GENETIC EFFECTS OF CHRONIC IRRADIATION GIVEN TO MICE THROUGH THREE SUCCESSIVE GENERATIONS

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GENETIC effects of ionizing radiations in man are of great concern to all people in the atomic age. However, since available data on man have been quite limited, information in various living organisms, especially in mammals, should be summarized and critically evaluated in order to assess the effects. The most comprehensive studies so far published on mutagenic effects of radiations in mammals were limited to those obtained with the specific-locus method in mice, mainly by W. L. RUSSELL and his group, and concerned largely with seven specific loci. Such studies, although indispensable for comparative purposes, examine only parts of the over-all genetic damage from radiation.

Thus, in recent years, information has been needed concerning mutation rates for the total chromosome set and those mutations which could be detected only by statistical means. The problem of the genetic effects of irradiations on mammalian populations for successive generations has been of special interest. The results of investigations of this type will be presented by the contributors to the present symposium. In the present paper the experiments were designed so as to obtain the rates of certain classes of mutations for a whole gamete of the mouse, and to search for mutations detectable only by statistical means. The relative importance and maintenance of various kinds of mutations in mouse populations will be discussed.

Three series of experiments have been carried out by the author and his collaborators, and parts of the results have been published elsewhere (SUGAHARA, TUTIKAWA, and TANAKA 1958; SUGAHARA, TUTIKAWA and TAKEDA 1959; SUGAHARA, OKAZAWA, TUTIKAWA, and MURAMATSU 1961; MURAMATSU, SUGAHARA and OKAZAWA 1963). In the first two experiments, three successive generations were chronically irradiated in order to accumulate recessive mutations. In the last experiment, one generation was exposed to a relatively large dose of single acute or chronic irradiation for comparison. The results of the three experiments will be summarized and critically discussed. Although the scales of the experiments were different, some results were consistent in the three experiments. However, since some contradictory results were observed, further study will be required to elucidate the nature of the difference.

In any case, the results obtained so far may suggest the significance of chronic irradiation on the fitness of mammalian populations, although a dose-rate effect similar to that reported by RUSSELL, RUSSELL, and KELLY (1958) might be presumed in the present study as well.

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MATERIALS AND METHODS

The principal scheme of breeding and irradiation used in the present study is taken from HALDANE (1956) as shown in Figure 1. However, in addition to scoring recessive lethals by means of HALDANE's method, other types of genetic effects such as changes in litter size, sex ratio, fertility, and juvenile death have been studied at every generation by statistical means.

The principle of HALDANE's method is simple, although the interpretation of the experimental results poses complex statistical problems, as has been discussed previously (MURAMATSU, *et al.*, 1963). As far as irradiation is concerned, a breeding scheme was designed to keep any recessive lethals induced by the irradiation in heterozygous condition. A multiple recessive stock (*gg*) of the mouse, irradiated and bred according to this scheme, may accumulate induced recessive mutations generation after generation. The accumulation would reach at its maximum at the P_1 generation. If induced recessives had some dominance in heterozygotes, or passed through hemizygotes, the rate of accumulation may be reduced.

Each P_1 mouse is crossed with a mouse carrying dominant alleles at the marked locus. The F_1 mice are sib-mated and all the marker genes segregate in F_2. If a lethal is linked to a marker gene in P_1, a homozygote for the marker in F_2 will tend to be homozygous for the lethal also and will appear with an abnormally low frequency.

The method used to estimate the number of presumed lethals linked with one of the marker genes in the P_1 was developed by HALDANE (1956). It is based on the frequency of abnormal segregation of marker genes using a predetermined probability of error (1 percent) and an estimate of total map length scanned.

If a lethal segregates but is not linked to any of the marker genes, the average F_2 litter size is expected to be reduced 6.3 percent but each marker will show the normal 3:1 Mendelian ratio. Furthermore, if the lethal has some dominance in heterozygous condition, the average litter size in F_1 will also be reduced. If a detrimental or semilethal exists in place of the lethal, increased morbidity may be observed.

