EXPERIMENTS TO TEST THE VALIDITY OF THE LINEAR R-DOSE/MUTATION FREQUENCY RELATION IN DROSOPHILA AT LOW DOSAGE[1]

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SINCE the discovery of MULLER (1927) and STADLER (1928) that X-rays induce mutations in organisms, a very large body of data has been accumulated by many workers dealing with the relationship of mutation frequency to dosage intensity. X-rays of various wave-lengths, radiations of radium, neutrons and ultra-violet light have all been employed as causative agents. It is not the intention here to review the voluminous literature which has grown up in this field. The reader may refer to the general reviews of SCHULTZ (1936), STUBBE (1937), and TIMOFÉEFF-RESSOVSKY (1937).

On the basis of the accumulated data on *Drosophila melanogaster*, on which most experiments have been conducted, and with allowance for variables not easily controlled from experiment to experiment, radiation geneticists are generally agreed that the r-dose/mutation frequency relation seems to be a linear one. Furthermore, experiments on fractionation of dosage and variation in time-intensity relationships would seem to indicate that this linear relationship should hold at very low dosages. While the extrapolation of the curve into the region of low dosages is a reasonable hypothesis, the experimental investigation of this part of the curve is of interest. From a theoretical point of view the validity of the extrapolation needs to be checked by observation. From the practical aspect of the effect of low dosage radiation on man this part of the curve is of special significance.

The lowest dosages thus far used have been 385 r for X-rays by OLIVER (1932) and 400 r for radium by RAYCHAUDURI (1941, 1944). The accumulation of sufficient data to be statistically significant for lower dosages than the ones reported is a considerable task. Here will be reported data on controls, 25 r, 50 r, 150 r, 500 r, 1000 r, 2000 r, 3000 r, and 4000 r. The collection of the data occupied the full time of three workers in the laboratory from October, 1943 to July, 1945. In addition to the main problem of induced mutation frequency at low dosages, some data are presented on the ratio of pure lethals to visibles,

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semilethals with visible effects, and semilethals without visible effects, the occurrence of lethal clusters in controls and such clusters at low dosages, the association of lethals with crossover modifiers at different dosages, and the distribution of lethals and visibles on the genetic map of the X chromosome in *Drosophila melanogaster.*

DESCRIPTION OF TECHNICAL METHODS EMPLOYED

The genetic procedure

Through the courtesy of DR. H. J. MULLER, now of INDIANA UNIVERSITY, whose cooperation and advice have been invaluable, a tool stock was secured for use in making tests of lethals and visibles in the X chromosome of control and irradiated flies. This stock, the fifth in a series of stocks, will be designated as "Muller-5." The complete formula for the stock is $sc^{SI}B$ In-S w^a sc^8. MULLER (written communication) explains the genetic content of the stock as follows: "The expression sc^{SI} · · · sc^8 refers to a chromosome whose left end is derived from the scute^{SI} inverted X chromosome and whose right end is from a scute^8 chromosome. Such a chromosome is easily obtained by single crossing over between the two scutes. Since these inversions are sensibly identical except for the fact that the left break of scute^{SI} is just to the left of it, this chromosome has two scute genes, scute^{SI} near the left end and scute^8 near the right end, but is otherwise sensibly identical with either a scute^{SI} or a scute^8 chromosome. "In-S" designates Inversion-S (S = SINITSKAYA), an inversion slightly smaller than and in very nearly the same position as "delta 49," which arose in the scute^{SI} chromosome by irradiation at the same time as the scute^{SI} itself arose. I made up this particular chromosome for the purpose of studying mutations in the female, because there is needed for this object a chromosome having (1) very little crossing over throughout its length, (2) suitable markers, both dominant and recessive, and (3) comparatively high viability and fertility. Condition (1) is here fulfilled by the two inversions, one included within the other, though rarely (less often, I believe, than in the case of ClB) double crossing over does occur: this usually separates the Bar from the rest, so if both Bar and apricot loci are followed, one can be virtually certain of what one has. Condition (2) has been provided by Bar and apricot. Condition (3) holds because the duplication of the scute locus largely prevents the lowering of fertility and viability that scute^8 or scute^{SI} alone would give."

For the purpose of the initial detection of mutations in the X chromosome and for their subsequent testing and retention in stock, we have found this chromosome distinctly superior for the following reasons to the ClB chromosome used widely in such experiments. Crossing over is practically eliminated throughout the length of the X chromosome. In a test experiment involving 30 cultures in which female parents were Muller-5/scute-crossveinless-vermilion-forked-carnation (see below), no crossovers were observed among 2622 male offspring. In other cultures, the rare double crossovers involving Bar have been seen on an average of only one in several thousand flies examined, but the presence of two markers readily identified these. The stock is viable in

homozygous form, and this gives it a distinct advantage over any ClB balanced stock in procuring large numbers of virgin females for initial crosses. Homozygous Muller-5 females have a lower viability and fecundity than females heterozygous for Muller-5 and any sex-linked mutant either lethal or visible, except a few weak dominants such as "Minutes." This makes possible the retention of any new lethal or weak recessive visible indefinitely in stock in a pseudo-balanced condition with Muller-5, provided the stocks are cultured in half-pint bottles and enough parents are used in each generation to eliminate the chance selection of only Muller-5 females when flies are shaken into a fresh stock bottle. Such stocks could, of course, be carried in smaller culture bottles if heterozygous females were selected as parents each generation.

The use of this stock in testing for either lethals or visibles which have arisen in the X chromosome of control or treated males is illustrated in figure 1.

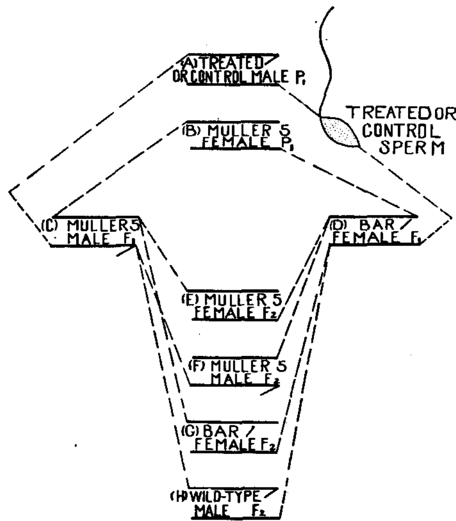

FIGURE 1. The Muller-5 technique for the detection of lethals and visibles in the X chromosome of *Drosophila melanogaster*. See text for full explanation.

Treated or control males (A) are mated to Muller-5 females (B). After the F_1 flies have been aged together for several days, each female is placed in a separate test culture bottle with one or more Muller-5 males. Four classes of F_2 flies generally appear in such a test culture, Muller-5 females (E) and males (F) which are apricot Bar, heterozygous apricot Bar females (G) which carry one X from the control or treated grandfather, and wild type males (H) carrying the X chromosome from the control or treated grandfather. If a new lethal has arisen in the control or treated sperm cell figured, all of class (H) will fail to appear. If a new visible has arisen, all of class (H) will show it. A further test of the lethal or visible is made by mating females (G) which carry the new mutation to their Muller-5 brothers (F). The new mutation can be carried indefinitely in this manner as the Muller-5 chromosome eliminates

crossing over. The occurrence of non disjunction males among the F_2 flies will never obscure the presence of a new lethal as such males will be Muller-5 and not wild type. The genetic set-up is particularly convenient because only at one stage in the entire procedure is it necessary to use virgin females, the P Muller-5 females (B). Even here the inclusion of an occasional non-virgin Muller-5 female has no serious consequences as her homozygous apricot Bar daughters are easily separated from the red heterozygous Bar F_1 females used for the test matings. This is an important item in a large scale experiment.

About one fourth of the lethals and visibles found were tested for linkage relations. F_2 females (G) or F_3 heterozygous Bar females were mated to the multiple recessive males, scute (0.0)-crossveinless (13.7)-vermilion (33.0)-forked (56.7)-carnation (62.5). Their non-Bar daughters, carrying the new mutant in one X chromosome and the recessive tester genes covering the genetic map in the other X chromosome, were mated to their Muller-5 sons. From five to seven such pair matings were made for each gene tested, and a lower limit of 200 males to be classified was set. The use of Muller-5 fathers in these tests again eliminated errors which might have arisen from non disjunction males.

Choice of stock for testing mutation rate

For irradiation work at low dosages, it was desirable to use a stock of flies with a low control mutation rate and one which would not prove highly variable. Canton-Special was tentatively chosen as the stock to be used, provided it showed on test a low control mutation rate suspected from previous experience. The following description is taken from BRIDGES and BREHME (1944). "A standard wild type phenotype, obtained from a stock collected in Canton, Ohio. Selected by BRIDGES. The salivary chromosomes were found by BRIDGES to be normal. The stock contains a recessive for multiple thoracic and scutellar bristles, which overlaps wild type in most of the flies but appears sporadically in strains in which Canton-S has been used in crosses." The stock had originally been made isogenic by BRIDGES, and about four years before these experiments were started, the process had been repeated by one of the present authors (C. S.).

During the autumn and winter of 1943, preliminary tests on control mutation rates in Canton-S and seven other stocks were run. As data accumulated, it became evident that Canton-S had a low natural mutation rate, considerably lower than the average of the other seven stocks and possibly as low or lower than any of the others. (For a discussion of the variability of spontaneous mutation rates, compare MULLER 1928b, DEMEREC 1937b.) The results of these tests are given in table 1. The figures for the seven stocks include about 3000 tests run during the spring and summer of 1944. As Canton-S is a wild type stock, it has the advantage over any stock homozygous for visible mutations which might mask new visibles; for instance, a stock containing white would eliminate work on all other eye colors at the white and other loci. During the course of these tests, a routine was gradually evolved whereby large numbers of test cultures could be run through on a uniform schedule. The detailed

TABLE 1

Preliminary tests on natural mutation rate in eight stocks of Drosophila melanogaster. Lethals and visibles arising in male germ cells.

