EMBRYONIC MORTALITY IN MOUSE POPULATIONS AFTER ELEVEN GENERATIONS OF SPERMATOGONIAL IRRADIATION

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WE have empirically approached the question of what will happen to the genetic constitution of an irradiated population of mice. That is, we have exposed populations of mice to radiation in a certain way for several successive generations and are now prepared to compare the progeny in the irradiated and nonirradiated populations by a variety of criteria. The empirical and analytical approaches to this question are complementary, not mutually exclusive. The approach we have used draws heavily upon the results of previous experiments of the analytical type (GREEN 1959, 1960). It has the potentiality of revealing any effect of long-continued irradiation of a genetic system, but it suffers from the disadvantage of not yielding by itself a clear-cut genetic interpretation of any difference that may be found. The analytical approach has the advantage of working with known types of mutations and aberrations, but the disadvantage of not being able to predict what will happen if those same changes are suffused into a population.

In 1956 we started 12 populations of mice each with 16 mated pairs per generation with the mates chosen at random from the mice available in each generation except that brother-sister matings were excluded. The mice of the 192 initial matings were all descended from a single pair in an inbred strain, C57BL/10Gn. For brevity this is called the "inbred" experiment and is denoted by RX-ML. The 12 populations represent all possible combinations of three levels of radiation exposure with four levels of inbreeding. The radiation exposures were 0, 50, and 100r applied only to the gonads of young adult males with bodies shielded, seven weeks before mating. The four inbreeding levels were determined by the number of interbreeding pairs of mice in sublines within each population. Another experiment with exactly the same radiation and breeding plan was started in 1958 with 192 matings between mice descended from a 4-way cross of the C3HeB/FeJ, C57BL/6J, DBA/2J, and BALB/cJ strains. This is called the "hybrid" experiment and is denoted by DX-ML. Further details about the plan of the experiments appear elsewhere (GREEN 1964a).

Waiting for seven weeks after irradiating the testes before making up the matings ensures that the sperm are descended from irradiated spermatogonia (OAKBERG 1957). This means, further, that any mutations induced in the spermatogonia and subsequently transmitted to the progeny will most probably be recessive and that a large fraction of these, maybe as many as 75 percent, will be lethal when homozygous (RUSSELL and RUSSELL 1959). The probability for two mutant alleles, identical by descent from a single mutation, to unite at fertiliza-

tion in a subsequent generation depends upon the effective breeding size or the inbreeding coefficient. For this reason we have arranged for four different levels of inbreeding in these small randombreeding populations. A further question is whether induced mutations, satisfying the usual criteria for recessives, may have some detectable and, possibly, deleterious effects in heterozygotes. Of course, the probabilities of heterozygotes also vary with the inbreeding coefficient (WRIGHT 1951).

OBSERVATIONS AND EXPERIMENTS

Differences between the 12 populations in each experiment may be searched for in an almost infinite variety of ways. These include measures of reproductive and physiological fitness and breeding tests for the presence of recessive visible and lethal mutations.

The first and most notable result of comparing the populations in both the "inbred" and the "hybrid" experiments is that the mice all look about alike or are well within the range of normal variation. There are not any markedly conspicuous differences in appearance of the mice from one population to another. In the "hybrid" populations there are, of course, several different coat colors derived from the different alleles at four coat-color loci in the double-cross hybrids. This is not to say that there have been no genetic changes in these populations, but merely that, by the criterion of the appearance of the survivors at weaning age and beyond, there are no striking differences.

The differences between the populations, if there are any differences at all, will probably be fairly small and expressed in subtle ways in generalized or quantitative fitness traits. We have compared the populations with respect to various measures of reproductive fitness, including incidence of sterile matings, sizes of first and second litters, and intervals of time between makeup and birth of the first litter and between birth of the first and second litters. There were no detectable differences between the populations in the first ten generations associated with the three levels of radiation exposure or the four levels of inbreeding (RODERICK and SCHLAGER 1964).

The failure to find an effect on reproductive performance measured by the first few litters rather than by the entire lifetime production of litters is not a consequence of the relatively low levels of radiation, 50 and 100r, used in these experiments. We have found that populations of mice, both "inbred" and "hybrid," can persist at least eight generations with an exposure of paternal gonads in each generation to a very large but substerilizing dose of X rays (GREEN and LES 1964).

