GENETIC EFFECTS OF IRRADIATION IN THE RAT

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MASTER

The need for knowledge of the effects of exposure to mutagenic agents has become more necessary in recent years as a result of the increased use of radioactive materials. Most of the experiments to detect and measure the effects of germ-cell irradiation on fitness and other quantitative traits in experimental mammalian populations were begun after 1954 when the prospect of increased radioactivity in the human environment resulting from radioactive fallout seemed very real. The motive for these experiments was to provide a basis for predicting the effects of radiation on the fitness of human and other nonexperimental populations.

The lethal equivalent theory, put forward by Morton et al. (1956), provided a method by which the entire genotype of the individual could be utilized to determine the genetic load of the population under study. The purpose of the main experiment to be described in this report was to apply a modified form of this theory to information from the descendants of irradiated rats. This should permit estimates of the rates of occurrence of various types of induced mutations and their effects on fitness.

In many experiments the organ irradiated is the testis. However, it was thought desirable to gain a knowledge of the effect of irradiation on both male and female germ cells. The cell stages chosen for irradiation were those considered to have the greatest potential effect on the genetic constitution of later generations, namely spermatogonia in males and oocytes in females.

The experimental animal used in these studies was the rat, mainly because of the availability of the animals and facilities. Pooling information from similar experiments with other species such as mice and swine, should broaden the base from which predictions of the effects of ionizing radiation on man could be made. This should decrease the error involved when extending the results from populations of experimental animals to human populations.

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The Effects of Cumulative Spermatogonial and Maternal Irradiation

Objective

The objective of these studies was to allow the accumulation of mutations over several successive generations of spermatogonial and maternal irradiation. The traits studied were fertility, litter size, sex ratio, weight at 21, 42 and 69 days of age, weight gains and age at sexual maturity. Litter size and sex ratio were used to determine the frequency of induced lethal mutations. The other traits, with the exception of fertility, were studied to obtain estimates of the effects of irradiation on the means and variances of these quantitative characters, the mutations affecting these traits being regarded as selectively neutral. In addition it was hoped that information would be obtained on the frequency of induced visible mutations.

Materials and Methods

The rats used in these studies came from a highly inbred line (MI_{14}) developed during an experiment to test the effectiveness of selection for ovarian response to a gonadotrophic hormone (Kyle and Chapman, 1953; Chung and Chapman, 1958). A highly inbred line was used in an attempt to minimize the initial genetic variability and to reduce the possibility of inbreeding depression in the progeny from sib matings.

Before the main experiment was begun, a series of preliminary experiments were run to determine a treatment age, dose rate and total dose which would allow a relatively high dose of irradiation with a minimum of somatic effects. This series of experiments also permitted determination of the time of the sterile period in the male following irradiation. Matings after the sterile period are believed to use sperm descended from irradiated spermatogonia (Oakberg 1963). The results of the preliminary experiments led to the use of a total dose of 450 roentgens given as 100, 150 and 200 roentgens at 10, 12 and 14 weeks of age, respectively. This was the largest tested dose that

could be given at a relatively young age without resulting in major somatic effects. The sterile period in the irradiated males was found to occur approximately eight to nine weeks after the last dose of irradiation.

There were two main X-ray treated groups in this experiment. In one group males were irradiated in each generation and, following the sterile period, were mated to non-irradiated daughters of irradiated males. In the second group females were irradiated each generation and immediately mated to non-irradiated sons of irradiated dams. It was assumed that the cells irradiated in females were diocyte oocytes (Ohno, Kaplan and Kinoshita, 1960, 1961). A total of nine generations of irradiation was given to each group and data were collected for five subsequent generations after irradiation was discontinued. Two non-irradiated control lines, derived from the same base population as the irradiated groups, were maintained to be contemporaneous with them. The four groups were maintained by restricted random mating to avoid chance homozygosity of mutations occurring during the experiment. The offspring of these matings are referred to as 'outbreds'. Full-sib matings were made in generations 3, 4 and 6 to 14 in the spermatogonial irradiated line and in generations 3, 4, 5, 7, 10, 12, 13 and 14 in the maternal irradiated line. The 'inbred' progeny of these matings will show the effects of mutations when homozygous. 'Inbred' animals were not irradiated or bred. In some generations animals were remated and the litters resulting from these matings are referred to as 'mating group (2)'. The design and mating procedure can be found in Figure 1 of Havenstein et al. (1968). Further details of procedures used can be found in Chapman et al. (1964).