STOCK	CHROMOSOMES TESTED	LETHALS	VISIBLES	% LETHALS
Pittsford, N. Y.	2,218	5	1	
Webster, N. Y.	1,913	3	1	
Oregon-R	1,923	2	1	
Muller-5	1,366	1	0	
white	1,080	3	0	
yellow-white-forked	764	2	0	
New Wilmington, Pa.	727	0	1	
Total of seven Stocks	9,991	16	4	.16
Canton Special	20,324	17	2	.08

account of this routine given in the following section appears justified both as a guide for other workers and as a source of intrinsically important information on the details of the present experiments.

Culture of Muller-5 and Canton special stocks

Each week on Friday ten or eleven half-pint cultures of Muller-5 were started. The usual molasses-cornmeal medium, enriched with dry brewer's yeast was used for these and all other cultures. Moldex was added to keep down mold growth. Carragar, a specially prepared extraction of the marine seaweed, *Chondrus crispus*, was substituted for agar because of the difficulties in securing adequate supplies of the latter. About fifty pairs of parent flies were used in each half-pint culture. On the following Tuesday, two double sheets of "Kleenex" tissue paper soaked in a suspension of about 100 g of baker's yeast in 600 cc of water were added to each half-pint culture. The stocks were kept in an incubator at 24–25°C and flies were emerging at this temperature 11–12 days after the cultures were started. Each Wednesday virgin females to be used as parents for one week's supply of flies for test matings were isolated. All flies were shaken out of the stock bottles Tuesday night and virgins collected Wednesday morning, or flies were shaken out Wednesday morning and virgins collected Wednesday afternoon. Each week 400 or 440 virgin females were isolated. With the culture methods outlined above this required about one half hour. The following record was kept to illustrate this point. From seven half-pint Muller-5 cultures, the flies were shaken out at 10:00 a.m. Newly emerged flies were shaken out at 3:30 p.m., etherized, and 625 virgin females, maximum age five and one half hours, isolated by 4:00 p.m. The Muller-5 females were placed in ten or eleven half-pint culture bottles, 40 flies per bottle and aged until Friday, two days, at which time the Canton-S control or irradiated males were added to these bottles. On Friday, flies which had emerged the two previous days in the MULLER-5 stock bottles were used to start fresh stocks and

the old ones discarded. Generally these flies were simply shaken into the new stock bottles, but about once a month the parents were selected under the binocular microscope to eliminate any round eyed males or heterozygous Bar females due to Bar reversion in the MULLER-5 stock.

Stock of Canton-S was started each week on Wednesday by shaking newly emerged Canton-S flies into six half-pint culture bottles. These flies were all shaken on Friday into one half-pint etherizer and etherized together. The bottles from which they had been shaken were set back as fresh stock and yeasted on Monday. The flies which had been shaken out and etherized were two to four days old, and from them 400 or 440 males were selected under the binocular microscope and placed in gelatin capsules, 20 flies per capsule, for irradiation or as controls. These males were examined to make certain that they showed no new visible mutations at the time they were isolated. After irradiation, the flies were immediately placed with the virgin MULLER-5 females, 40 males per half-pint bottle, and each bottle marked with the dosage received. The Canton-S stock was thus carried in six lines and the flies from the six lines thoroughly mixed and sampled for each week's lot of parent males to be irradiated or to serve as controls. The Canton-S stocks were purposely raised under somewhat crowded conditions in order to eliminate semilethals which might arise in the stocks. Such semilethal flies are generally weaker than wild-type, and by some larval and adult crowding, it was hoped that they would largely be killed off. It may be argued that crowding the Canton-S stock might have affected in some way the natural mutation rate. This might be true, but this possible objection seemed to be outweighed by the necessity of eliminating as far as possible any semilethal males which might otherwise be used as parents for the experimental cultures.

On Tuesday, the parent flies from the ten or eleven crosses of MULLER-5 females to Canton-S males started the previous Friday were shaken into a fresh set of marked half-pint culture bottles and the first set provided with Kleenex soaked in yeast suspension. On Thursday, the parent flies from the second set of cultures made up on Tuesday were discarded and these cultures yeasted.

As the F_1 flies (C) and (D) of figure 1 began emerging, they were shaken into marked half-pint aging bottles. These F_1 flies generally started emerging twelve days after the cultures were started, that is, on Wednesday. Emergence from the two sets of cultures spread over about one week's time; the first flies emerging after having been aged for several days were used on the following Monday for the pair mating test cultures of which the flies (E), (F), (G), and (H) of figure 1 were the offspring. All test matings for a given week were made from the offspring of one lot of irradiated and control males. From 40 to 60 half-pint aging bottles were used each week. It was found that the use of aging bottles cut the number of failures in the pair mating test cultures from around ten percent to less than five percent. Females aged with MULLER-5 males were generally already impregnated and ready to lay fertile eggs when placed in the test culture containers. The small amount of work involved in aging the flies was well repaid in results obtained.

The test cultures

During the period from December 1, 1944, through June, 1945, approximately 127,000 test cultures were made up; of these, 121,353 (an average of 17,336 per month) developed and were examined for lethals without etherization (see below). In the earlier part of the work, fewer test cultures were reared as they were all etherized and examined both for lethals and visibles. The following routine was followed for the period mentioned above. Test cultures were reared in one ounce culture bottles ("creamers"). The medium was the same as that used for half-pint cultures. Creamers were arranged in rows on tin trays; three fourths of these trays held 95 creamers each and one fourth held 120 creamers. Hot medium was poured into four trays of creamers each morning and afternoon, a total of 810 creamers per day. On cooling, the culture bottles were inoculated with a drop of heavy yeast suspension and the four trays placed in the refrigerator without capping the bottles. The lot made up in the morning was used that afternoon and the lot made in the afternoon was used the following morning. On Saturday only one lot of culture bottles was prepared and these bottles were used the following Monday morning.

When a tray of creamers was removed from the refrigerator, it was covered with cheese-cloth. Flies from the aging bottles were etherized and males and females separated and placed in dry vials where they could easily be re-etherized. One female and one or more males were placed in each creamer which was immediately covered with a paste-board creamer cap in which three short cuts were made with a dental scalpel for ventilation. As soon as creamers were removed from the refrigerator, the air within them began to warm up with consequent drop in relative humidity and evaporation of moisture from the food surface. Few flies became stuck in the medium and there was less contamination with mold than if paper or other materials had been added. Failure of a culture to develop was, however, generally due to the parent female becoming mired in the food medium, often in the spot of yeast. In order to avoid this as much as possible, the yeast was placed at one side of the creamer. Actually, many trays came through with no failures, and the percentage of failures was definitely correlated with atmospheric humidity. A library card was placed on each tray with date and X-ray dosage marked in two ways to avoid errors in recording. The trays were then placed in order on shelves in open cabinets in the laboratory and not disturbed until the cultures were examined for lethals exactly two weeks after they were made up. Parent flies were not removed, for their phenotypes were such that they did not interfere with the identification of the lethals. Thus at all times there were 88 trays and 8,910 test cultures arranged in sequence on the cabinet shelves.

Examination of test cultures for lethals

On Monday through Friday eight trays of test cultures per day were examined for lethals and on Saturday four trays. As the cultures were arranged in rows on the trays, they could always be examined in the same order. On the average, more than 100 flies were present per culture. These were examined by

placing the creamer on its side under the low power of the binocular microscope, magnification 7×, and checking for wild type males which could readily be identified at this magnification. The 810 cultures could be examined accurately in this manner in less than two hours. Each culture without offspring was set out of the tray and also each culture with no wild type males, a potential lethal bearing culture. The total number of fertile cultures and of failures was recorded on the card, and this card set in a small tray with any potential lethal cultures or filed if there were none of these present. Potential lethal cultures were set back for two more days and then the flies etherized and each class of flies recorded on a lethal card together with the date on which the lethal was found. At this time two new cultures of three heterozygous apricot Bar females and five MULLER-5 males were made up to test the lethal through another generation. A later count of the original lethal culture and counts of the two F_3 generation tests were made and recorded on the lethal card. In case of linkage tests, heterozygous apricot Bar females from the F_3 cultures were mated to the multiple recessive males mentioned above. The few cultures coming through with less than a dozen flies were scored as failures and were not included in the total. With the food medium enriched with brewer's yeast, the number of such cultures was extremely low and could not have introduced any large error. However, to be sure that such low yielding cultures were not due to some dominant effect of a lethal carrier, many cultures of a few flies containing no wild type males were tested through another generation using the heterozygous Bar females present. The results of such tests indicated that there was no selection against lethal bearing chromosomes by the discard of the small group of cultures with only a few flies per culture. In such cultures, generally one or both parents were found dead, thus explaining the low yield. The procedure outlined above was specifically designed for the discovery of pure lethals, which experience had shown was by far the largest class of mutants that could be recovered rapidly and with little subjective error. No attempt was made to save semilethals and a culture was discarded as soon as one wild type male was seen. As such a male could not be due to nondisjunction and as crossovers were practically eliminated, no pure lethals would be discarded on this criterion. All of the checking for lethals without etherization of flies was done by the senior author.