Samples of mice from several generations have been set aside for measuring lifespan. The experiment is not complete and no results are reportable as yet.

One test for differences between the populations has given suggestive results. Samples of mice from each of the 12 populations of the "inbred" and "hybrid" experiments were set out after 0, 3, 6, and 9 generations of spermatogonial irradiation for measures of lifetime reproductive performance. This test is different from those done with the pairs of mice used to propagate the populations since

the propagating pairs are allowed to breed only for a few months during which they produce one, two, or three litters. The lifetime test of reproductive performance showed that the "inbred" populations and possibly also the "hybrid" populations are different after nine generations of spermatogonial irradiation. The "inbred" populations receiving 100r per generation reproduced for a shorter period of time, produced fewer litters, and a smaller total number of progeny than did the control (0r) populations. The populations receiving 50r of spermatogonial irradiation each generation were intermediate between the 100r and the 0r populations. The mean litter size was not noticeably affected, nor was the mean interval of time between litters. Crudely stated the results suggest that the progeny of irradiated mice wear out sooner than the progeny of nonirradiated mice in a lifetime test of reproductive performance. The results with the "hybrid" populations may later turn out to be the same. At the end of nine generations the issue is in doubt (GREEN 1964b).

We have not carried out breeding tests to detect the presence of recessive visible and recessive lethal mutations using the methods developed by SNELL (1935), CARTER (1957), and HALDANE (1956, 1957). It is not likely that we shall be able to do so without additional facilities since all tests require at least two generations and large numbers of progeny for each tested parent, even after the appropriate testing stocks have been synthesized.

The presence of recessive lethals may be detected, possibly more efficiently, by estimating the proportion of dead fetuses in litters about 15 to 17 days of gestation in brother-sister matings in irradiated lines relative to controls. This paper will be devoted to describing the results of an experiment to do so. The results suggest that in these populations of mice the proportions of zygotes surviving to implantation, the proportions of implanted zygotes surviving to produce fetuses, and the proportions of fetuses surviving until birth are relatively unaffected by 11 generations of 50 and 100r of spermatogonial irradiation. Further the slight variation in level of inbreeding within populations appeared to make no difference, although a large difference between "inbred" and "hybrid" mice appeared as expected.

MATERIALS AND METHODS

Mice: Excess males and their female sibs, not needed for propagating the RX-ML and the DX-ML populations, were saved for this experiment from six of the "inbred" populations and from six of the "hybrid" populations in the 11th generation after spermatogonial irradiation was started. All mice were born in the period from June to September, 1963. None of the males or females of this experiment were exposed to radiation. They are descendants of irradiated or control parents. Female mice of the C57BL/6J strain were also used in this experiment. They were not irradiated, had no history of ancestral irradiation, and were unrelated to the RX-ML and DX-ML mice.

The six populations of RX-ML and the six populations of DX-ML represent all combinations of two levels of inbreeding, denoted by S3 and S4, and three levels of radiation D1, D2, and D3 (GREEN 1964a). The S4 populations were propagated as single lines with 16 pairs per generation with random mating excluding brother-sister matings. The S3 populations each contain two lines with eight pairs per generation. The S3 populations are thus slightly more inbred than the S4 populations. The three radiation dose levels were 0, 50, and 100r, respectively, applied to the gonads of male ancestors in each generation with bodies shielded.

Matings: Each male of each DS population, both "inbred" and "hybrid," was mated to one sister and also to one unrelated female of the C57BL/6J strain. The objective was to have each brother-sister mating matched with an outcross mating as a control. This was realized in 832 or 416 pairs of females in 902 matings in the experiment as a whole. It will be convenient to denote the matings as:

- RX-RX: brother-sister matings within the "inbred" populations;
- RX-C57: outcrosses of males of the "inbred" populations with females of the C57BL/6J strain;
- DX-DX: brother-sister matings within the "hybrid" populations;
- DX-C57: outcrosses of males of the "hybrid" populations with females of the C57BL/6J strain.

All mice were of breeding age, but none had previously been mated. Females were introduced to the home pen of the male and examined for copulation plugs daily thereafter to time the pregnancy as accurately as needed for this experiment. Some females became pregnant without our having observed copulation plugs. All females were dissected between 15 and 18 days of pregnancy as estimated from the date of the plug or from the abdominal swelling of the females. The females were coded so that the dissector had no knowledge of the type of mating. All females were black nonagouti except that the females of the DX-DX matings had a variety of coat colors.

