be important determinants of the variations in sex ratio (KING 1918), the mechanisms involved have not been precisely described in mice

(HOWARD, MCLAREN, MICHIE, and SANDER 1955; WEIR, HAUBENSTOCK and BECK 1958), nor have generally accepted theories been found to cover the differences between or within other mammalian species (GINI 1951; DAHLBERG 1951; MCKEOWN 1956–57; LINDAHL and SUNDELL 1958).

SUMMARY

CAF_1 male mice, irradiated with 250 kv X-rays, were mated during the post-sterile period with unirradiated BALB/cCrgl females. For absorbed doses in the range 0–720 rads, the slopes of the following regression lines were not significantly different from zero: males per litter, females per litter, progeny per litter, and sex ratio (both for data at birth and at weaning).

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