Until mid July, 1944, all control and radiation test cultures were etherized and examined under the binocular microscope for both lethals and visibles. As this method required much more time, all three workers helped. During this period another step was included in the routine. About five days after the test cultures were made up, parent flies were removed and a small piece of Kleenex paper soaked in yeast suspension was put in each creamer. This increased the yield somewhat, and the presence of the paper made it easy to shake out adult flies at the time of etherization since, especially in warm weather, carrager medium becomes soft, and it is difficult to shake flies from the culture. Lethals and visibles found were tested in the same way as described above for lethals.

During the period from December 1944 to July 1945, one of the assistants (DR. M. HARLAND) also examined most of the cultures from flies receiving

radiation of 150 r or more for visibles after they had first been checked for lethals. These cultures were etherized in checking for visibles. Problems involved in scoring both lethals and visibles will be discussed below.

The X-ray treatments

In an ideal radiation experiment, all lots of flies receiving different dosages would be irradiated on the same apparatus on the same day and the output of the apparatus checked during the radiation with a dosimeter which had itself been standardized immediately before the radiation. Obviously, these conditions could not be adhered to in a large scale experiment involving eight different dosages and hundreds of thousands of test cultures. It was impossible to predict at the beginning of the experiment how many points on the dosage curve could be profitably investigated. It has been the present aim to spread the low dosages over as many lots of flies and as much time as practicable and to spread the control lots over the entire period of the experiment.

A lot refers to control or irradiated flies used for one week's test cultures. These may therefore be referred to as weekly lots. Table 2 gives the number

TABLE 2

Dosages and other conditions of radiation and numbers of lots of males irradiated at different dosages.

DOSAGE	WEEKLY LOTS	K.V.	M.A.	T.S.D. IN INCHES	r/MIN.
Control	70				
25-r	43	90	3.0	18.5	15–22
50-r	11	90	3.2		10
50-r	45	90	3.0	18.5	15–22
150-r	12	90	3.2		10
150-r	31	90	2.0	18.5	15–22
500-r	14	90	3.0	18.5	15–22
1000-r	5	90	3.2		10
1000-r	20	90	3.0	7.25	91–100
2000-r	2	90	3.0	7.25	91–100
3000-r	2	90	3.0	7.25	96
4000-r	1	90	3.0	7.25	96

of weekly lots for each dosage. The weekly lots at 50 r, 150 r, and 1000 r given at 10 r per minutes were irradiated on a machine whose output was checked with a dosimeter at six-week intervals. In all, these lots account for 7,535 test cultures at 50 r, 13,595 test cultures at 150 r, and 3,938 test cultures at 1000 r. For all of the other lots, which include the bulk of the work, dosimeter readings were taken each week at the time the flies were irradiated. As this work was a small part of a much larger radiation program, the dosimeters were being checked from time to time for accuracy. In spite of this fact, small errors in dosimeter readings occur. It is felt that these errors would tend to be equalized by the large numbers of treatments given at dosages from 1000 r down. Data

secured at dosages above 1000 r had relatively little bearing on the main problem being investigated.—At the beginning of the experiment and before any flies were irradiated, some 20,000 Canton-S control cultures were reared but not recorded in weekly lots.

The mutational category chosen for quantitative study

It seems pertinent at this point to consider briefly the kinds of mutations occurring in Drosophila and their adaptability to a quantitative study of the type undertaken, and to define carefully the category of mutation chosen.

Mutations occur in both autosomes and sex chromosomes, and there is abundant evidence that more of them are occurring in the autosome complex than in the X chromosome. However, there is no breeding procedure by which newly arisen autosomal mutants can be found as easily and effectively as can sex-linked mutants by the method outlined in figure 1. Although the data are as yet fragmentary, it would seem that comparative studies on sex-linked and autosomal mutation should make possible a transfer of findings on X chromosome mutation rate to the mutation picture in the autosomes.

Mutations are known to occur at different stages in the development of the organism. The study of mutation rate in mature sperm has certain technical advantages when it comes to securing large quantities of valid data. In extensive experiments on natural mutation rate, MULLER (1945, 1946) has shown that "overall mutation frequency is two or three times higher in the first crop of spermatozoa, accumulated during a male's pre-imaginal life, than in those of a week later derived from a non-virgin male," (MULLER 1945). It should be noted that all of the Canton-S spermatozoa used in our control and radiation experiments were from males a week or less old.

Mutations may be somewhat arbitrarily grouped into the following overlapping categories: dominant lethals, recessive lethals, recessive lethals with visible dominant defects, sterility mutants, recessive semilethals without visible effects, recessive semilethals with visible effects, deleterious or lowered viability mutants without visible effects, deleterious or lowered viability mutants with visible effects, recessive visibles, dominant visibles, and iso-alleles (mutants whose presence can only be detected by special genetic or environmental tools).

CATCHESIDE and LEA (1945) review the data on dominant lethals in *Drosophila melanogaster* and present new data. While these can be shown by statistical means, using relatively high X-ray dosages, to have a higher incidence than any of the other categories quantitatively studied, their use in experiments at low dosages is out of the question because of relatively high control variables. Furthermore, while they are well adapted to the study of certain theoretical problems, their significance can hardly be compared with that of certain of the other categories.

Iso-alleles of the normal alleles present in the original stock, may and probably do, constitute the largest group of genetic changes, but the obvious difficulties in demonstrating their presence eliminates them from consideration. Another large group of mutations is represented by genes with lowered viabil-

ity. Their occurrence was first noted by MULLER (1928a) and their abundance proven by MULLER (1934), KERKIS (1935, 1938) and TIMOFÉEFF-RESSOVSKY (1934). These authors came to the conclusion that the class of viability mutants is from two to three times as numerous as the lethals. More recently, IVES (1945) and DOBZHANSKY and SPASSKY (1944) have reported similar results. This heterogeneous group of genetic changes, readily influenced in expression by genetic and environmental modifiers, is not amenable to quick detection and objective classification and is therefore not favorable for the kind of quantitative study here undertaken.

Visibles, both dominant and recessive, occur in much smaller numbers than any of the previously discussed categories or than pure lethals. Furthermore they are subject to the traditional objection that their discovery depends upon the training of the investigator and is less objective than the discovery of pure lethals where the proper genetic tools are used. In any case, the time involved in finding them is very much greater than that in finding pure lethals.

This leaves lethals and semilethals with and without visible effects. Unfortunately for a purely objective study, there is a sizeable borderline group between the pure lethals and semilethals. In this group are found mutants which may in one generation or culture appear to be pure lethals while in another generation or culture either by the vagaries of statistical sampling or through changed culture conditions a few flies may come through and they must then be scored as semi-lethals. About half or a little over half of these extreme semilethals which we have found show visible effects. This visible effect forms an objective criterion for classification. The other semilethal group grades into the lowered viability class and an arbitrary criterion of classification needs to be set up to separate them. Such criteria are either not accurate because of varying numbers of flies per culture or if accurate, are laborious and time consuming, an important factor to be considered.

Second generation tests of all lethals found were also made. In spite of the fact that 65 of them appeared as pure lethals in the first generation count but as semi-lethals in the second generation count, they have been included with the 914 lethals which behaved as pure lethals in both generations and the calculations based on the total number of 989 lethals of which a small number behaved as semi-lethals in the second generation. This has been done because the criterion of selection in the first generation has been a purely objective one, the total absence of all wild type males. For reasons stated in a later section, we have not included those semi-lethals with visible effects in which a few flies came through in the first generation.

In the present experiments, while the Canton-S stocks, from which the chromosomes being tested were derived were kept at constant temperature, $24^{\circ} \pm 1^{\circ}C$, the experimental test cultures reared for the detection of lethals were kept on open shelves in cabinets in the laboratory, where rather wide fluctuations in temperature occurred at different seasons of the year. This procedure resulted in some lots of experimental cultures being reared under temperature conditions where the emergence of the flies in the cultures was further advanced at the time the cultures were examined than in other lots.

Furthermore, certain mutants behave as semi-lethals at one temperature and as lethals at another temperature (see DOBZHANSKY and SPASSKY 1944). Many examples of mutants were found which behaved as lethals in the first generation and as semi-lethals in the second generation. Some which appeared as lethals in the two test generations acted as semi-lethals in later linkage tests. As explained above, all mutants which were scored as pure lethals in the first generation were included in calculations of mutation rate. However, had the cultures been reared at constant temperature, a rather large group of semi-lethals and delayed emergence mutants with visible effects, discovered through the total absence of the semi-lethal or delayed emergence class when the cultures were first examined, could have been included in the totals. Our experience with such mutants would indicate that the inclusion of these classes would increase by ten percent or more the total mutations discovered, a considerable figure when low mutation rates constitute a limiting factor in these studies. In any case, test cultures should be reared at constant temperature to cut to a minimum inaccuracy in objective scoring of that class of mutants which is semi-lethal at one temperature and lethal at another.