The presence of a sex-linked recessive lethal in a P_1 (*gg*) female will result in a disturbance of the sex ratio among her offspring. This effect may be detected by comparing the sex ratio among offspring of P_1 females with the sex ratio of offspring of P_1 (*gg*) males. To rule out the possibility of a maternal effect, these ratios are also compared to those obtained in the unirradiated control series.

Litter size, sex ratio, and sterility have been studied at all irradiated generations and their shift has been analyzed generation after generation, on assumptions similar to those mentioned above.

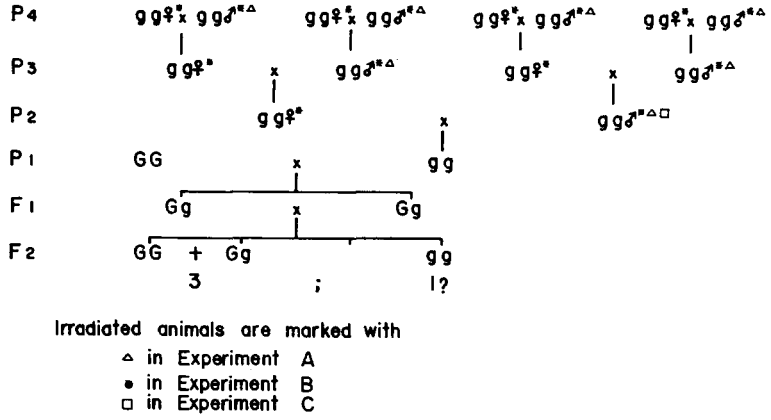

FIGURE 1.—Scheme of irradiation and breeding.

The characters such as litter size and sex ratio may tend to fluctuate due to various causes mostly unknown. For example, changes according to birth order may be recognized in some cases. Thus analytical tests of these values on birth order have been carried out, if necessary, to exclude these biases.

There were three experimental series. The first one (Experiment A) was a pilot experiment to test the validity of the above principles, especially of HALDANE's method. The second one (Experiment B) was the main experiment to study the genetic effects of radiation under conditions as similar as possible to those which threaten humans, i.e., chronic irradiation with a low dose rate from conception to reproduction during successive generations. The last one (Experiment C) was undertaken to study the dose-rate effect observed with the specific locus method in the present experimental system. Although the experiment was not completed, the preliminary results seemed interesting and will be presented here.

Animals used and irradiation conditions are given in Table 1. In Experiment A, adult males were irradiated and mated with unirradiated females immediately after irradiation in an unirradiated mouse colony. In Experiment B, both sexes were irradiated from conception to reproduction, being reared and bred in a radiation field. In Experiment C the gonads of adult male mice were exposed to X irradiation for a single acute exposure, and whole-body irradiation by gamma rays was administered for a chronic exposure as in Experiment A.

Chronic radiation dose was measured with Toshiba pocket chambers placed in the same position during irradiations as the mice. The pocket chambers were calibrated for gamma rays with a Victoreen condenser chamber. Xrays at 180 kvp were used for acute irradiation, with a dose rate of 96r/min as measured by a Radocon dosimeter at 50 cm from the target.

For chronic irradiation mice were kept free in an aluminum cage, fed laboratory chow, and given tap water ad libitum. For acute irradiation, mice were inserted into a plastic tube with their tails fixed in position and the upper two-thirds of the body shielded with lead 3 mm thick.

In Experiment B, the irradiation was started with eight females in which vaginal plugs were observed the day before. The mice were irradiated from conception through birth to adult and designated the P_4 generation in Figure 1. Males and females were caged separately after weaning. To obtain the succeeding generation, mating was done by placing a male and a female from different parents in a cage after they had received the desired dose. After birth and weaning of first litter, the pair was removed from the radiation field and kept in the mouse colony to obtain further litters. Thus the gametes for the later litters received radiation for about 40 days longer than those for the first litter. The data from successive litters were pooled for the initial analysis, but treated separately in exact analysis. Similarly, P_3 and P_2 mice were obtained by mating nonsibs from the first litters of the previous generation. Then, P_2 mice were mated in the mouse colony outside of the radiation field to obtain P_1 mice.

In the other experiments mating was started in the mouse colony outside the radiation field immediately after termination of irradiation. In each case, mating of P_2 mice was continued until the prescribed number of P_1 recessive mice was obtained. The time relations between irradiation and mating may be important for surmising the stage of gametogenesis in question.