A discussion of the results of this experiment will take the form of sections considering the effects of the various types of mutations.

a) Dominant and recessive autosomal lethals.

Genetic damage resulting from irradiation may be due, in part, to

mutations with detrimental effects on viability. An attempt has been made to determine the frequency of viability mutations from data on litter size at birth and postnatal survival. In order to obtain meaningful estimates of mutation rates from litter size data it is necessary to make certain assumptions about the expression of the induced mutations. The assumptions made in this study are that viability mutations induced by irradiation consist only of dominant lethals and completely recessive detrimentals and lethals, and that these mutations are not epistatic to each other. Evidence from studies on mice (Russell and Russell, 1959) supports the former assumption which is the more critical of the two.

The traits used as measures of dominant and recessive autosomal lethals, induced and spontaneous, were litter size at three different stages: - one day after birth, 21 days of age and 69 days of age. Litter size at one day of age was considered in two ways: - total number of young found (live and dead) and number of live young. Litter size at this stage can be considered as a function of prenatal and perinatal deaths. Litter sizes at later ages will include postnatal deaths in addition to those before and at birth.

Details of the theory and methods of analysis can be found for the spermatogonial irradiated line in Taylor and Chapman (1969a) and for the maternal irradiated line in Havenstein and Chapman (1968). The method of analysis differed for the two lines. In both lines the means, adjusted for parity, age of dam and age of dam squared, were calculated within generation, mating group and mating system ('outbred' or 'inbred').

In the spermatogonial irradiated line the differences between the control and irradiated adjusted means were regressed on the amount of heterozygous induced, homozygous induced and homozygous spontaneous exposure put to test

in a weighted regression analysis, to estimate the mutation rates to induced dominant lethals, sex-linked and autosomal recessive lethals and spontaneous recessive lethals. A test for non-linearity indicated that the regression model accounted for a significant amount of variation in each of the four dependent variables. In the maternal irradiated line, estimates of the number of induced autosomal recessive lethals affecting the four traits, i.e., total young and number live at one day of age, number at 21 and 69 days of age, were made for each generation in which full sibs were mated. These estimates for each trait in the different generations were tested for heterogeneity and pooled using the method described in Havenstein et al. (1968).

The mutation rate estimates can be seen in Table 1. Only one estimate, that to induced dominant lethals - $1.4 \pm 0.6 \times 10^{-4}$ - was significant.

All estimates were in good agreement with comparable estimates in mice. The finding that estimates based on litter size are not consistently smaller than those based on embryonic mortality tends to support the assumption that litter size is a true reflection of differential embryonic mortality. The data also indicates that most lethals kill in the prenatal period. There was no evidence for the occurrence of mutations with mildly detrimental heterozygous effects.

b) Sex-linked recessive lethals.

The weighted regression method, outlined in the previous section, was used to calculate the frequency of induced sex-linked recessive lethals in the spermatogonial irradiated line. The estimates based on total number of young and number of live young at one day of age and number at 21 and 69 days of age can be seen in Table 1. An alternative method, utilizing sex ratio of live young at one day of age, was used to estimate the number of sex-linked lethal mutations induced in both the spermatogonial and maternal

TABLE 1.

Estimates of mutation rates to various classes of mutation in the spermatogonial and maternal irradiated lines.