Dissections: Pregnant females were killed with carbon dioxide. The abdomen was opened, the horns of the uterus spread out, and the ovaries exposed while still in their capsules. After the capsule was ruptured the ovaries were examined under a dissecting microscope to facilitate counting the number of corpora lutea. Each uterus was slit lengthwise to reveal the living and dead fetuses and the deciduomata or "moles." The fetuses were preserved in Tellyesniczky's fluid for later examination of external features.

Records: Each dissection yielded the following information: Experiment: "inbred" RX-ML or "hybrid" DX-ML; Dose-size: DS13, 14, 23, 24, 33, 34; Mating: brother-sister or outcross; Number of corpora lutea, C ; Number of "moles," M ; Number of dead fetuses, D ; Number of living fetuses, L . The sum $T = M + D + L$ estimates the number of implantations and $C - T$ estimates the number and $(C - T)/C$ the proportion of preimplantation deaths; M/T , the fraction of implantation deaths; and $D/(D + L)$, the fraction of prenatal deaths among those fetuses surviving into the postimplantation period.

The data were treated in two ways, one disregarding the pairing of two females (sister and unrelated) with each male so as to use the data from all 902 dissections; the other taking account of the pairing and using only the 416 matched pairs. The data were transferred to punched cards and all analyses were carried out on an IBM 1620 computer. Several analyses were done after transforming the ratios to angles (arcsin transformation), both weighted and unweighted. The results summarized in the next section are all based upon the unweighted untransformed ratios since neither transformation nor weighting produced changes in the interpretations.

RESULTS

Before the relationship of radiation exposures and inbreeding levels to embryonic mortality can be examined, it is necessary to see if any of a number of associated uncontrollable variables has influenced the results.

Associated variables: We estimated the ages of development of the fetuses by comparing features of their external development with GRÜNEBERG's (1943) standard. If the fetuses of one group were more frequently observed at a later age than another, this might influence the frequencies of deaths, especially late deaths. An analysis showed that this variable was not important. The average age of the litters at the time of examination was very close to 15 days in all groups. Marked variation in the ages of the dams could likewise bias the results. In general the groups had about the same distributions of ages of the dams. The few exceptions were sporadic.

The regression of the number of corpora lutea on age of the dam was not significant in all but one of the 24 groups of females. In the outcross of RX-ML males to C57BL/6J females in DS14 the regression coefficient was 0.017 ± 0.008 corpora lutea per day of dam's age. It is apparently best to regard this as a random statistically significant result.

Radiation exposure: The mean numbers of corpora lutea, "moles," dead fetuses, and living fetuses in each of the 24 groups of females are shown in Table 1, along with the numbers of pregnant females dissected. The most striking thing about the data is that the mean numbers of corpora lutea *C* in the outcrosses are consistently lower than the mean numbers in the brother-sister matings. This may mean that, in this measure of reproductive potential, the "inbred" RX and "hybrid" DX females in general are superior to females of one of the standard inbred strains of mice, C57BL/6J. Further, because of the narrow range of variation in *C* from an average of 8.8 to 9.7 in the outcrosses, we gain a measure of

TABLE 1

Mean numbers and standard errors of mean numbers of corpora lutea, "moles," dead fetuses, and living fetuses in pregnant females of brother-sister and outcross matings in six "inbred" RX and six "hybrid" DX populations