As for the mice, the NH strain maintained in the National Institute of Genetics, Misima, was used in Experiments A and C as a recessive stock. The mice of NH strain are homozygous for three recessive genes, *aa*, *pp*, and *ss*. A special stock homozygous for six recessive genes was used in Experiment B. This stock was produced by K. TUTIKAWA, National Institute of Genetics, when he was at The Jackson Laboratory in 1957, brought back to Japan, and tentatively designated as "wavy" by him. Four genes out of the six, i.e., *aa*, *bb*, c^{ch} c^{ch}, *and se se*, were used as markers. Before its use in the present experiment, the "wavy" stock had been maintained in the National Institute of Genetics through several generations of sib-mating with forced heterozygosis for the c^{ch} and *p* loci. As wild stock, strain CBA mice were used in Experiments A and B and strain C3H in Experiment C.

With the unirradiated controls, matings were performed in Experiment B according to the scheme in Figure 1, but without irradiation. In the two other experiments, unirradiated multiple recessive mice were mated with wild mice to obtain F_1 and F_2 progeny as controls. An epidemic

of infectious catarrh occurred in the stock colony during Experiment C, and might have disturbed the experimental results.

TABLE 1

Animals used and irradiation conditions

Irradiated animals	Experiment A	Experiment B	Experiment C	
	Adult males	Males and females from conception to reproduction	Gonads of adult males	Adult males total body
Dose	P_4: 167r for 30 days P_3: 167r for 31 days P_2: 225r for 47 days	Approximately 34.4r for about 80 days in each generation	600r (acute)	597.3r (chronic)
Irradiated generations	3 generations successively	3 generations successively	1 generation	1 generation
Dose rate	5–8r/22-hr day	About 0.43r/22-hr day	96r/min	8.3r/22-hr day
Radiation	Co^{60}-γ ray	Scattered γ radiation from Co^{60}	180kvp X ray	Co^{60}-γ ray
Strain	NH (*aa, pp, ss*)	wavy (*aa, bb, $c^{ch}p/c^{ch}p$, dse/dse*)	NH (*aa, pp, ss*)	

RESULTS

Breeding behavior of mice in successively γ irradiated generations. Analysis of the breeding behavior was based on some of the following items: (1) mean litter size, (2) deleterious effect on fertility, i.e., incidence of sterile pairs, (3) sex ratio.

Mean litter size: Mean litter size in the two chronic irradiation experiments is shown in Figure 2 based on pooled data. In Experiment A, litter size showed a slight decrease after irradiation as expected. However, in Experiment B a gradual increase in litter size was observed with successive generations in the irradiated as well as in control series, as will be reported in detail elsewhere (MURAMATSU, SUGAHARA, and OKAZAWA, in preparation). These tendencies were significantly larger in the irradiated series than in the control. The regression coefficients were 0.198 ($.10>P>.05$, not significant) in the control series and 0.400 ($.05>P>.02$, significant) in the irradiated series.

However, since it was found that litter sizes differed significantly according to their birth order, an exact comparison was made based on litters of the same order. Matings which produced two litters or more (except in P_4 where there was only one litter) were selected, and mean litter sizes and their variances were analyzed for each litter order of the selected matings. A significant increase in litter size was observed in both the irradiated and control series as in the pooled data. The differences between irradiated and control are statistically significant in the second litters of P_3 and P_1 and in the first litter of P_2.

Incidence of sterile pairs: In the present investigation, a sterile pair was defined as a pair with no progeny during a mating of six or more months duration. In Experiment A, some cases were observed in every generation as expected, since the dose and dose rate were relatively high. The incidence of sterility, however, decreased generation after generation as shown in Table 2. In Experiment B, no such effects were observed in generations P_4 and P_3. However, in the P_2 and P_1 generations, a higher incidence of sterile pairs (17.8 percent P_2 and 4.0 percent in P_1) was found in the irradiated series than in the control series (2.6 percent in

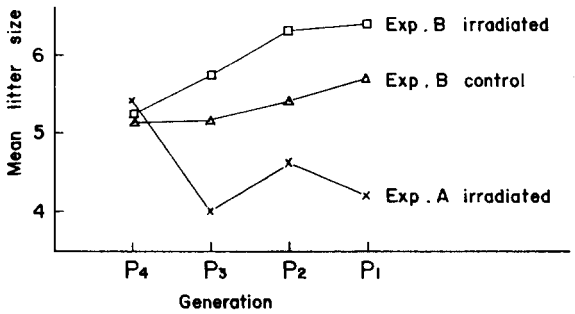

FIGURE 2.—Changes in mean litter size in successive generations under irradiation in Experiments A and B.