Class of mutation	Mutation rate [*]									
	Total number born		Number born alive		Number at 21 days		Number at 69 days		Visible abnormalities	
	Male line	Female line	Male line	Female line	Male line	Female line	Male line	Female line	Male line	Female line
Induced										
Dominant lethals (/gamete/roentgen)	1.2±0.5	-	1.4±0.6	-	1.4±0.8	-	0.9±0.9	-	-	-
Sex-linked recessive lethals (/X chromosome/roentgen)	2.2±1.3	-	1.6±1.4 [†] 1.6±0.6 [‡]	- -0.2±0.4 [‡]	0.8±1.9	-	2.0±2.2	-	-	-
Autosomal recessive lethals (/gamete/roentgen)	0.0±0.6	0.5±1.2	1.0±0.8	1.6±1.3	1.2±1.1	1.5±1.8	1.6±1.3	1.4±2.2	-	-
Visibles (/gamete/roentgen)	-	-	-	-	-	-	-	-	7.5±3.8	-
Spontaneous										
Autosomal recessive lethals (/gamete/generation)	68.9±57.3	-	-10.7±61.7	-	-22.8±85.3	-	-39.3±99.9	-	-	-
Visibles (/gamete/generation)	-	-	-	-	-	-	-	-	0.2±0.1	-

^{*} All mutation rates have been multiplied by 10⁴.[†] Estimated by the method of Taylor and Chapman (1969a).[‡] Estimated by the method of Havenstein et al. (1968).

irradiated lines. This method is detailed in Havenstein et al. (1968). The estimates are given in Table 1. The estimate from the spermatogonial irradiated line - $(1.61 \pm 0.59) \times 10^{-4}$ - is highly significant, but the comparable estimate from the maternally irradiated line - $(-0.15 \pm 0.38) \times 10^{-4}$ - is not significantly different from zero. However, the two estimates do not differ significantly from each other.

It should be noted that the frequency of recessive lethals induced on the X-chromosome is larger than the frequency of this class of mutations for the rest of the genome considered as a whole. The reasonably close agreement between these estimates and those from the mouse (Luning, 1963; Searle, 1964) would support the assumption that the change in the sex ratio is not due to chance alone, although it may be that the change is not due only to the action of sex-linked lethals.

c) Visible mutations.

This class of mutations may be considered to have a more serious effect in the human population than those which act before birth. The latter class will cause abortions whereas the former, if detrimental, could cause serious defects to appear in children surviving until after birth, which is a more tragic circumstance. It was therefore felt that this type of mutation merited study.

During the spermatogonial irradiation experiment six visible mutations occurred in the irradiated line and three in the control. A description of these mutations can be found in Taylor (1968). All mutations are thought to be inherited as recessives. Estimates of the mutation rate to spontaneous visibles and to induced visibles were calculated. The spontaneous mutation rate estimate is $(0.75 \pm 0.38) \times 10^{-3}$ /gamete/generation and the estimate of the induced mutation rate is $(1.59 \pm 0.65) \times 10^{-5}$ /gamete/roentgen. These values are probably underestimates of the true mutation rates to visibles as

minor abnormalities will probably not be detected. Coat color mutations would not be detected in this line as most animals observed for mutations were albino. In addition, mutations with dominant deleterious effects on fitness would probably be eliminated in heterozygotes and would not be detected.

One mutation, designated paralyzed (pl), was detected in the line exposed to maternal irradiation.

d). Mutations affecting quantitative traits.

This class of mutations was studied in an attempt to determine if irradiation could induce variation in a quantitative trait which could be utilized for genetic improvement in a selection program. The traits studied were body weight at 21, 42 and 69 days of age in males and females and age at vaginal opening, considered as an indication of sexual maturity in the female.

As the effects of irradiation on quantitative traits in mammals have not been established, it is necessary to make certain assumptions about the mode of gene action. The main assumptions made were that the mutations affecting the quantitative traits under study do not have an effect on fitness, are not sex-linked and do not show epistasis. It is also assumed that genotype-environment interaction is not important for the range of conditions in this experiment.

The method of analysis was similar for the male and female irradiated lines. Details can be found for the male line in Taylor and Chapman (1969b) and for the female line in Davison, Havenstein and Chapman (1970).