LETHAL MUTATION RATE IN CONTROL AND IRRADIATED MALES

Table 3 presents the data secured during the course of the experiment on lethal mutation rate in controls and males irradiated at the several dosages mentioned in the introduction. In the first column, the dosages are given; in the second column, the number of test cultures reared following each dosage; in the third column, the number of tested chromosomes carrying at least one lethal. If lethals are produced at random throughout the test material, it is clear that at high dosages and high mutation rates some chromosomes will have two lethals induced simultaneously. It has been pointed out by GOWEN and GAY (1933) that the apparent mutation rate at high dosages will therefore fall below the value expected on the linearity rule unless corrections for test chromosomes carrying two or more lethals are made. Obviously the chief interest here is in mutation rate at dosages where this correction factor will be negligible. However, at the high values, 1000 r and up, no attempt has been made to introduce this correction factor for the following reason. It must be remembered that, except where linkage tests have been made, the breeding procedure described above has been such that the mutational complex of a chromosome is not separated into its constituent components. This is due to the fact that all crossing over is eliminated. Therefore, at the higher dosages the observed number of apparently pure lethals will be somewhat increased by cases where two semi-lethals or lowered viability mutants present in the same chromosome cause no males of the class carrying the chromosome in question to appear even though some would have come through if either one of the semi-lethals had been present alone. This might be due to an interaction and lowered viability effect of the two semi-lethals in the same organism. It might also result from the lowered chance of observing one individual in the sample represented by the flies in one test culture. For example, if 50 wild type flies were expected in a culture, then with two semi-lethals, each with a viability of

ten percent of normal, the number of flies of this class expected would be 0.5; and with two lowered viability mutants, one with 30 percent and the other with 20 percent of normal viability, the expected number of flies coming through would be three. Obviously, in some individual cases with this expectation, none would actually emerge. Since semi-lethal and lowered viability mutants together make up a group of considerable size, the observed mutation rate at high dosages will thus tend to be increased. As the above factors act in opposite directions, though not necessarily cancelling one another out, it has been

TABLE 3

Summary of data on lethal mutation rates for controls and for different dosages of x-rays.

DOSAGE	N	n	MUTATION RATE %		
Control	73,901	72	.0788	.0974	.1220
25-r	51,907	88	.1396	.1695	.2080
50-r	31,560	77	.1985	.2439	.3034
150-r	23,195	74	.2586	.3190	.3983
500-r	6,634	87	1.0802	1.3114	1.6087
1000-r	6,977	147	1.8102	2.1069	2.4667
2000-r	2,755	130	4.0276	4.7187	5.5691
3000-r	2,029	132	5.5535	6.5057	7.6737
4000-r	1,843	182	8.6462	9.8752	11.3391

N = Total number of chromosomes tested.

n = Total number of chromosomes in which lethals were found.

Column 5 gives the lethal mutation rate observed in percent.

Columns 4 and 6 give the percentage values of which the observed mutation rates would be the upper and lower five percent fiducial limits (five percent in both tails jointly).

deemed unprofitable to attempt to introduce a correction factor where the highest dosage used is 4000 r. It is of interest to note in the paper of GOWEN and GAY (1933) that of the six points on each of two curves for the percent of chromosomes without recessive lethals, the last two points (highest radiation) fall below the fitted curve. Such a result is not surprising when a correction was made for chromosomes bearing two lethals and no counter correction for double semi-lethals and double lowered viability mutants.

Column 5 gives the lethal mutation rates expressed in percent of lethal bearing chromosomes found in the tests for controls and at different dosages. It was intended to proportion the test cultures at different dosages in such a way that the standard error would be about the same percent of the mutation rate for such dosage and for the controls. Actually on this criterion about twice as many data have been secured at dosages from 1000 r up as at the lower dosages and controls. No attempt was made to secure exactly the same number of mutants or to keep the standard error/mutation rate ratio a constant for all dosages studied for this would inevitably have resulted in selective error toward the end of the experiments.

Columns 4 and 6 give percentage values of which the observed lethal mutation rated would be the upper and lower five percent fiducial limits (five percent in both tails jointly). Thus, if 0.0974 percent represents the observed control mutation rate, then a true rate of 0.0788 would be expected once in forty times to result in an observed value as large as, or larger than 0.0974. Similarly, a true rate of 0.1220 would be expected once in forty times to result in an observed value as low as, or lower than 0.0974. The calculated values were obtained by the following method suggested by DR. DONALD R. CHARLES:

"Let n′/N represent a "universe" mutation rate and n/N the mutation rate in a sample of N. The standard error of n′ is

$$\sqrt{n'\left(1-\frac{n'}{N}\right)}$$

By ordinary theory, the upper five percent fiducial limit of n′ is

$$n' + 1.96\epsilon$$

But from STEVENS (1942) this limit is actually

$$n' + 1.96\epsilon + c$$

c being a correction. We want to find an n′ such that

$$n' + 1.96\epsilon + c = n$$

For any particular sample n and c are known; the equation can thus be solved for n′."

On the basis of the experimental evidence, it would seem that an X-ray dosage even as low as 25 r produces a significant rise in mutation rate over that found in the controls.

Figure 2 shows the fit of a straight line curve to the data secured. The vertical lines represent the statistical limits at the five percent level of significance. The curve is based on all the data reported in table 3, which give a control mutation rate of 0.1004 percent per chromosome tested, and an induced mutation rate of 0.00215 percent per r-unit of radiation per chromosome tested. Only the control, 25 r, 50 r, and 150 r points are plotted on this curve. On the basis of this figure, it is not unreasonable to conclude that the lethal mutation rate in mature sperm of the Canton-S stock is directly proportional to the X-ray dosage for values as low as 25 r. The low value at 150 r must be considered in connection with the entire experiment. As eight dosages and control values have been studied, it is not surprising to find one point on the curve considerably out of line. Figure 3 is a log dose-mutation rate plot made up from data in table 3.

CONTROL VALUES AND LETHAL CLUSTERS

At X-ray dosages of 1000 r and up the induced mutation rate is relatively so much higher than the control rate that the latter plays a very minor role in the experimental procedure and the interpretation of data. This is by no means the case at low dosages in the range of 25 r to 50 r where the control mutations may equal or exceed in number those produced by the radiation.

It is therefore important to collect a large body of control data and to collect these data in so far as possible at the same time and under the same environmental and genetic conditions as the radiation data in order to reduce errors from control fluctuations to a minimum.

The data on both controls and each dosage group were collected and filed in weekly lots as explained above. These lots were in turn made up of sub-lots consisting of the test cultures on each tray, an average of a little less than 100 cultures per tray. The filing of the data in this way made it possible to observe and test cases in which the lethal distribution among control and low dosage cultures appeared to be non-random. This procedure led to the discovery of several cases of what we shall designate as "lethal clusters" among the controls and the low dosage tests. As this matter has an important bearing on the collection of data in low dosage experiments, some specific cases will be presented in detail.

Among 1,726 test cultures examined in the forty-second lot of controls, there were seven lethals found where, on the basis of the control rate established, about 1.7 lethals were expected. Four of the lethals were found in two trays, two per tray, and all were late pupal lethals. As not more than 20 percent of all lethals are pupal, this made their chance association even less likely. It was thought that these might have come from a Canton-S male carrying a semi-lethal X chromosome, but subsequent tests showed that this was not the case. In table 4 the data are presented. It will be noted that the linkage tests placed

TABLE 4

Lethal cluster: 42nd lot of controls.

CULTURES MADE UP	NO. CULTURES	NO. LETHALS	NO. FLIES PER LINKAGE TEST	LOCUS
12-14-44 (Tray 8)	91	2	(a) 340	22.7
			(b) 335	25.3
12-14-44 (Tray 9)	90	2	(a) 332	23.2
			(b) 248	23.4

Counts demonstrating that these are pure lethals (1 F_1, 2 F_2 cultures).

LETHAL	APRICOT BAR ♂♂	+♂♂	APRICOT BAR ♀♀	+/APRICOT. BAR ♀♀	TOTAL
8a. 12-30	79	0	78	162	319
8b. 12-30	76	0	77	191	344
9a. 12-30	68	0	68	185	321
9b. 12-30	94	0	105	150	349
Totals	317	0	328	688	1,333

the loci of these four lethals at 22.7, 25.3, 23.2, and 23.4 on the genetic map of the X chromosome. This fact, in conjunction with their phenotypic character

(late pupal) clearly indicates that they are one and the same lethal. As there were three creamer cultures of each lethal reared, this really amounted to 12 test cultures and 1,333 flies reared with not a single wildtype male coming through. In addition a half-pint mass culture of each of the four lethals was reared and several hundred flies from each culture examined with no wild type males appearing. It seems well established that this was a pure lethal and not a semilethal and that the four lethals found actually came from four sperm cells derived from the same Canton-S male and all carrying the same lethal which must have arisen in the germ tract of this male. The male might possibly have been a gonosomic mutant (SIDKY 1940), the germ cells all lethal bearing and the body cells non-lethal bearing. Such an explanation is in principle the same as that suggested, the lethal mutation having arisen at an earlier stage in the ontogeny of male.

Among 804 test cultures examined in the forty-second lot at 50 r, twelve lethals were found. At the mutation rate established for this dosage, about two lethals were expected in the 804 cultures. Three of these lethals were found in one tray of 119 cultures and four in a tray of 89 cultures. These seven were run through linkage tests and three cultures of each were raised. All proved to be pure lethals and the linkage tests showed that six of the seven were probably identical. The data on this case are given in table 5. The other five lethals in this lot were not tested for linkage relations. Some or all of them may have been identical with the six shown to be the same. Such a cluster of pure lethals can best be accounted for by assuming that a lethal arose early in the germ tract of one of the 80 Canton-S males used as grandparents of the flies in the 804 test cultures. These lethals would then be control lethals and not due to the radiation. although they appeared in the 50 r tests.