TABLE 2

Incidence of sterile pairs in successive generations under and after irradiation in Experiments A and B

	Generation of pairs			
	P_4	P_3	P_2	P_1
Experiment A				
Number of pairs tested	12	20	13	31
Number of sterile pairs	3	3	1	0
Experiment B				
Irradiated series				
Number of pairs tested	15	17	45	227
Number of sterile pairs	0	0	8	9
Control series				
Number of pairs tested	15	17	38	202
Number of sterile pairs	0	0	1	1

P_2 and 0.5 percent in P_1). These increases are statistically significant ($\chi^2 = 4.89$, $P<.05$ for P_2, $\chi^2 = 5.63$, $.02>P>.01$ for P_1).

Sex ratio at birth: Sex ratios at birth in P_4, P_3 and P_2 generations are shown in Table 3. It is sometimes found that in mice there may be some differences in sex ratio among litters of different birth order (ALBERT, TOMSON, and SMALL 1961). Although such a change was found in Experiment B, no consistent change was found in our control matings. We therefore present the pooled data, but changes in sex ratio as related to birth order remains to be studied more carefully.

Some differences between generations as well as between the irradiated and control series were observed, but these were not statistically significant.

From the results of breeding according to the scheme in Figure 1, analysis of the breeding behavior was made on some of the following items: (1) incidence of abnormal segregations in F_2, i.e., HALDANE's method, (2) mean litter size at birth, (3) juvenile death, and (4) sex ratio.

Haldane's method: The numbers of presumed lethals linked with one of the marker genes in the P_1 estimated according to HALDANE's method are shown in

TABLE 3

Sex ratio at birth in successive generations under irradiation in Experiments A and B

	P_3		P_2		P_1	
Generations	Number of mice	Sex ratio	Number of mice	Sex ratio	Number of mice	Sex ratio
Experiment A						
irradiated	88	0.570	185	0.547	189	0.505
control	253	0.538	...	....	...	....
Experiment B						
irradiated	481	0.528	543	0.501	524	0.505
control	326	0.546	422	0.519	777	0.501

TABLE 4

Results of Haldane's method

	Experiment		
	A	B	C
Irradiated generations	3	3	1
Dose rate	chronic	chronic	acute
Effective dose per gamete of P_1 recessives	297.5r	103.4r	300r
Number of suspected lethals			
irradiated	1	9	3
control	0	4	0
Total map length swept*			
irradiated	1,504.1	19,643.7	2,228.0
control	1,108.8	21,680.9	804.4
Incidence per total autosome set			
irradiated	1.213	0.836	2.456
control	0	0.337	0
Mutation rate per autosomes/r	4.3×10^{-3}	4.8×10^{-3}	8.2×10^{-3}

* Length in centimorgans.

Table 4. Criteria for assuming the presence of a linked recessive lethal gene and chromosome length swept are the same as those of CARTER (1959). For details see SUGAHARA *et al.* (1961). The incidence of recessive lethals in a total autosome set were calculated using 1824 centimorgans as the total autosomal cytogenetic map, according to SLIZYNSKI (1955).

Mean litter size at birth: Since it was recognized in the course of the study that offspring of P_1 recessive males and those of P_1 recessive females showed some differences in their reproductive performance, these two groups were subdivided into male line and female line respectively. They correspond to the offspring of reciprocal crosses.

Mean litter sizes at birth in F_1 and F_2 based on the pooled data for birth order are shown in Table 5. In the F_1 the increase in litter size was consistently observed in irradiated female lines in chronic irradiation experiments. On the contrary, the increase in mean litter size in male lines was observed in the acute X irradiation. In the control of any experiment, there was no significant difference between reciprocal crosses in the F_1.