The data from the male line were adjusted for parity, age of dam, litter size at birth and at 21 days of age by a multiple covariance technique. In addition to these covariates, the data from the female line were also adjusted for age of dam squared and litter size at one day of age. The effects of induced and spontaneous mutations of the means of the quantitative traits

were estimated by making orthogonal comparisons within each generation and mating group. The differences were then regressed on the amount of heterozygous induced, homozygous induced and homozygous spontaneous exposure put to test in a weighted multiple regression analysis. In the analysis of the male line data from generations 1 through 14 were used. Only data from those generations in which the females were exposed to irradiation were utilized in the analysis of the female line.

Significant heterozygous effects of irradiation on the means of these traits were found in the offspring of irradiated sires and the offspring of irradiated dams. Induced mutations tended to increase body weight and decrease age at vaginal opening in heterozygotes and decrease body weight and increase age at vaginal opening in homozygotes. These results are consistent with overdominance of the induced mutations.

Two methods were used to test for an increase in variance as a result of irradiation. One method compared the within litter variances in the control and irradiated groups. Comparisons were made within mating system for each mating group. An increase in variance in the 'outbred' comparisons would be due to half the variance resulting from heterozygous effects, while an increase in the 'inbred' comparisons would be due to homozygous and heterozygous effects of induced mutations. In the second method, the regression of offspring on parent was calculated within generation, treatment group, mating system, sex of offspring and sex of parent. The differences between the regression coefficients of the irradiated and control lines within each group were regressed on the cumulative irradiation exposure in a weighted regression analysis. This should indicate if irradiation has increased the additive genetic variance.

Analysis of within-litter variances and regressions of offspring on parent in the male irradiated line indicated that irradiation had increased the variability in the irradiated line compared to the control. Data from

the female irradiated line did not provide evidence of a larger within-litter variance or a larger regression of offspring on parent in the irradiated line when compared to the control.

The results from the line exposed to cumulative spermatogonial irradiation were considered sufficient to warrant undertaking an experiment to study the effectiveness of selection for six-week body weight. This experiment is in progress.

e) Fertility.

A study of fertility in the female irradiated line indicated that irradiation of female rats resulted in a decrease in the number of females producing live litters in the irradiated line when compared to their contemporaneous controls.

In addition to the main experiment outlined above, three other experiments have been carried out using animals from the spermatogonial and maternal irradiated lines. These are: -

1. A study of the genetic effects of cumulative irradiation in the female rat on prenatal survival and development to the twelfth day of gestation (Wardell, 1965).

The rats used in this study were taken from the line exposed to maternal irradiation and the corresponding control line. Havenstein (1965) found that, after seven generations of maternal irradiation, females from the irradiated line showed a consistent decrease in litter size at one day of age when compared to contemporary control females. The objective of this investigation was to autopsy female offspring of seventh (Experiment 1) and eighth (Experiment 2) irradiated and control generations during gestation in an attempt to determine at what stage of fetal development these differences originated. The dams in this experiment were not irradiated in an attempt to remove somatic effects resulting from irradiation and so enable a more accurate examination of genetic effects due to irradiation in previous generations. Descendants

of the line exposed to maternal irradiation will be referred to as 'irradiated'.

Estimates of fetal mortality were obtained by counts of the number of live and resorbed embryos in the left and right horns of the uterus on the twentieth day of gestation, by measurement of the number of implantation sites and from a measure of the number of preimplantation losses given by the difference between the number of corpora lutea and the number of implantations. These estimates were used to measure the effect of radiation induced autosomal and sex-linked recessive lethals. The genetic effects of irradiation on two quantitative traits, ovulation rate of the dams, as measured by corpora lutea counts, and the weight of the fetuses on the twentieth day of gestation, were also studied. Corpora lutea counts were only available in Experiment 2.