In the forty-sixth lot at 50 r, three lethals appeared in one tray of 89 test cultures. Linkage tests placed their loci at 0.0, 28.0 and 30.4. Apparently two were identical and the third at a different locus. Two lethals appeared in a tray of 94 cultures in control lot 67. They proved to be identical, the linkage tests placing their loci at 29.1 and 29.2. In the third lot at 50 r two lethals were found in a tray of 89 cultures; the locus of both by linkage tests proved to be at 52.0. In the fifth lot at 150 r in a tray of 94 cultures two mutants were found; one gave no wild type males in the test culture and the other gave one. The linkage tests placed these at 23.7 and 24.4 respectively. During the linkage tests, a few flies came through showing extreme rough eyes, coarse and irregular bristles, and coarse textured wings sometimes nicked. This is apparently an extreme semi-lethal allele of goggle, locus 25 in the X chromosome (BRIDGES and BREHME 1944). It is unlikely in the latter case that one of the Canton-S males irradiated at 150 r was goggle as these flies were examined for mutants, and such an extreme phenotype would not seem likely to be overlooked. It seems more probable in the light of evidence from the other cases that this represented a visible cluster.

The following case, however, illustrates the fact that a visible might be overlooked in selecting Canton-S males for irradiation. In lot 54 at 150 r out of 830 test cultures, fourteen were found showing wavy wings. These cultures

TABLE 5

Lethal cluster: 42nd lot of 50-r

CULTURES MADE UP	NO. CULTURES	NO. LETHALS	NO. FLIES PER LINKAGE TEST	LOCUS
3-6-45 (Tray 3)	119	3	(a) 119	38.4
			(b) 143	38.9
			(c) 267	37.8
3-6-45 (Tray 4)	89	4	(a) 258	36.6
			(b) 205	0.0
			(c) 234	39.5
			(d) 207	38.4

Counts demonstrating that these are pure lethals (1 F_1, 2 F_2 cultures).

LETHAL	APRICOT BAR ♂♂	+♂♂	APRICOT BAR ♀♀	+/APRICOT BAR ♀♀	TOTAL
3a. 3-20	89	0	76	90	255
3b. 3-20	103	0	79	128	310
3c. 3-20	92	0	80	144	316
4a. 3-20	142	0	108	183	433
4b. 3-20	122	0	91	154	367
4c. 3-20	125	0	111	184	420
4d. 3-20	135	0	131	164	430
Totals (4b. omitted)	686	0	585	893	2164

were found in four trays out of the nine 150 r trays for that week. There were 354 cultures in these four trays. On the average about ten test cultures should come from one male fly. In this case it seems probable that all fourteen female parents of these test cultures showing wavy wings had obtained their wavy-bearing X chromosome from the same male. As some wavy males approach the normal in phenotype it could easily have been overlooked when the Canton-S males were selected for raying. This case, however, may represent another visible cluster. The linkage test of the mutant placed it at locus 40.7. The mutant fits the description of wavy, locus 41.9 (BRIDGES and BREHME).

From the evidence presented above, it seems clear that mutation clusters, groups of germ cells bearing an identical mutation which has arisen at an early stage in the germ tract, are likely to form an appreciable proportion of the control or natural mutations recovered. The time at which the mutation occurs in the germ tract need have no bearing per se on mutation rate. But it *will* have a definite bearing on the structure of samples taken from mature germ cells. In small Drosophila populations, this phenomenon could play a role in the high incidence of certain mutant genes. In the present problem, it has a definite bearing on the method of sampling the germ cells from a stock of flies in which mutation rate is being studied. In this study, between 20,000 and

25,000 Canton-S males were used to secure the sample of sperm cells for the 200,801 test cultures. If the experiment were to be repeated on the same scale, about four times this many males would probably be used. Ideally, one male should be used for each F_2 test culture. However, this would practically double the work so that half as many data could be collected in the same time. A reasonable balance should be struck between the work involved in the sampling method and that applied to the collection of test data so that the sampling errors may be kept small and the data secured may be as extensive as possible. It seems clear in the present experiment that each lethal test culture found in a demonstrated lethal cluster must be scored as a separate lethal as each test culture from a non-lethal cluster of germ cells is scored as a separate non-lethal. Obviously there is no method of demonstrating the test cultures forming non-lethal clusters, but by implication they must be there.

We believe that the body of data presented in this report is sufficiently large so that the sampling error from control lethal clusters does not seriously invalidate the conclusions drawn. It is not unlikely, however, that some of the variations in control mutation rate in different stocks and in the same stock at different times as reported by various workers can be accounted for on the basis of insufficient or faulty sampling. Even where linkage tests did not demonstrate any lethal cluster, the inclusion of a disproportionately high number of germ cells from non-lethal clusters in one experiment might give a mutation rate lower than normal. It seems clear that where control data are needed, the method of sampling the germ cells must be given careful consideration. LURIA and DELBRÜCK (1943) discuss the sampling problem involved in determining mutation rate in bacteria, and AUERBACH and FANO (1943) consider the same problem in dealing with the scoring of mutations arising from chemical treatment of Drosophila larvae.

The mutation rate at low dosages

As will be seen from table 2, only two weekly lots of flies were irradiated at 2000 r and 3000 r and only one at 4000 r. As much work has already been done at these dosages, the present purpose was only to secure some additional data at these dosages as a general check on the low dosage experiment. From all of our data, assuming a linear r-dose/mutation rate relation, the control mutation rate value has been calculated as 0.1004 per cent and the mutation rate per r-unit as 0.00215 percent. From these data, it appears that the control or natural mutation rate is doubled by a dosage of 46.7 r.

Owing to the possibility of errors at the high dosages due to the small number of irradiated lots at these dosages and consequent chance of errors in dosimeter readings and to the inaccuracies introduced by multiple lethals and semilethals in some chromosomes, it was decided to calculate the control and radiation mutation rate on the basis of the data collected at 0 through 1000 r radiation. These data were less likely to be subject to errors in dosimeter readings as all radiations at 1000 r and lower were carried out on many weekly lots of flies (see table 2). They were relatively free from errors due to multiple mutations in chromosomes tested owing to the low induced mutation rates.

Calculations on the basis of these data gave a control mutation rate of 0.1039 percent and a mutation rate per r-unit of 0.00197 percent. These figures indicate a doubling of the control mutation rate at a dosage of 52.7 r. The two sets of values check closely, the low induced mutation rate at 150 r and the high rate at 4000 r, accounting largely for the minor differences in the figures.

In figure 2 the solid line represents the curve based on the assumption of a linear r-dose/mutation frequency relation and calculated from all the data, while the broken line represents the curve calculated from the data for controls through 1000 r radiation. On the basis of this curve, the 150 r mutation value shows a deviation from the expected at about the five percent level of signifi-

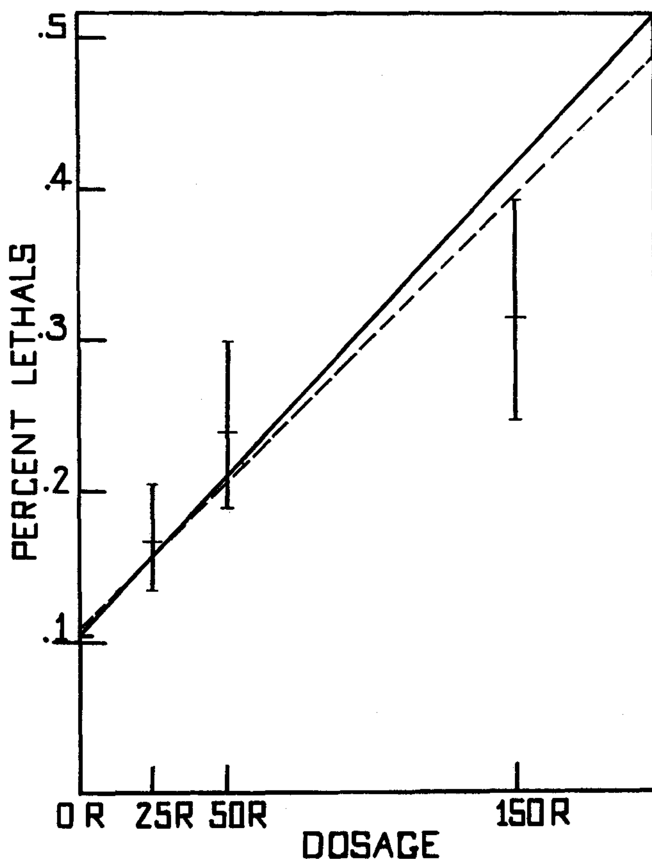

FIGURE 2. The linear relation between r-dose and lethal mutation rate. The solid line is based on all data in table 3; the broken line is based on the control through 1000 r data, omitting the 2000, 3000, and 4000 r data. The data points for control, 25, 50, and 150 r are shown as small central horizontal marks. The ends of the vertical lines represent values of which the observed would be the upper and lower five percent fiducial limits.

Calculated mutation rates (maximum likelihood) from all data: control, 0.1004%; induced, 0.00215% per r.