In the F_2, effects of radiation on mean litter size were rather contradictory in the three experiments. Mean litter size in F_2 is generally increased as compared with that in F_1 in the control as well as in the irradiated series. In Experiment B, litter sizes in the irradiated series were significantly smaller than those of the control, but the situation was reversed in the acute X-ray experiment (Experiment C).

The last result is quite unexpected. Before any appropriate interpretation is given, further experimentation would be required. In Experiment A and the chronic-γ experiment of Experiment C, no significant difference between irradiated and control series was observed.

TABLE 5

Mean litter sizes at birth and their standard error in F_1 and F_2 generations

	Irradiated series	Control series
Experiment A		
F_1 female line	6.52‡ ± 0.67	... ...
male line	4.47 ± 0.46	... ...
F_2 female line	7.60 ± 0.20	7.36 ± 0.75
male line	7.56 ± 0.27	6.95 ± 0.33
Experiment B		
F_1 female line	6.68‡ ± 0.14	6.08 ± 0.14
male line	6.09 ± 0.20	6.40 ± 0.13
F_2 female line	7.04† ± 0.06	7.50 ± 0.02
male line	7.31† ± 0.06	7.52 ± 0.05
Experiment C—acute X-ray experiment		
F_1 female line	5.33 ± 0.18	5.06 ± 0.14
male line	7.06* ± 0.24	5.23 ± 0.19
F_2 female line	7.48* ± 0.13	5.82 ± 0.10
male line	8.83* ± 0.14	6.03 ± 0.09
Experiment C—chronic γ experiment		
F_1 female line	5.71 ± 0.44	... ...
male line	5.20 ± 0.25	... ...
F_2 female line	5.61 ± 0.21	... ...
male line	6.04 ± 0.15	... ...

* Significantly larger than control.
† Significantly smaller than control.
‡ Significantly larger than male line.

Juvenile death: Percent mortality before weaning was compared in the irradiated and control series. In Experiments B and C, a statistically significant increase due to ancestral irradiation was observed, but not in Experiment A (Table 6). The increase was much higher with acute irradiation than with chronic irradiation. The reason for the failure to find a significant difference in early mortality in Experiment A may be a relatively high mortality in both series due to an epidemic of disease or to some unknown causes. The loss of whole litters occurred very rarely.

Sex ratio: Detailed data on sex ratio with regard to birth order in Experiment B were presented previously. In the present paper, a comparison of pooled data between male and female lines are shown in Table 7. Sex ratio fluctuated from case to case. However, sex ratios in female lines in most cases were smaller than in male lines. The significant differences observed so far were those between male and female lines in the irradiated series of Experiment B and in the acute X-ray experiment of Experiment C. This is in good accord with the expectation based on the assumption of induced sex-linked recessive lethals. However, as pointed out by LÜNING (1963), the difference between irradiated and control series is not significant in any of lines.

Sex ratio in small litters (less than five offspring) was analyzed in various cases

TABLE 6

Percent juvenile death in F_1 and F_2 generations

		Irradiated series		Control series
Experiment A				
F_2	female line	9.75		13.10†
	male line	8.33		7.74
Experiment B				
F_1	female line	4.77*		2.15
	male line	4.87*		1.89
F_2	female line	1.86*		0.81
	male line	1.20*		0.61
Experiment C				
		Acute X-ray	Chronic γ	
F_2	female line	10.86*	3.86*	1.12
	male line	7.94*	23.10*	1.59

* Significantly higher than in control.
† Including one case of 12 losses out of 13 offspring in a litter.