Four groups of females were studied in each experiment. Non-irradiated females from the irradiated line were mated to non-related males from the same line, these matings being termed 'outbred' (RO). Descendants of the irradiated line were also sib-mated, these matings being referred to as 'inbred' (RI). Appropriate matings were made within the control line to form corresponding 'outbred' and 'inbred' groups (CO and CI, respectively). The effects of irradiation on survival, other than those due to the action of induced recessive lethals, will be expressed in both the irradiated outbred (RO) and the irradiated inbred (RI) groups when compared to the control outbred (CO) and control inbred (CI) groups, respectively. Any difference in survival due to induced recessive lethals will be shown in the CI-RI comparison due to a decrease in survival in the RI group. Any effect of inbreeding, independent of irradiation, will be expressed in both the irradiated and control inbred groups. It should be noted that the inbreeding level of the dams in the inbred groups differed in the two experiments, being 25% in Experiment 1 and 0% in Experiment 2. Thus, for each survival trait, the difference between the control outbred and irradiated outbred groups (CO-RO) should provide a control comparison for the difference between the control and

irradiated inbred groups (CI-RI).

The traits measured on the dam were adjusted for age of dam, age of dam squared and age of dam cubed, the latter two variables being used in an attempt to correct for any non-linear effects of age of dam. In addition to the three age of dam effects, fetus weight was also adjusted for the number of live and resorbed embryos in the uterus of the dam.

The analyses of survival traits did not indicate that radiation induced autosomal recessive lethals had decreased survival in litters within the irradiated inbred group. Sex ratio was used as a measure of radiation induced sex-linked lethals. In Experiment 2 a significantly lower percentage of males was found in the irradiated group compared to the control. This is expected if induced sex-linked lethals are acting. However, on closer examination, it was found that this difference resulted from an increase in the percentage of males in the control group rather than a decrease in the irradiated group.

No effect of irradiation on the corpora lutea counts was found in Experiment 2. This does not agree with the finding of Carter and Lyon (1961) of a significantly smaller number of corpora lutea in the paternal grand-daughters of irradiated male mice.

Twenty-day fetus weight was decreased in the irradiated line compared to the control in both experiments, but this decrease was only significant in the experiment using the offspring of animals exposed to eight generations of maternal irradiation (Experiment 2). If it can be assumed that there is no carry-over of somatic effects from irradiation of granddams and previous female ancestors, this decrease in fetus weight may be explained by a detrimental effect of radiation induced mutations. These mutations appear to be additive or dominant in expression, as inbreeding had the same effect in the irradiated and control lines. The difference between the results from the two experiments may not be ascribable to the additional generation of irradiation. There were other differences between the experiments such as level

of inbreeding, parity and age of the dams and the numbers of females mated.

2. The linkage relationships of irradiated induced mutants, spontaneous mutants and marker genes (Yen, 1971).

To date the number of known mutants in the rat is not large and genetic mapping of the chromosomes in this species has not been extensive. Four linkage groups have been established, only two of which contain more than two genes. The discovery of four mutations in the male irradiated line, two in the maternal irradiated line and two in the control line offered the opportunity to study the linkage relationships of these mutations and certain marker genes present in the stocks maintained in the laboratory.

None of the mutations detected in the irradiated and control lines closely resembled descriptions of previously reported mutations in the rat and so were considered as previously unknown loci. The four induced mutations in the male irradiated line were chubby (cb), masked (mk), spastic (sp) and vibrissaeless (vb), and the two occurring in the male control line were skinny (sk) and crawler (cr). A description of the phenotypic characteristics of these mutations is given by Taylor (1968). A single visible mutation was found in the maternal irradiated line. This mutation was given the name paralyzed (pl). The rear limbs of affected young are paralyzed. It appears that all the above mutations are inherited as recessives. However, in most cases, less than expected 25% of affected offspring are found from matings of two heterozygotes indicating that these mutations tend to depress viability when homozygous.