Experiment	DS	Mating type	Females	<i>C</i>	<i>C - T</i>	<i>M</i>	<i>D</i>	<i>L</i>
"Inbred" RX	13	B × S	38	11.1 ± 0.4	2.4 ± 0.4	2.0 ± 0.3	$.11 \pm .06$	6.7 ± 0.5
		Outcross	36	8.9 ± 0.3	0.9 ± 0.2	1.4 ± 0.2	0	6.5 ± 0.4
	23	B × S	23	10.9 ± 0.4	1.7 ± 0.4	1.5 ± 0.2	$.04 \pm .04$	7.7 ± 0.5
		Outcross	28	9.5 ± 0.3	1.7 ± 0.4	1.0 ± 0.2	$.07 \pm .05$	6.7 ± 0.4
	33	B × S	27	11.6 ± 0.4	2.0 ± 0.4	1.6 ± 0.3	$.11 \pm .06$	7.8 ± 0.4
		Outcross	34	8.8 ± 0.2	1.0 ± 0.3	1.6 ± 0.2	$.06 \pm .04$	6.1 ± 0.4
	14	B × S	19	10.4 ± 0.4	2.2 ± 0.6	1.5 ± 0.4	0	6.8 ± 0.6
		Outcross	28	9.3 ± 0.2	1.3 ± 0.3	1.4 ± 0.3	$.04 \pm .04$	6.6 ± 0.3
	24	B × S	21	10.1 ± 0.4	1.9 ± 0.4	1.6 ± 0.4	$.05 \pm .05$	6.9 ± 0.6
		Outcross	26	9.5 ± 0.4	2.1 ± 0.4	0.7 ± 0.2	$.12 \pm .06$	6.6 ± 0.4
	34	B × S	26	10.1 ± 0.3	0.8 ± 0.2	2.0 ± 0.3	0	7.5 ± 0.4
		Outcross	34	9.1 ± 0.3	1.4 ± 0.3	1.4 ± 0.2	$.03 \pm .03$	6.3 ± 0.4
"Hybrid" DX	13	B × S	48	11.7 ± 0.4	1.6 ± 0.4	1.7 ± 0.3	$.15 \pm .05$	8.5 ± 0.4
		Outcross	50	9.4 ± 0.3	1.8 ± 0.3	1.4 ± 0.2	$.10 \pm .04$	6.1 ± 0.4
	23	B × S	46	11.1 ± 0.2	1.5 ± 0.3	1.7 ± 0.3	$.15 \pm .06$	7.9 ± 0.4
		Outcross	44	9.5 ± 0.2	1.1 ± 0.2	1.8 ± 0.2	$.14 \pm .08$	6.4 ± 0.4
	33	B × S	42	11.0 ± 0.3	0.9 ± 0.2	1.9 ± 0.3	$.33 \pm .13$	8.0 ± 0.4
		Outcross	41	9.2 ± 0.2	1.4 ± 0.3	1.8 ± 0.2	$.07 \pm .05$	6.0 ± 0.4
	14	B × S	46	10.6 ± 0.3	1.1 ± 0.3	1.4 ± 0.2	$.11 \pm .06$	8.2 ± 0.4
		Outcross	46	8.9 ± 0.3	1.2 ± 0.3	1.3 ± 0.2	$.11 \pm .09$	6.3 ± 0.4
	24	B × S	52	11.9 ± 0.2	1.2 ± 0.3	1.2 ± 0.2	$.21 \pm .07$	9.5 ± 0.3
		Outcross	54	9.2 ± 0.3	1.4 ± 0.2	1.4 ± 0.2	$.02 \pm .02$	6.4 ± 0.3
	34	B × S	46	13.0 ± 0.4	2.2 ± 0.4	1.3 ± 0.2	$.13 \pm .05$	9.5 ± 0.3
		Outcross	47	9.7 ± 0.2	1.1 ± 0.2	1.4 ± 0.2	0	7.2 ± 0.3

DS=dose and size. D1 is 0r, D2 is 50r, and D3 is 100r of spermatogonial irradiation in each generation. S3 is 8 pairs and S4 is 16 pairs per population per generation.
C=Number of corpora lutea; *M*=Number of "moles"; *D*=Number of dead embryos; *L*=Number of living embryos;
T=*M* + *D* + *L*, number of implantations; *C - T*=Number of unfertilized ova and preimplantation losses.

FIGURE 1.—Schematic results to show possible effects of spermatogonial irradiation on the number of live fetuses L and on the number of corpora lutea C in brother-sister and outcross matings and to show the differences between the two types of matings in mortality rates at three levels of spermatogonial irradiation.

confidence in the technical accuracy of the results as a whole. Figure 1, with imagined not actual results, will help in interpreting the results in Table 1. For instance, the mean number of corpora lutea C might decrease as radiation increases in brother-sister matings but should be constant in all outcross matings. The mean number of living fetuses L might decrease in both the outcross and brother-sister matings, but more rapidly in brother-sister matings, as radiation increases. The differences between the ratios, $(C-L)/C$, should, under these imagined conditions, increase as radiation increases.