TABLE 7

Sex ratio at birth in F_1 and F_2 generations

		Male lines		Female lines		Increase (+) or decrease (—) in female lines
		Number of mice	Sex ratio	Number of mice	Sex ratio	
Experiment A						
F_1	irradiated	77	0.506	187	0.427	—
	control	435	0.636	633	0.548	—
F_2	irradiated	685	0.519	1428	0.540	+
Experiment B						
F_1	control	1158	0.532	1071	0.509	—
	irradiated	1170	0.528	1362	0.479	—*
F_2	control	8073	0.502	7806	0.493	—
	irradiated	8442	0.495	8581	0.497	+
Experiment C						
F_1	control	314	0.519	263	0.544	+
	acute X irrad.	917	0.513	1200	0.500	—
	chronic γ irrad.	52	0.577	80	0.550	—
	control	977	0.524	1181	0.465	—***
F_2	acute X irrad.	1202	0.541	2654	0.498	—**
	chronic γ irrad.	181	0.536	432	0.530	—

* .05>P>.02. ** .02>P>.01. *** .01>P>.001.

on the assumption that smaller litters might have resulted from embryonic death of males due to sex-linked recessive lethals. However, no significant differences were observed between irradiated and control series nor between irradiated male and female lines. The standard error of the sex ratio may be too large for these comparisons in experiment of this scale.

DISCUSSION

The present series of experiments was undertaken in order to obtain the induced mutation rates for a whole gamete of the mouse and to demonstrate mutations detectable only by statistical means as a clue to the estimation of the genetic burden of ionizing radiations in man. Recently papers have appeared dealing with the same questions (RUSSELL 1957; CARTER 1957; CARTER and LYON 1961; LÜNING 1960, 1963; SPALDING and STRANG 1962a, b; SPALDING, STRANG and LESTOURGEON 1963; MCGREGOR and NEWCOMBE 1961; MCGREGOR, JAMES and NEWCOMBE 1960; EHLING and RANDOLF 1962; BROWN, KRISE, PACE and DEBOER 1964; STADLER and GOWEN 1964). However, in most of the cases reported so far, the breeding systems adopted appeared to be inadequate for the proper accumulation of recessive genes, which were presumably most frequently induced by radiation. Thus various types of genetic effects were suggested, but the mutation rate for these traits could not be estimated in most cases.

In the present paper, recessive lethal mutation was searched for by means of HALDANE's method. Furthermore, changes in reproductive integrity induced by ancestral irradiation were followed generation after generation; some of these changes may be assumed to be the results of the mutations induced by radiation.

From the estimate of the effectively accumulated doses of radiation, based on the assumptions of the inheritance of the induced mutations, mutation rates for a whole gamete per unit dose of radiation were calculated for various criteria. Radiation doses accumulated in a gamete in P_1 recessive mice are the sum of the dose for all generations where both parents were irradiated, and half the sum where one of the parents was irradiated. Disturbance due to slight dominance of recessive genes was disregarded. Accumulation of mutant genes in an X chromosome is a little more complicated and should be followed for each case.

As for the effects of radiation in the course of chronic irradiations in successive generations, changes in mean litter sizes in Experiment B are significant and interesting. In general, a reduction of litter size may be expected in the irradiated series. Contrary to expectation, mean litter sizes increased in successive generations as radiation doses accumulated, while only a slight but nonsignificant increase was observed in the control series. The increase was not only observed on pooled data but also confirmed by exact analysis for litter order.

It is generally observed that hybrid animals produce litters considerably larger than those produced by inbred strains. The results in F_1 and F_2 in the present report are in good accord with this general observation.

The "wavy" stock was maintained by sibmating for several generations, and the increase of litter size corresponded to the generations of noninbred matings in both series. These results may suggest that there is a certain heterotic effect caused by nonsibmating, but that this is accentuated in the irradiated series by the additive effect of radiation-induced recessive mutations. However, hyperovulation by radiation (RUSSELL and RUSSELL 1955; HAHN and MORALES 1964) and increase of implantation rate by low dose of X radiation (SATO 1962) may be an alternate or supplementary explanation of the phenomenon.

Direct effects on fetus or embryo were not observed, but these effects, if any, may be obscured by the above factors.