Six marker genes have been identified in three lines of rats (MI_4 , $Curly_2$ and four-way crossbreds) maintained in this laboratory. These genes are albino (c), hooded (h), non-agouti (a), brown (b), pink-eyed yellow (p) and straight (cu₂). Four of these genes had previously been mapped. Genes c and p are located in linkage group I, the distance between them being given

as 19 crossover units (Robinson, 1965), gene b in linkage group II and gene a in linkage group IV.

Appropriate matings were made to obtain segregation information on the six marker and seven mutant genes, and to expose unknown recessive genes. All information used in this analysis was based on identifiable genotypes, these genotypes being identified by phenotype, pedigree and progeny test. Data from single backcross-intercross matings were analyzed to obtain information on the segregation of marker and mutant genes, and to test for evidence of linkage between any of these genes. The chi square test was used to test for deviations from expected segregation ratios, due to factors such as differential viability and misclassification, and for deviations from those ratios expected on the basis of independent assortment. The maximum likelihood method was used to estimate the proportion of crossing over.

Only one linkage value (p) differed significantly from a value of 0.5. This linkage was between genes a (non-agouti) and b (brown) - the value of p was 0.23 ± 0.095 . The 'significance' of the linkage between a and b must necessarily be considered in the context of the number of paired comparisons (37) made between non-allelic genes in this study. The number of 'significant' linkage values expected due to chance alone, if each pair of genes tested was independent, is approximately one. Previous work has indicated that b is located in linkage group II, while a is in linkage group IV.

Analysis of the sexes separately showed that crossing over occurred as frequently in dihybrid females as in dihybrid males.

3. A study of the effect of irradiation on avoidance learning.

With the increase in exposure to mutagenic agents, interest has become focused on the effect of irradiation on the genetic component of human intelligence. Estimates of the extent of the change have been made but generally these involved extrapolation from traits such as bristle number in *Drosophila*

to intelligence in man. Such extrapolation was necessary as studies of the effects of irradiation on 'intelligence' in more closely related species, such as mammals, are lacking.

Gilbert and Graham (1964) studied the effects of prolonged gamma irradiation on the ability of rats to run an elevated T-maze. They found that rats exposed to 3r/week for thirteen weeks had a significantly faster mean running speed than the controls, but there was no difference in the number of errors in the irradiated and control groups. They concluded that changes in motivation, i.e., drive strength, and not changes in learning ability, were responsible for the observed behavioral differences. To our knowledge, experimental data on the effect of X-irradiation on the genetic components of 'learning' in mammals come from only two sources. Newcombe and McGregor (1964) studied maze learning in rats from lines in which males had been irradiated for up to twelve generations and found that maze learning ability was reduced in the irradiated animals compared to the controls. Willham (1969) studied the effect of paternal irradiation on avoidance learning in swine. He found no significant differences in avoidance learning between the irradiated and control animals. However, only one generation of irradiation was given, so there was no accumulation of induced mutations in these animals.

The behavioral trait chosen for study was avoidance learning in which animals learn to associate the onset of a noxious stimulus (an electric shock) with a neutral stimulus (a green light) given a few seconds earlier. This trait was chosen for study for three reasons. First, it has been established that this behavioral trait has a genetic basis. Bignami (1964) selected for avoidance learning in rats and found that the number of conditioned avoidances was increased in a line selected for high rate of avoidance learning and decreased in that selected for a low rate of avoidance learning. The finding that bidirectional selection can produce strains of animals with striking

behavioral differences indicates that there is heritable variation in this trait. This is also indicated by a number of studies on inbred lines of mice (Royce and Covington, 1960; Collins, 1964; Schlesinger and Wimer, 1967; Henderson, 1968; Bovet, Bovet-Nitti and Oliverio, 1969) and rats (Wilcock and Broadhurst, 1967). These workers showed differences in avoidance learning between inbred strains. Henderson (1968) also obtained an estimate of the heritability for the acquisition of a conditioned avoidance response. From studies of crosses between inbred lines he obtained a heritability estimate of 0.21 for this trait.