With one exception there are no discernible trends in any of the means of C , M , D , and L relative to the amount of spermatogonial irradiation. The exception is in the increase in C in the "hybrid" DX brother-sister matings in DS14, 24, and 34. The means of C are 10.6, 11.9, and 13.0 for the "0r," "50r," and "100r" groups, respectively, and the differences exceed twice their standard errors in each case. On the surface this appears to mean that spermatogonial irradiation for 11 generations enhances the output of corpora lutea of nonirradiated daughters. But this result did not occur in any of the other three sets of populations. Therefore it appears best to give it no importance.

To pursue the question of differences between the brother-sister and the outcross matings and to take advantage of the paired data, ratios were computed for each female of the number of preimplantation losses to the number of corpora lutea, $(C-T)/C$; of the number of implantation losses or "moles" to the number of implantations, M/T ; and of the number of dead fetuses to the number of dead and living fetuses, $D/(D+L)$. Then the differences between the ratios for the females of the brother-sister matings and the females of the outcross matings were found and averaged. The mean differences for the three ratios are given in Table 2. It may be seen at once that, while one of the differences is individually significant, no pattern of differences emerges. They do not increase as spermatogonial irradiation increases. The 9.3 percent difference in the ratios of preimplantation losses in the DS13 of the "inbred" RX is a difference between nonirradiated brother-sister and outcross matings. No comparable difference appeared

TABLE 2

Mean differences and standard errors of mean differences between the paired brother-sister and outcross matings in percentages computed from the numbers of corpora lutea, "moles," dead fetuses, and living fetuses in pregnant females of matings in six "inbred" RX and six "hybrid" DX populations

Experiment	DS	Pairs	(C-T)/C	M/T	D/(D+L)
"Inbred" RX	13	34	9.3 ± 3.2	2.6 ± 4.2	3.2 ± 2.9
	23	21	-4.1 ± 6.4	6.1 ± 3.6	-1.5 ± 1.0
	33	24	3.8 ± 5.9	-4.2 ± 4.0	1.1 ± 1.1
	14	19	10.0 ± 6.8	4.8 ± 6.4	0
	24	20	-5.0 ± 5.7	8.3 ± 4.4	-1.0 ± 1.9
	34	25	-9.3 ± 4.5	4.4 ± 5.5	-0.6 ± 0.6
	13	48	-7.1 ± 4.5	-2.9 ± 4.5	0.1 ± 1.0
	23	43	3.8 ± 4.6	-4.9 ± 3.1	-0.5 ± 1.5
	33	40	-5.8 ± 4.2	-4.2 ± 4.0	2.4 ± 1.4
"Hybrid" DX	14	45	-3.7 ± 4.0	-4.2 ± 3.3	-1.4 ± 2.4
	24	51	-6.1 ± 3.4	-5.6 ± 2.7	1.8 ± 0.7
	34	46	2.9 ± 3.9	-3.8 ± 2.7	1.2 ± 0.5

Symbols defined in Table 1.

in any of the other three control contrasts. Thus no importance is attributed to this one.

Given the three ratios above and their differences in brother-sister and outcross matings, one may readily find, to a reasonable approximation, many other ratios by making use of the required relationships between these variables. For example, since $T = M + D + L$, the ratio of number of embryonic deaths to implantations is

$$\frac{D}{T} = \frac{D}{D+L} \cdot \frac{D+L}{T} = \frac{D}{D+L} \left(1 - \frac{M}{T}\right).$$

For this reason the ratios M/C , D/C , L/C , D/T , and $L/(D+L)$ are omitted from Table 2 even though they were all computed directly from the observations. Of these various other comparisons, two were just barely significant, but suggest no general result.

Inbreeding: The difference in the amount of inbreeding due to numbers of breeding pairs being either 8 or 16 (i.e., S3 or S4) in each generation appears not to have affected the measures of embryonic death in these populations of mice as readily seen in both Tables 1 and 2.

General: The ratio of the number of preimplantation losses to the number of corpora lutea averages about 15 percent even in vigorous hybrid stocks and the ratio of the number of losses of all types to the number of corpora lutea commonly exceeds 20 percent in untreated stocks (RUSSELL and HUNSICKER 1964; LÜNING quoted in AMOROSO 1963). There appear to be no unusual features of the data of this experiment in these respects.