Another significant effect observed during irradiation was an increase in sterility. This harmful effect was observed in the P_2 and P_1 generations of Experiment B, but in Experiment A it was highest in the first generation after chronic irradiation of 167r and gradually declined in later generations. Generally, sterility is regarded as a dominant genetic effect resulting from chromosome aberrations, but in the irradiated generations the direct effect of ionizing radiation on the reproductive organ should be added to this. In this respect there are indications in Experiment B that some of the P_1 recessives, which themselves were not irradiated, inherited a dominant sterility gene from their parents. Mutation rate to this type of gene may tentatively be calculated at 5.1×10^{-4}/r, provided that all sterility genes in P_1 recessives were induced in either of their parents. However, the results indicating the gradual changes in the incidence of sterile pairs in successive generations, though the directions of the changes were contrary to each other in the two experiments, may suggest a more complicated mechanism of the maintenance or elimination of sterility genes than mere dominance. The shift of sex ratio observed in Experiment A has been discussed previously (SUGAHARA, TUTIKAWA, and TANAKA 1958).

As far as the accumulated mutations in the P_1 recessives are concerned, quite consistent results were obtained for recessive lethals by HALDANE's method. However, the results seem to be different from those of CARTER (1959). Higher rates were obtained with acute irradiation than with chronic irradiation, though the difference is not statistically significant. Since in Experiment A, matings were done immediately after irradiation for 30 to 45 days, most of the gametes at issue might have been irradiated at postspermatogonial stages. In Experiment B, differences in radiosensitivity of various stages of gametogenesis and of different sexes have been disregarded in calculating mutation rates for the sake of convenience. It may be more probable that the mutation rate obtained corresponds rather to that of postspermatogonial cells than to that of spermatogonia. In Experiment C, both presterile and poststerile matings were done to obtain P_1 recessives. Three presumed lethals came from poststerile matings, though no differences were observed in other traits in the F_1 and F_2. It has been generally assumed that the mutation rate in postspermatogonial cells is higher than that in spermatogonia.

Reduced litter size after several generations of acute X irradiation in mice or rats was observed by PROSHINA (1961) and LÜNING (1963), but others reported no effect on litter size after many generations of chronic γ irradiation (BROWN, KRISE, PACE, and DEBOER 1964; STADLER and GOWEN 1964).

In the present investigation a significant decrease in litter size was observed in the F_2 generation of Experiment B but not in other cases. On the contrary, an increase was observed in the F_1 generation of female lines in the chronic irradiation experiment. This may be explained by assuming a heterotic maternal effect in recessive P_1 females. An increased litter size in the F_1 of the male line in the acute irradiation experiment suggests some heterotic effect on embryos themselves. However, LÜNING (1963) suggested as a possible explanation of the in-

crease that the litter size varied with the birth order, with a maximum in the second and third litters. In order to test this possibility, litter size and order were compared in these cases. It was found from this comparison that the increases observed were mainly due to the increased litter size, but not entirely.

In any case, before final conclusions can be reached on the existence of heterotic effects of induced recessive mutant genes, further study would be required on the control of litter size, especially since the reduction of litter size expected could not always be demonstrated. The data in Experiment B were interpreted previously assuming recessive lethals with a slight dominant effect in the heterozygote (MURAMATSU *et al.*, 1963), and the mutation rate was estimated. Similarly, CARTER (1957) estimated mutation rate to recessive lethals for a whole gamete based on the reduction in litter size in backcross mating with chronically irradiated ancestors. Contradictory results in the present investigation may reduce the importance of these estimates. It may be possible, however, that the patterns of mutations induced by acute and chronic irradiation are quite different in these respects, and that the results of chronic irradiations are really consistent.

As for early mortality after ancestral irradiation, LÜNING (1963) reported increased mortality in mice concluding the importance of maternal effect, and PROSHINA (1961) reported a significant increase in rats after single or divided X irradiation for many generations. RUSSELL (1951) and CHARLES, TIHEN, OTIS, and GROBMAN (1961) reported an increase in juvenile death rate after paternal X irradiation. The results of the present investigation seemed to be in accord with their results. A significant increase in juvenile death rate was observed when the control rate was low but not when the control rate was rather high. Dose-rate dependency was observed in juvenile death as well. The deleterious effect of mutations may be masked in the latter case by some harmful environmental factors. Since most of the juvenile death occurred only in parts of litters, it may be indicated that the increased death is not a maternal effect but rather due to higher morbidity of offspring from irradiated ancestors. Thus, the mutation rate was estimated on the pooled data of Experiments B and C as reported in part previously (MURAMATSU *et al.*, 1963).