A second reason for choosing avoidance learning to study the effects of irradiation on 'learning' ability is because of the type and constancy of the motivation involved. Third, this trait can be easily measured on relatively large numbers of animals.

The apparatus used to study avoidance learning was a shuttle box, which consists of two grid floor compartments separated by a door. The door can be lowered or raised. The neutral or conditioned stimulus (CS) is a green light and the noxious or unconditioned stimulus is an electric shock delivered through the grids which form the floor of the compartment. Each opportunity the animal is given to learn the association between the light and the shock is termed a trial. Photocells in the compartment walls monitor the position of the rat in the shuttle box and indicate the occurrence of an avoidance or an escape response, defined as movement, by the rat during a trial, from one compartment to another which terminates the neutral stimulus given prior to the shock (avoidance response) or the grid shock (escape response).

The animals used in this study came from the line exposed to nine generations of spermatogonial irradiation and its corresponding control. In the irradiated line no irradiation had been given for a number of generations prior to the beginning of the experiment, but most of the induced mutations

should still be present as close inbreeding had been avoided in this line.

A preliminary experiment was set up to study such variables as shock intensity, CS-UCS interval and intertrial time. Each rat was given 40 trials (a session) on 5 consecutive days - a total of 200 trials. The criterion to be used in deciding on the levels of the variables to be used in the main experiment was that the animals should learn to avoid the shock in 50% of the trials given during a session. By choosing conditions giving 50% learning rather than maximizing the amount of learning possible, it was hoped that the probability of detecting differences in avoidance learning between the irradiated and control lines would be enhanced. It was found that this criterion was met at the fourth session when the conditions were a shock intensity of 0.3 milliamps, a CS-UCS interval of 10 seconds and an intertrial time of 30 seconds.

This experiment is still in progress. Animals which are 'outbred' with respect to the induced mutations have been tested. These animals are assumed to be heterozygous for the mutations induced by irradiation. Seventy males and seventy females from the irradiated line and the same number from the corresponding control line were tested. Each rat was given four sessions under the conditions determined from the preliminary experiment. The inter-session time was 23 to 25 hours.

In females a difference of 6.4% in learning ability during the fourth session, measured as the percentage of avoidance responses, was found between the irradiated and control lines, the irradiated females avoiding more often than the controls. This difference is significant at the 5% level. The percentage of avoidance responses in the fourth session was larger for males from the irradiated line compared to the controls, but this difference was not significant. Induced mutations, when heterozygous, would appear to enhance the learning of this behavioral task in females.

If a parallel can be drawn between this behavioral trait and the quantitative traits, such as body weight, studied in this irradiated line and its control, induced mutations will tend to show overdominance. Thus it may be expected induced mutations may have a detrimental effect on avoidance learning when homozygous. To test this, animals from the irradiated and control lines have been sib-mated to produce 'inbred' offspring. These 'inbreds' will only be homozygous for 25% of those genes heterozygous in their 'outbred' parents. The effect of inbreeding on these animals should be small as the lines originated from a highly inbred base. The testing of 'inbred' animals is in progress.

Genetic Effects of Acute Spermatogonial X-irradiation (Chambers, 1971)

This experiment was initiated to study the frequency of occurrence of different classes of mutants induced by a single dose of acute spermatogonial irradiation. The design of the experiment can be seen in Figure 1. ✓

The mating plan chosen was a combination of systems designed for the detection of various types of mutational effects. Induced dominant lethal mutations will be expressed in the first filial generation. Hemizyosity allows induced sex-linked recessive mutations to be expressed in male progeny of the second filial generation. Mating of full sibs can be used to increase the level of inbreeding and so the expression of autosomal recessive mutations. Induced mutations in this class will not be brought to expression before the third filial generation.

Haldane (1956) outlined a method in which marker genes were used to scan the genome for recessive lethal mutations. A line homozygous for a number of recessive markers and with a history of exposure to irradiation is mated to a non-irradiated line homozygous for the complementary marker alleles. The progeny of these crosses are sib-mated and segregation of the marker genes observed in their offspring. Deficiencies of offspring showing the recessive phenotype will occur in those sibships in which both parents carry a recessive lethal mutation closely linked to a marker locus.