Calculated mutation rates (maximum likelihood) from 0 through 1000 r: control, 0.1039%; induced, 0.00197% per r.

cance, and the 50 r value shows a deviation in the opposite direction and almost as great. If the control lethal clusters reported for the 50 r material had been present in the 150 r cultures the deviation of these two points from the fitted curve would have about the same values. The deviation of the mutation rates at the different dosages from the expected values may well be explained as due to chance, particularly with the factor of control lethal clusters taken into consideration. An examination of figure 3 and of published curves on the

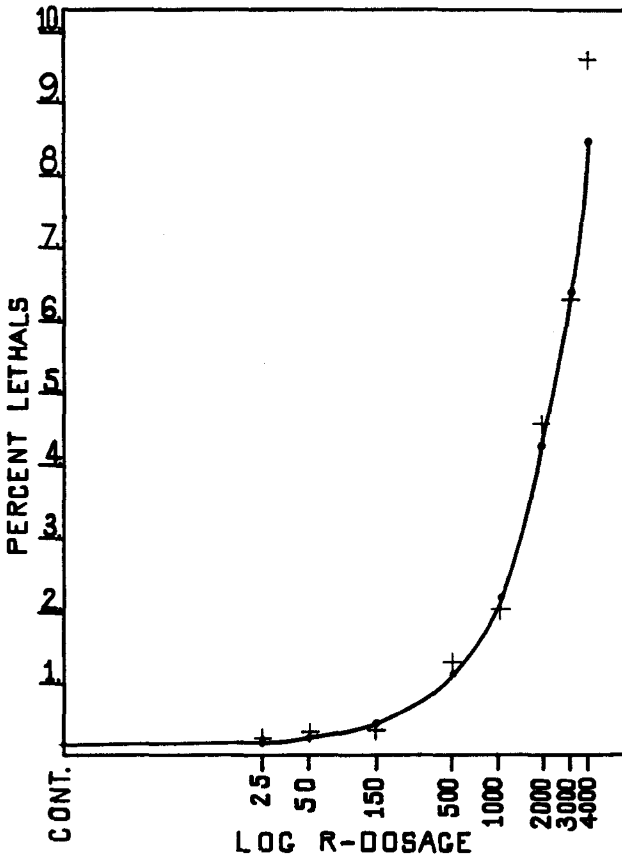

FIGURE 3. A log dose mutation rate plot constructed from all the data in table 3. The crosses above or below the theoretical points on the curve mark the actual mutation rates observed at the different dosages.

linear r-dose/mutation relation at high dosages will show that here, also, chance and unknown variables cause considerable fluctuations from the theoretical values to be expected. On the basis of control through 1000 r data the facts are consistent with the interpretation that the linear r-dose/mutation frequency relation holds at low dosages.

Let us now consider certain differences between our findings and those of previous workers. The somewhat lower than average mutation rate which was found in both control and irradiated flies needs comment. It is possible that

the genetic background in Canton-S is such that certain mutations which would act as lethals in some stocks behave as semilethals here because of the general vigor of the stock. Many of the experiments on the lethal mutation rate have been done on multiple mutant stocks less vigorous than Canton-S.

Furthermore, in our experiments the use of yeast-enriched medium tended to bring through some flies, which, on a poorer medium, would all have died before the adult stage. In this connection a survey of the earlier literature on the induction of mutations by X-rays shows that in individual radiation experiments almost invariably inadequate data were secured to establish control mutation rates within reasonably narrow limits. This is no reflection on early workers as the high dosages employed showed a tremendous increase in the induced mutation rates even if the control rates were in error by a factor of ten or more. However, when the small lots of control data from separate experiments are combined into larger lots representing control data collected at about the same time in the radiation work, an interesting comparison may be made. The earliest control data, those reported by MULLER and ALTENBURG (1919) give a very high mutation rate. The summary of control data(SCHULTZ, 1936) excluding the early data of MULLER and ALTENBURG, and based on 15 different experiments totaling 26,145 X chromosomes tested gives a control rate of 0.18 percent. DUBININ (1946) published a table on control mutation rates in which two sets of data are on a scale sufficiently large to be of some validity. In this table, data of TIMOFÉEFF-RESSOVSKY published in 1940 on eight lines of flies and 68,221 chromosomes tested give a mutation rate of 0.14 percent and data of DEMEREC (1937) on 12 lines and 13,612 chromosomes tested give a mutation rate of 0.10 percent. It is quite possible that improvement in culture conditions, particularly by the addition of brewer's yeast to the culture medium, have resulted in the apparent lowering of control mutation rates by bringing through a sizeable group of semilethals, which would be scored as lethals under poorer culture conditions. The criteria used in scoring lethal mutations will also have a bearing on the apparent lethal mutation rate, and these criteria should be carefully stated for comparative purposes.

In our experiments, though the mutation rate/dosage curve is linear throughout the range of dosages tested, the mutation rate per r is even lower in comparison to that reported by TIMOFÉEFF-RESSOVSKY (1937) than is the control rate in comparison to his control rate. Consequently our data show a doubling of the control rate at an exposure of about 50 r in contrast to the figure of 35 r published by TIMOFÉEFF. Possibly this discrepancy is due to differences in dosimetric measurements. Quite a different factor, however, may be responsible. Recently MULLER (1946) reported that "experiments involving tests of some 200,000 X chromosomes for 'spontaneous lethals' demonstrate that the time rate of mutation varies greatly among different types of normal cells, representing different stages in the germinal cycle. Overall mutation frequency is two or three times higher in the first crop of spermatozoa, accumulated during a male's pre-imaginal life, than in those of a week later, derived from a non-virgin male." It seems likely that many of the earlier control data were collected from flies of varying ages while the

present data come exclusively from flies of one week or less in age. As the figure for the doubling of natural mutation rate at 35 r was based exclusively on relatively high radiation data, the control mutations occurring in the irradiated material would have a negligible effect on the results. MULLER'S experiments carried on at the same time and on about the same scale as the present ones, make possible an explanation of the apparent discrepancy between the present findings and those previously reported.

In any case the present data clearly indicate that, for radiation with X-rays, dosages as low as 25 r produce mutations as drastic in their effects and in the same proportion to the dosage as do exposures to high dosages. If an extrapolation is permissible, one may assume that there exists no tolerance dose below which mutations are not induced. The total effect of X-rays on the induction of point mutations in a fruit-fly population would depend, then, on the mean value of r-units to which the individuals of the population are exposed. In other words, the total effect on the germ-plasm of a population of 100,000 individuals would be as great if each individual received 1 r at the beginning of the reproductive period as it would be if 1,000 individuals each received 100 r and the others none. If the data on flies can be shown to have transfer value to man, then widespread exposure of large numbers of the human race during the reproductive period even to low dosages of X-rays must be looked upon as a serious hazard. It should also be kept in mind that for all practical considerations mutation of the germ-plasm is an irreversible process. The new mutation, once it occurs, cannot be cured or alleviated. As most transmitted mutations, however deleterious their effects, are recessive, the importance of the induction of a mutation is not to be reckoned in terms of its effect on one individual, but rather in respect to its possible effect through transmission to many individuals over a large time scale. A final evaluation of these problems will have to take into account the data published simultaneously by CASPARI and STERN (1948) and will have to await still further investigations.

APPENDIX A. VISIBLES AND VISIBLE-LETHAL RATIOS

Table 6 contains the data on the occurrence of pure lethals, semilethals without visible effects, semilethals with visible effects and visibles. No data are given for the flies subjected to the 25 r dosage as none of these cultures were examined for visibles. In the table the pure lethal class contains those mutants tested through two generations without showing any wildtype males. In addition to those listed, there were 78 mutants of this class found in the 25 r lots. In testing these lethals, 253,773 flies were etherized and classified, an average of 277.6 flies per lethal tested. The semilethals without visible effects are for the most part cultures which were set back because no wild type males had emerged when they were first examined and in which no wild type males were present when the first counts were made on them. Many semilethal and lowered viability mutants were observed but not recorded for the reasons given above. Therefore the figures on this group should not be taken as a quantitative measure of the total numbers of this type of mutant occurring.

TABLE 6

Lethal-visible ratio at different dosages.

DOSAGE		LETHALS (PURE)	SEMI LETHAL NON-VISIBLES	SEMI-LETHAL VISIBLES	VISIBLES	CORRECTED VALUE FOR VISIBLES AND SEMI-LETHAL VISIBLES	RATIO LETHALS TO VISIBLES
4000 r	# cultures examined	1,843	1,843	1,843	1,627		
	# mutants found	163	20	11	22	33.3	4.9:1
3000 r	# cultures examined	2,029	2,029	2,029	1,871		
	# mutants found	125	8	8	24	24.1	5.2:1
2000 r	# cultures examined	2,755	2,755	2,755	1,472		
	# mutants found	119	15	12	5	20.9	5.7:1
1000 r	# cultures examined	6,977	6,977	6,977	1,206		
	# mutants found	137	10	14	3	31.0	4.4:1
500 r	# cultures examined	6,634	6,634	6,634	6,328		
	# mutants found	81	5	5	9	14.3	5.7:1
150 r	# cultures examined	23,195	23,195	23,195	17,099		
	# mutants found	67	9	6	6	14.1	4.8:1
50 r	# cultures examined	31,560	31,560	31,560	7,535		
	# mutants found	74	4	3	1	7.2	10.3:1
Control	# cultures examined	73,901	73,901	73,901	36,319		
	# mutants found	70	7	10	3	16.1	4.3:1
Totals		836	78	69	63	159.0	5.2:1

Note: Figures in column 6 derived from the expression $\frac{(N-L)v}{N'}+slv$.

N = total cultures examined for lethals; N′ = total cultures examined for visibles; L = total cultures in which lethals or semi-lethals of either kind were found; v = visibles found; slv = semi-lethal visibles found.

All semilethals which showed visible effects were classed with the visibles in calculating total visible mutation rate. This would seem to be the most objective procedure, for it is possible on the basis of viability to arrange a continuous series of visibles ranging from extreme semilethal types at one end to mutants with a viability equal to most wild stocks at the other. A criterion for separating lethals from semilethals was set as the inability of any flies to emerge from the pupa case. When flies can be shaken out of the culture bottle, they are considered as non-lethal even though they may die soon after emer-

gence. Several interesting types of this sort have been seen. In the 40th lot at 25 r, two semilethal visibles of particular interest have been found. In one, the tarsal joints were absent from all legs. In another, the flies on emergence showed complete lack of muscular coordination and soon died.