There have been many arguments for and against the usefulness of sex ratio as a possible indicator of genetic effect. Contrary to rather consistent results on man, mouse geneticists have failed to obtain a consistent result from their material. In the majority of experiments with mice only males were irradiated, and the existence of sex-linked dominant lethals has been studied (for example, see KOHN 1960). Recently LÜNING (1963) studied the shift of sex ratio after four to six generations of gonadal irradiations in random mating, and STADLER and GOWEN (1964) after ten generations with chronic γ irradiation in brother-sister matings. They could not find any effect of radiation on sex ratio.

The present results may indicate nothing about this point, since the values are scattered so widely. This may be rather natural because of the large random variation expected in an experiment of this scale. However, from the comparison of male and female lines, a significant decrease was observed in three cases, i.e., in the F_1 of Experiment B and in the F_2 of acute X irradiation and its control in

TABLE 8

Estimates of mutation rates to recessive lethals and sterility

Method	Lethal effective in the period	Assumption for dominance	Acute or chronic irradiation	Mutation rate	Based on experiment
HALDANE's method	From conception to weaning	Complete recessive	chronic	4.3×10^{-3}/r/total autosomes	A
	From conception to weaning	Complete recessive	chronic	4.8×10^{-3}/r/total autosomes	B
	From conception to weaning	Complete recessive	acute	8.2×10^{-3}/r/total autosomes	C
Litter size in F_2	From conception to birth	With slight dominance	chronic	5.0×10^{-3}/r/total autosomes	B
Early death rate	From birth to weaning	Partial dominance	chronic	5.2×10^{-4}/r/total autosomes	B
		Complete recessive	acute	4.2×10^{-3}/r/total autosomes	C
		Complete recessive	chronic	7.6×10^{-4}/r/total autosomes	C
Sex ratio at birth	From conception to birth	Sex-linked complete recessive	chronic	4.0×10^{-3}/r/X-chromosome	B
	From conception to birth	Sex-linked complete recessive	acute	1.3×10^{-3}/r/X-chromosome	C
Sterility	. . . . .	Partial dominance	chronic	6.8×10^{-4}/r/gamete	B

Experiment C. The result in Experiment B is in accord with the expectation assuming the induction of sex-linked recessive lethals by radiation. However, the mutation rate calculated from the shift of sex ratio seems to be too large and may suggest a complicated relation between radiation and sex ratio. The results in Experiment C are quite puzzling. In any case, the induction of sex-linked recessive lethals may be more probable than that of sex-linked dominant lethals but should be studied further.

Estimates of mutation rates to recessive lethals based on the present results are summarized in Table 8. According to HALDANE's method, lethals include all dead in the period from conception to weaning, but according to other methods they include death in only a part of this period. The degree of assumed dominance is slightly different in various criteria adopted. These estimates should be taken as very approximate, except for those obtained by HALDANE's method, because they are based on many different assumptions temporarily employed in the various cases.

It is concluded that the dose-rate dependency of induced mutation rate may be suggested in the rate of mutations to recessive lethals for a whole gamete, and that the harmful effect of ionizing radiations on mammalian populations may be indicated at a very low dose rate. Although a heterotic effect of induced mutations may be suggested in some cases, more consistent results indicate the induction of recessive lethals with a slight dominance in the heterozygote.

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SUMMARY

Three series of experiments designed to obtain rates of certain classes of mutations for a whole gamete of the mouse, and to search for mutations detectable only by statistical means on the same mouse, were summarized. In two experiments three successive generations were irradiated with chronic γ radiation in order to accumulate recessive mutations. Autosomal recessive lethals, detected by means of HALDANE's method, changes in litter size, sex ratio, and percent juvenile death were studied in their offspring. In another experiment, adult male mice were irradiated with acute X radiation and chronic γ radiation for one generation. The scheme of breeding and irradiation was taken from HALDANE (1956).

A consistent result was obtained from HALDANE's method. The result indicated a mutation rate to recessive lethals of 4.3 to 4.8×10^{-3} per autosomes per r and the existence of a dose-rate effect. Results on other items were rather inconsistent except for juvenile death, but suggested complicated genetic effects of radiations on mammalian populations. Further study will be required to elucidate the nature of genetic effects detectable only by statistical means.

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