Figure 1.

Mating design for estimation of effects of various classes of mutations induced
by spermatogonial x irradiation

The specific locus method outlined by Russell (1951) was used to study the mutation rate to recessive visible mutations. This method is a type of 'test cross' in which recessive alleles are used to allow expression of any visible phenotypic changes of dominant alleles induced by irradiation. The necessity of inbreeding to allow expression of autosomal recessive visible mutations is thus avoided.

Modification of the Haldane and specific locus methods was necessary for the inclusion of both methods in the experimental design. The former system requires irradiation of the recessive marker line, while, in the latter, the dominant line is exposed to irradiation. Thus both lines were irradiated. This relaxed the restriction that each line may carry either only dominant alleles or only recessive alleles. In the Haldane method, crossing individuals from the two lines must be delayed until the first generation following irradiation. This delay reduced the efficiency of the specific locus method, one litter now testing a gamete with a history of irradiation instead of each individual representing a test of an exposed gamete.

The effects of induced mutations on three quantitative traits - body weight at 21 days of age and body weight gains from 21 to 42 and 42 to 69 days of age - were also studied.

The two lines of rats used to initiate the experiment had complementary alleles at four marker loci. The MI_4 stock was homozygous for albinism (c), hooding (h), straight vibrissae (cu₂) and non-pink-eyed yellow dilution (P). The Cu_2 stock was homozygous for color (C), non-hooding (H), curly vibrissae (Cu₂) and pink-eyed yellow dilution (p).

The experiment had two 'replicates'. In the first 'replicate', the treatment was a single acute dose of 600 roentgens of testicular irradiation. A long period of sterility occurred following this treatment. To avoid this, the second 'replicate' was treated to a total dose of 450 roentgens of whole body irradiation, given as 100, 150 and 200 roentgens at 10, 12 and 14 weeks

of age, respectively.

A consistent reduction in litter size at one day of age in the irradiated line compared to the control was found in the first and third generations following irradiation. In the first generation dominant lethals will be brought to expression, while autosomal recessive lethals will be expressed in the third generation. There was some evidence that litter size in the second generation was reduced as a result of irradiation. The consistency of the treatment effects in the two replicates indicated that the biological effects of a unit of irradiation on litter size were similar in spite of the total dose and exposure interval differences. Inconsistency between the replicates for the effects of irradiation on 21-day litter size would suggest that later measurements are unreliable indicators of irradiation damage.

Dominant lethal mutation estimates ranged from $(1.18 \pm 1.50) \times 10^{-4}$ to $(3.30 \pm 2.93) \times 10^{-4}$ mutations/gamete/r. Estimates of autosomal recessive lethal mutation rate, based on litter size at one day of age, ranged from $(8.35 \pm 7.64) \times 10^{-4}$ to $(9.08 \pm 3.33) \times 10^{-4}$ mutations/gamete/r. These estimates are about five times higher than estimates obtained in other studies with rats and mice. It is possible that, due to the method of estimation, lethality caused by induced reciprocal translocations has inflated these estimates. Analysis of sex ratios did not indicate a reduction in the proportion of males as a result of irradiation.

Segregation of marker genes in F_2 progeny was analyzed for distortion in an attempt to estimate autosomal recessive lethals. Regions adjacent to the marker genes were scanned for these effects. However, variation in marker gene segregation was too large to permit detection of any effects of irradiation by this method.

No specific locus mutations were observed. The efficiency of this method was reduced by a generation delay in making the test cross. This delay was

necessary because of the incorporation of the Haldane technique into the experimental design.

One visible mutant was observed in the third generation following irradiation. The phenotypic features of this mutant and the apparent semi-sterility of its female relatives indicated that it was probably due to a reciprocal translocation.

No consistent effect of irradiation on the quantitative traits studied was found.

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