Since during the second year of work most of the cultures were not etherized and examined for visibles, it has been necessary to estimate the number of visibles occurring from the sample of cultures which were examined. In all, 73,457 cultures have been etherized and examined for visibles in addition to those cultures etherized as potential lethals and found to contain semilethal visibles. As may be seen from the table, the estimates of visible-lethal ratios at the different dosages are not based on samples of equal significance. However, the total data on visibles seem sufficiently extensive to justify their inclusion. Based on a total of 836 pure lethals and 159 visibles (of the latter 132 were discovered and the others calculated from the samples of cultures actually examined), the over-all lethal-visible mutation ratio is 5.2:1. Furthermore, the recorded ratios are quite similar for controls and the different dosages. This result is subject to a considerable statistical error so that the ratios may be intrinsically somewhat different. Such a difference would be expected since it is known that X-ray induced lethals consist of two classes, namely, cytologically visible small deficiencies and cytologically unrecognizable changes, while spontaneous lethals are composed nearly exclusively of the latter group. It will be noted that the ratio of 10.3:1 in the case of the 50 r dosage is based on a relatively small number of cultures examined considering the low rate of expected mutation. SCHULTZ (1936) tabulates the lethals and visibles found in radiation experiments by five different workers. They found 1,189 lethals to 161 visibles, a ratio of 7.4:1. The somewhat lower ratio obtained in the present experiment may be due to a difference in criteria of classification or it may arise from an inherent difference in stocks. In any case, the order of magnitude is the same.

As the total mutation rate is composed of the sum of the mutation rates at the different loci, a comparative study of mutation at different loci is of interest. Obviously, sex-linked lethals are not adapted for such a study as there is no general way by which two such lethals can be tested for allelism although ALIKHANIAN (1937) and SHAPIRO (1937) were able to make such tests for loci which could be "covered" by the presence of a deleted X chromosome in addition to the undeleted X chromosome of lethal-bearing males. A comparative study of visible mutation rate could best be made by using an attached-X chromosome stock and examining individual males for new sex-linked visibles. However, it seems worthwhile to include the data we have secured from over 70,000 test cultures examined. While new sex-linked mutants have been found as individual males in cultures examined, these have not been tabulated, and all cases mentioned are of cultures in which all of the Canton-S males showed the visible. Each recurrence represents a new mutation at the locus; in the case of the goggle locus, the two mutations recorded are probably a semilethal visible cluster. Of the total of 148 visibles and semilethal visibles found (these include some semilethal visibles at 25 r not recorded in table 6) 47

were identified by their distinctive phenotypic appearance and by linkage tests as representing mutations at known loci in the X chromosome. Most of the others were probably also mutations at known loci, but in the absence of an easily identified and distinctive phenotype, no attempt has been made to associate them with known loci. Thus many recessive minute bristle types have been found, but as this phenotype is found at several X chromosome loci it is difficult to identify them. Table 7 presents the data on the mutants identified,

TABLE 7

Record of visible mutations found at identified loci.

VISIBLE	LOCUS	X-RAY DOSAGE IN r-UNITS FOR EACH RECURRENCE	TOTAL RECURRENCES	LOCUS MUTATIONS IN % ALL VISIBLES	
				THIS EXPERIMENT	COMPILED FROM BRIDGES-BREHME
chlorotic	0.0	50; 4000	2	1.4	0.3
yellow	0.0	500; 2000; 3000	3 (1)	2.7	8.6
yellow achaete	0.0	50	1	0.7	0.6
scute	0.0	4000	1	0.7	8.5
rough eye	0.5	2000	1	0.7	0.2
broad	0.6	150	1	0.7	1.1
prune	0.8	150; 150	2	1.4	1.4
oval	17.5	1000	1	0.7	0.2
singed	21.0	4000; 4000; 4000	3	2.0	3.9
goggle	25.0	150; 150	2	1.4	0.3
lozenge	27.7	150; 500; 1000; 1000	4	2.7	3.1
vermilion	33.0	cont.; 5000	2	1.4	3.1
miniature	36.1	cont.	1	0.7	3.9
dusky	36.2	3000	1	0.7	1.6
furrowed	38.3	cont.; cont.; 500; 3000	4	2.7	1.4
wavy	41.9	150	1	0.7	0.6
garnet	44.4	500	1	0.7	3.3
scalloped	51.5	3000; 4000	2	1.4	0.5
uneven	54.4	1000	1	0.7	1.0
rudimentary	54.5	cont.; 150; 500; 1000; 1000; 2000; 3000	7	4.8	4.6
forked	56.7	4000	1	0.7	5.2
fused	59.5	150; 500; 1000; 4000	4	2.7	1.7
22 Loci			47	31.8	55.7

giving the recurrence and the dosage at which each mutant appeared. It is to be remembered that at least some of the mutants occurring in the radiated material may be control mutations and not due to the radiation. No quantitative data for the white and Bar loci are given as these loci for technical reasons could not be investigated accurately in the genetic set-up used. Several muta-

tions to the Notch deficiency have been found but are not listed in the table. The table includes a column showing the percentage of the total number of visibles which has occurred at each locus, and another column for comparison compiled from data on visibles in the report of BRIDGES and BREHME (1944) based on over 600 visible mutations at sex-linked loci. (The Bar and white loci were omitted in making this tabulation.) In general, the visible mutation picture in the two sets of data seems rather consistent. Visibles have been found recurring repeatedly at certain loci known from previous work to have a relatively high mutation rate. Possibly a more accurate comparison could be made if the percentages were doubled for each locus for about half of the visibles which have been included were semilethal visibles, which are not so likely to have been seen, or, if seen, may have remained unanalyzed in previous work. It would appear that the present mutation rates at the yellow, scute, miniature, garnet and forked loci are lower than those reported before, but higher at the furrowed, rudimentary, and fused loci. However, the numbers are too small to be significant. Of the 47 mutants listed in the table, five occurred in the controls. This is the same proportion of control visibles to radiation visibles as that found among all the visibles recorded in table 6. On the basis of the relatively constant visible-lethal ratio at different dosages as indicated by data in table 6, it may be inferred that approximately five more of the visibles listed in table 7 from the radiation lots are actually control visibles. It would seem that most of the visibles listed in table 7 were induced by the radiation. There is no way of knowing which of the specific visible mutations occurring in the radiation experiments were really spontaneous control visibles. Without this knowledge and with consideration of the relatively low number of visibles, it is premature to speculate whether or not relatively highly mutable loci, producing visibles even under control conditions, have a high induced visible mutation rate following irradiation. This is a problem worthy of future investigation. Extensive experiments on specific loci may show that some react differently from others in the relative numbers of control and induced visibles occurring. This, however, would be no proof that the total dosage mutation curve for induced visibles differs from that for induced lethals. Loci yielding lethals may also react differentially from others to radiation. The latter point would be more difficult to establish owing to the difficulty of identifying lethal loci. However, the problem might be attacked by studying lethal mutation rates at loci in which visible alleles occur. In this connection, it is of interest to note that in the present material, among those lethals tested by linkage tests and not found to be associated with crossover inhibitors, eleven lethals were found so close to the scute locus as to give no crossing over among about 200 flies per test. Two such lethals were found near the crossveinless locus, three near the vermilion locus, eight near the forked locus, and none near the carnation locus. Thus, of 194 lethals not associated with crossover inhibitors and tested for linkage, 24 occurred in the regions of the five marker loci. Of the 148 visibles found, 11 were located at these five loci or gave no crossing over with them. Had all semilethal visibles been tested, it seems likely that several more visibles would have been found so close to these loci as to give no crossing

over with them. The numbers are too small to establish any relation of mutation rate to dosage at the individual loci, but the data indicate that induced and control lethals and visibles both occur at specific loci. In the course of linkage tests, both visibles and lethals have been found associated with crossover inhibitors particularly abundant at high dosages. While no cytological studies of these cases have been made, they probably represent gross chromosome rearrangements.

On the basis of the facts presented in Appendix A the tentative conclusion was reached that the same X-ray dosage/mutation rate relation holds for sex-linked visibles as for sex-linked lethals.

APPENDIX B. THE LINKAGE MAP AND CROSSOVER MODIFIERS

The linkage relations of 205 lethals and 39 visibles were studied by the method outlined in the section on genetic procedure. In addition, the locus of a number of recurrent visibles was determined by crossing them directly to flies from stock of what appeared to be the same visible or an allele. In the linkage tests 60,515 males were classified for the five marker genes used and in the case of visibles, for the visible being located. This made an average of 248 males per linkage test. In only three cases were less than 100 flies classified, in 18 between 100 and 150 flies, in 27 between 150 and 200, and in all the rest over 200. In figure 4, the loci of 229 out of the 244 mutants studied are shown arranged in the order of their distribution on the genetic map of the X chromosome and in vertical columns representing their distribution in regions of five crossover units in length. Each locus has been assigned by calculation from the numbers of crossovers with the marker genes on either side of the mutant studied and by correcting the map distances thus determined by comparison with the standard map distance between the two marker genes involved. Where a mutant was found to lie in a region containing a significant crossover reducer it has not been included in the figure. The greatest number of mutants was found at the extreme left end of the map, and the smallest number in the adjacent region 5–10. Other peaks occur in regions 20–25 and 55–60. Most of the mutants within the two highest peaks are found at or immediately adjacent to the scute and forked loci respectively. On the whole, the distribution of mutations along the length of the X chromosome which was found agrees well with the distribution described by earlier workers. MULLER (1928a) was the first to provide such data. On the basis of extended observations he returned later to the same problem (MULLER and PAINTER 1932). Similarly, OLIVER (1932) has studied the distribution of 233 lethals in a radiation experiment and finds peaks at or near the scute and forked loci. SLIZYNSKA and SLIZYNSKI (1943) have investigated the distribution of 89 chemically induced sex-linked lethals from experiments of AUERBACH and find a peak in region 0–5, and a low point in region 5–10. They state: "The lowest frequency was found between 5 and 10 due to sudden drops around locus 10." It is interesting to note in this connection that the longest blank region in the present map is from region 9.4 to region 12.7, a map distance of 3.3 units. It should be kept in mind that the figures in the data here