DISCUSSION

From this experiment we conclude that low doses, 50 and 100r, of spermatogonial X irradiation repeated for 11 generations in "inbred" and "hybrid" mice appear not to have affected measures of population quality based on embryonic mortality in litters from matings of nonirradiated brothers and sisters compared with outcrosses. A number of associated uncontrolled variables—variation in ages of the fetuses at time of observation, variation in ages of the dams at mating, regression of number of corpora lutea on age of dam—appear not to have biased the comparisons between the samples of matings from the various populations of mice. The internal consistency of the results suggest that no other unusual causes of variability or of level of response influenced the rates of embryonic mortality.

We need to stress that this is an empirical test of the effects of spermatogonial X irradiation. We take it for granted that irradiation induces gene mutations and chromosomal aberrations in spermatogonia, that some of these at least pass through meiosis and enter zygotes, that some of them have deleterious effects in homozygotes and possibly also in heterozygotes, and that selection is steadily acting against deleterious alleles to remove them from the genetic makeup of a population. If all of the above assumptions could be stated more precisely and more inclusively, a theoretical model could be constructed to guide the design and interpretation of such experiments. As it is, we must face the fact that no testable model can be devised at the present time. Yet we need to know what effect radiation will have on the genetic constitutions of experimental and nonexperimental animal populations. Therefore, empirical studies such as this are the only means now available.

To emphasize the scope and limitations of this experiment we shall discuss the questions of the accumulated genetic dose of radiation, the expectations of deleterious effects in various types of matings, and a sketch of a theoretical approach to the issue of the effects of radiation on the genetic constitution of populations.

Accumulated dose: The accumulated dose of radiation in an exposed population depends upon the number of generations, the magnitude of the dose, and the sex or cell population exposed. If spermatogonia are exposed as in this experiment to a dose d and the sires are mated to nonexposed dams, the progeny having received only one irradiated genome have a radiation dose equivalent to $d/2$. If the gonads of the males are again exposed to a dose d seven weeks before mating and if these males and the nonsister female relatives are randomly mated, the next generation of progeny will have an accumulated dose of d . In general if d is the dose and n is the number of generations of the progeny, the accumulated dose is $nd/2$. In this experiment, 11 generations of exposure to 50r and 100r may be regarded as producing accumulated doses of 275 and 550r, respectively. If both sexes had been irradiated, the accumulated dose would have been nd or 550 and 1100r, respectively. If males in each generation descended from irradiated sires had been irradiated and mated to unrelated females without any irradiated ancestry, the accumulated dose would form a series $d/2, 3d/4, 7d/8, \dots, d(1-(1/2)^n)$ which clearly approaches d asymptotically as n increases.

The accumulated dose in a population does not have the same genetic meaning as the accumulated dose for an individual. That is, an accumulated dose of 550r spread over 11 generations is not expected to be genetically equivalent to 550r applied as an acute dose to the spermatogonia of one individual, because deleterious mutations will have been selected against in each generation. The point of an empirical study is to try to find out what is the genetic equivalent of any given dose accumulated over n generations rather than given to animals of a single generation.

Types of matings: The effects of single, cumulative, or any other schedule of doses in a population may be searched for in an endless variety of ways. In this experiment we have used a very limited type of effect (embryonic mortality) in only one type of contrast between matings (brother-sister vs. outcrosses). If the results suggest that an experiment of greater scope is desirable, three types of matings might be useful for each different radiation dose level including the 0r dose.