4.9												
4.9												
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1.6												
1.5												
1.3											60.0	
1.2				24.7							59.8	
1.0				24.5							59.6	
0.9				24.4							59.6	
0.9				24.2							59.4	
0.9				24.1							59.2	
0.8				23.7			39.3			54.9	59.0	
0.8				23.7			39.5			54.8	58.4	
0.7				23.5			38.9			54.3	57.3	
0.5				23.4			38.8			53.9	57.1	
0.4				23.4	29.2		38.6			53.5	56.7	
0.4				23.3	29.1		38.4	44.5		53.3	56.7	
0.3				23.3	29.0		38.4	44.4		52.9	56.7	66.0
0.3				23.2	28.5	34.8	38.4	44.1		52.5	56.7	66.0
0.0			19.8	22.7	28.3	33.9	38.3	43.3		52.3	56.7	66.0
0.0			19.7	22.5	28.0	33.9	38.3	43.2	49.8	52.1	56.7	66.0
0.0		14.8	19.2	22.5	27.6	33.5	38.2	42.8	48.8	52.0	56.7	65.2
0.0	9.4	14.8	19.2	22.4	27.4	33.0	38.1	42.8	48.7	51.7	56.7	64.8
0.0	8.0	14.6	18.8	21.5	27.3	33.0	37.8	42.8	48.6	51.3	56.7	64.1
0.0	6.1	14.1	18.8	21.4	27.3	33.0	37.0	42.4	48.5	51.1	56.6	63.9
0.0	6.0	14.1	18.7	21.2	27.2	32.3	36.6	42.2	47.1	50.8	56.4	63.9
0.0	5.9	13.7	18.7	20.8	26.7	32.1	36.6	41.5	46.8	50.8	55.5	63.5
0.0	5.8	13.7	17.7	20.8	26.2	32.1	36.6	41.3	46.2	50.4	55.4	63.0
0.0	5.7	13.1	16.4	20.7	26.1	30.6	36.2	40.9	45.7	50.4	55.4	62.5
0.0	5.3	13.0	15.8	20.4	26.0	30.4	35.9	40.7	45.6	50.3	55.3	60.8
0.0	5.1	12.7	15.4	20.2	25.3	30.2	35.2	40.1	45.1	50.1	55.2	60.8

FIGURE 4. Graphic record of locus determinations of 229 lethals and visibles from control and radiation experiments to show high and low mutation rate regions along the genetic map of the X chromosome.

presented are based on the actual crossover counts and do not mean that there are as many as 172 loci in the X chromosome which can be separated by crossing over. As some recurrent visibles have been mapped more than once and fall near but not at the same locus according to the linkage tests, chance variations of the same type have almost certainly occurred in the case of some lethals tested.

During the course of the linkage tests, it was noted that crossing over seemed high in the left end of the chromosome, the region from scute to vermilion. A separate linkage test was therefore run, involving 30 cultures of Canton-S/*sc cv v f car* females to Muller-5 males. Tabulation of the 2,790 males gave

15.7 percent crossovers between *sc* and *cv*, 23.4 percent between *cv* and *v*, 26.6 percent between *v* and *f*, and 5.7 percent between *f* and *car*. The standard map distances (BRIDGES and BREHME 1944) for these regions are *sc-cv*, 13.7; *cv-v*, 19.3; *v-f*, 23.7; and *f-car*, 5.8. It will be noted that the left end of the X chromosome gives relatively high crossover values in the present test.

Using the map derived from this experiment instead of the standard map, the crossover values for each of the four map regions in each of the 244 linkage tests of lethals and visibles were examined for significant deviations

TABLE 8

Crossover suppressors associated with lethals and visibles at different X-ray dosages.

DOSAGE	LETHALS TESTED	CROSSOVER SUPPRESSORS REGIONS 1, 2, 3, 4	VISIBLES TESTED	CROSSOVER SUPPRESSORS REGIONS 1, 2, 3, 4
Control	32	0	5	0
25 r	4	0	0	0
50 r	21	1-2-3-4	2	0
150 r	48	3-4	7	0
500 r	8	0	4	0
1000 r	77	1-2-3; 1-2-3; 1-2-3-4	8	3-4; 1-2-3-4
2000 r	3	0	3	0
3000 r	9	3; 4; 1-2-3 1-2-3-4; 1-2-3-4	7	2; 3
4000 r	0	0	6	1; 1
Totals	202	10	42	6

Note: *sc-cv*, region 1; *cv-v*, region 2; *v-f*, region 3; *f-car*, region 4. 1-2-3-4 indicates crossing over suppressed, or nearly so, throughout the chromosome from scute to carnation.

from the values given above. Significantly high crossover values were found repeatedly in the two regions to the left of vermilion, a portion of the X chromosome which has generally been found relatively free from variations in crossing over (see BRIDGES 1915; PLOUGH 1921; KIKKAWA 1934). However, no attempt was made to analyse these cases further, and it is not known whether they were due to genetic or environmental modifiers or both.

Of more interest in connection with the present study were a number of crossover suppressors, which eliminated or very strongly reduced crossing over. Table 8 presents the data. It should be stated that linkage studies were undertaken only to demonstrate that the lethals and visibles secured were actually different from one another and not to study crossover suppressors. Most of this work was done the first year, and few lethals at dosages above 1000 r have been analyzed for linkage relations. However, it will be noted that of the 16 crossover suppressors reported, nine were associated with lethals or visibles

at 2000 r or above. Only 28 mutants at these dosages were studied for linkage relations. Five more of the crossover suppressors were found among 85 lethals and visibles from the 1000 r lots and only two from the 131 lethals and visibles at dosages of 500 r or less. LEA and CATCHESIDE (1945), after reviewing the evidence from data of TIMOFÉEFF-RESSOVSKY (1939), OLIVER (1932), DEMEREC (1937a) and DEMEREC and FANO (1941), came to the conclusion that gross chromosome rearrangements, which have been shown to increase approximately as the 3/2 power of the dosage (see MULLER 1940), do not in themselves constitute a separate class of lethals, but rather that lethals, of which a large class occur at chromosome breakage points are therefore often associated with gross rearrangements at high dosages. The data in table 8 indicate that visibles also tend to be associated with gross chromosome rearrangements at high dosages. Very extensive data of PATTERSON, STONE, BEDICHEK, and SUCHE (1934) on reciprocal translocations in Drosophila indicate that over two-thirds of 466 cases studied were not viable in homozygous condition under the culture conditions employed by these workers and were therefore associated with or were recessive lethals or semilethals. The presence of a large group of chromosome rearrangements after high X-ray dosages makes it difficult to determine actual mutation rates because of the chance of two or more mutations being associated with such a rearrangement and therefore not easily separable. For a study of the dosage/mutation relation, however, this problem becomes less serious at dosages on the order of 1000 r.

SUMMARY

1. Using a tool stock supplied by DR. H. J. MULLER, 200,801 X chromosomes of mature sperm of a Canton Special stock of *Drosophila melanogaster* were tested for the origin of new lethal mutations. Of these chromosomes. 73,901 were controls, 51,907 were subjected to X-rays at a dosage of 25 r. 31,560 at 50 r, 23,195 at 150 r, 6,634 at 500 r, 6,977 at 1000 r, and smaller numbers at 2000 r, 3000 r, and 4000 r.

2. From these tests it is concluded that even for X-ray dosages as low as 50 r and 25 r the linear r-dose/mutation frequency relation holds. From all of the data collected, on the basis of this linear relationship, the control lethal mutation rate in the Canton Special stock tested was calculated as 0.1004 percent and the induced mutation rate per r as 0.00215 percent. When only data at dosages of 1000 r and lower were included, the control mutation rate was calculated as 0.1039 percent and the induced mutation rate per r as 0.00197 percent.

3. The present data indicate that the control mutation rate was doubled at an exposure to about 50 r. The apparent discrepancy between this figure and that of 35 r derived from earlier X-ray experiments may be explained on the ground that sperm cells of young males, which MULLER has shown give a high control rate, were used exclusively in this experiment.

4. Several cases of control "lethal clusters" were found, and are interpreted as due to mutations occurring in early stages in the germ tract. The problem of sampling sperm cells is discussed in this connection.

5. The implications of the findings in connection with the exposure of large numbers of the human race to low dosages of X-rays are discussed briefly.

6. A parallel study of visible mutation rates, as reported in Appendix A, has given an over-all lethal-visible mutation ratio of 5.2:1. The data on visibles are not inconsistent with the view that the linear dosage/mutation relation holds at low dosages for this class of mutations as well as for lethals.

7. In Appendix B, linkage studies on 229 lethals and visibles are reported. Relatively high mutation rates both for lethals and visibles were found in genetic chromosome map regions 0-5, 20-25, and 55-60 and a relatively low rate near 10. Mutations in control chromosomes and in those subjected to low dosages of X-rays were rarely associated with crossover suppressors, while mutations in chromosomes receiving high radiation were frequently associated with crossover suppressors.

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