The three types of matings may be distinguished by considering the matings to assess the genetic makeup of any given male whether from an irradiated or control population. First, if a male is mated to a randomly selected unrelated female, a fraction a of the eggs may fail to produce live births because of alleles with deleterious effects in heterozygotes contributed by either parent, because of failure of some ova to be fertilized, and because of other unspecifiable genetic and nongenetic factors. This fraction will vary with the choice of animals but may be regarded as the irreducible minimum of reproductive failures in a given experiment. The difference between two males or between two groups of males, one experimental (e), one control (c), with respect to a may be regarded as the effect of one irradiated genome in an outcross. Second, a male may be mated to a randomly selected female from his own generation or population. The zygotic failures and embryonic deaths a will be augmented by a fraction b attributable to two nonidentical genomes, the degree of dissimilarity depending upon the inbreeding coefficient F of the population. If $x = a + b$, the difference $(x_e - x_c) - (a_e - a_c)$ measures the increment in embryonic deaths due to deleterious alleles in two irradiated genomes, provided F is quite small. This difference will also contain any effect of "genetic background" on the expression of mutant alleles, some of which may be expressed in heterozygotes of one type (within-population matings, say) but not in heterozygotes of another type (outcrosses, say). Third, a male may be mated to a randomly selected sister. The embryonic deaths will be further augmented by a fraction c , due to the action of mutant alleles with lethal effects when homozygous. If $y = a + b + c$, the difference $(y_e - y_c) - (x_e - x_c)$ will chiefly measure the effects of homozygous lethals, provided the inbreeding coefficient F of the population prior to the brother-sister mating is quite small. Higher mortality rates in any embryonic stage in brother-sister matings compared with outcross matings in the 0, 50, or 100r groups may be due to homozygosity of recessive lethal mutations, to heterozygosity of numerous mutant alleles having cumulative effects on lethality, or to differences in expression of single mutations on different genetic backgrounds.

In this experiment, only two of the three types of matings were carried out.

Thus we may compute differences of the type $a_e - a_e$ and $\gamma_e - \gamma_e$ and so estimate $(b_e - b_e) + (c_e - c_e)$, but not be able to separate the effects of homozygous lethals from heterozygous lethals. However, since the differences between the brother-sister and outcross matings were neither significant nor showed any interpretable pattern of differences, the question need not be further pursued.

Theoretical expectations: The difficulty of developing an adequate theoretical model to predict the effects of irradiating spermatogonia in successive generations arises from the bewildering array of types and degrees of genetic effects (GREEN 1959, 1960). It is this that has led to the necessity of an empirical approach. Nevertheless it may be useful to set forth the requirements for a theoretical model to help in seeing the need for further information and mathematical development. WRIGHT (1950, 1955) has developed the theory to the point of predicting the effects on fitness (somehow measured) of dominant, recessive, and semidominant deleterious alleles at single loci when the mutation pressure on the gene frequency is sustained for any specified number of generations.

We believe the following are needed: (1) Estimates of the total number N of loci capable of mutating to alleles that are in turn capable of surviving and of passing through meiosis into functional gametes and of having deleterious effects in heterozygotes and in homozygotes. (2) Estimates of the probabilities u_i ($i = 1, 2, \dots, N$ loci) of mutation at each locus or at least an estimate of an average mutation rate for the genome as a whole, $u = \Sigma(u_i)/N$. (3) The probability distributions of x mutations in gametes, zygotes, matings, and progeny in outcrosses, random matings, and brother-sister matings. If the mutations were randomly distributed the distribution of x mutations in the N loci of gametes, for example, would be $P(x) = (e^{-m} m^x)/x!$, where $m = N u d_c$, the mean number of mutations per gamete. (4) Estimates of the selective values s_i of mutations at each locus and an average selective value for genotypes in gametes and in zygotes. (5) Estimates of the genetically effective cumulative radiation doses d_c in outcrosses, random matings within irradiated lines, and brother-sister matings following two or more generations of exposure of spermatogonia, oogonia, or other reproductive cells to ionizing radiation.

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SUMMARY

Males of six populations of "inbred" mice and of six populations of "hybrid" mice were mated with their sisters and with females of an unrelated inbred strain. The six populations in each group represent three levels of spermatogonial X irradiation and two levels of inbreeding. Spermatogonia were exposed to 0, 50, or

100r of X rays in each generation, and the inbreeding coefficient was varied but controlled within limits by fixing the number of mated pairs per generation as either 8 or 16. The populations were propagated for 11 generations with random mating excluding brother-sister matings.

The females, both sister and unrelated, were killed at about 15 days of pregnancy and the numbers of corpora lutea, "moles," dead fetuses, and living fetuses counted to see if any differences in embryonic mortality attributable to radiation exposure, inbreeding level, or both had developed between the populations. The mice in the litters from the brother-sister matings each received two irradiated or control genomes, whereas the mice in the litters from the outcrosses received only one irradiated or control genome.

No differences between the populations related to radiation or inbreeding experience were apparent in rates of embryonic losses prior to implantation, immediately after implantation, or long after implantation. The total number of females dissected was 902; the average number was 37.6 per set.

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