THE RELATIONSHIP BETWEEN RADIATION DOSE AND DOMINANT VISIBLE MUTATION RATE IN DROSOPHILA MELANOGASTER[1]

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IT has recently been shown (IVES 1958, 1959) that over a broad dosage range, extending well above previously studied dosage levels of Cobalt-60 γ radiation, the relationship between rate of sex-linked lethal mutations and radiation dose is apparently linear. Concurrently tests were made of the frequency of easily seen visible mutations, chiefly autosomal dominants, at ten dosage levels in the .5 kr to 10 kr range. The purpose of this report is to present the results of those tests and to relate them in particular to the findings in the sex-linked lethal tests.

MATERIALS AND METHODS

The stocks used in this test were the same highly inbred Oregon-R wild type which was used in the X-lethal tests, and the marker stock known as *hes* carrying seven recessive third chromosome visible mutations. The Oregon-R stock had been inbred for 150 generations when the tests with *hes* began, but was mass mated during the period of the tests. A new derivative from the inbred Oregon-R line was substituted every 20 to 25 generations in order to minimize the effects of cumulative variability in the stock. It proved impractical to inbreed the *hes* stock, which was carried in small mass matings of about ten pairs of parents. Occasional clusters of mutations appeared during the experiment. They were usually traceable to the *hes* stock. That indicates the desirability of using inbred stocks in this kind of study whenever it is possible to do so.

The *hes* mutants are hairy, thread, scarlet, peach, curled, stripe, sooty. The basic symbols for those loci are *h, th, st, p, cu, sr, e*. They are described in BRIDGES and BREHME (1944).

In the general procedure of these tests, wild type males were raised at 25°C and were collected from the earliest flies emerging in a culture, within 12 hours after eclosion. Aged then on well yeasted medium for two days separated from females, they were irradiated with Cobalt-60 γ rays from a source which contained 430 curie at the beginning of this work. Intensities of radiation ranged between 100r and 250r per minute. Adjustments were made weekly for isotope degradation. Immediately after the treatment the males were mated for three days to *hes* females. For doses under 6 kr (the indicated dose is that which was delivered to the surface of the fly) the mating consisted of one male to two or

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three females. For doses of 6 kr and higher the mating was two or three males with four or five females.

The F_1 of these matings were raised in 95 × 25 mm shell vials containing about 10cc of a standard medium made from cornmeal, molasses, brewers yeast and agar. The medium contained methyl parasept as a mold preventative and was dusted very lightly with a few particles of powdered live yeast. Overcrowding of larvae in controls and tests of low doses was minimized by the use of only two female parents which were shaken to fresh cultures as often as was needed, generally daily. To increase the yield in tests of high doses the females were shaken to a second culture for an additional laying period at the time when the male parents were removed.

Control and low-dosage tests were carried out simultaneously and were randomized so that the F_1 could be classed without a bias toward either type of test. In tests of higher levels of radiation, three or more adjacent dosages were randomized similarly. The different dosage levels were tested alternately in time in order to minimize variations due to possible shifting classification standards. All classing was done by the author.

F_1 flies were examined under 9 × 2.3 binocular magnification and included all flies emerging through the 18th day after egg laying began. All the offspring from one male parent, or from a group of male parents, were tabulated together in order to detect clusters of mutant flies whose mutation was not related to the radiation treatment. All members of a cluster were examined routinely for the presence of any other visible mutation.

The F_1 were scored for any bilateral abnormal phenotype which was noticed while examining the flies for mutations of the *hes* group. Only wing venation was examined in addition to the parts affected by the *hes* genes. An abnormal phenotype had to be describable in specific verbal terms in order to be scored as mutant.

Most commonly found were variants of the small bristle phenotype called Minute, loci for which are distributed through all the major euchromatic areas. Minutes constituted 50 percent of the mutant phenotypes found in the radiation tests. Mutants of the *hes* group amounted to only 14 percent of the total. The remaining 36 percent were a wide variety of phenotypes which have been classed under one general heading, "other bilaterals." They will be referred to as OB.

About 20 percent of the OB were phenotypes closely resembling Delta (*Dl*), Hairless (*H*) and aristopedia (ss^a). Other well known phenotypes encountered repeatedly were sex-linked Notch (*N*) and second chromosome Plum (bw^V).

In addition to all of these mutations, another class, unilaterals or partially mutant flies, was recorded. It proved impossible to score such cases with a consistency approaching that achieved in scoring bilateral mutants. Therefore, no data will be presented for them at the present time.

Most of the *hes* mutants were tested for simple allelism (not pseudoallelism) by backcrossing to *hes*. Many of the more "attractive" OB, including the five specific types mentioned above, were tested for their dominant inheritance and

linkage group. When the mutant flies were fertile, which they were about half the time but less so when occurring in tests of doses above 5 kr, allelism was nearly always indicated for the *hes* mutants and dominant inheritance for the OB. Rarely was there positive evidence of a dominant mimic mutation in the tests of *hes*-like mutants. When the tested OB were females and when a mutation was linked to the third chromosome, it was generally observed that crossing over was reduced or absent in the region carrying the mutation.

It was not possible to make careful systematic tests of all mutant F_1 flies. It requires rigidly controlled environmental conditions to determine critically by such tests the proportions of sterile *vs.* fertile, allelic *vs.* nonallelic, and hereditary *vs.* nonhereditary mutants. An example of one uncontrolled variable in the present tests which would at least have weakened the value of such systematic tests in this work was the condition of the culture medium in which the F_1 larvae grew. It was possible to minimize crowding effects in controls and low doses, but at high doses the larval population was usually well below optimal size so that excessive drying and darkening of the medium occurred before the larvae pupated. While no evidence was obtained that this food condition produced phenocopies among the F_1, it may well have been a factor in the fertility and fecundity of the F_1, in particular the mutant F_1. It is not known what net effect such conditions may have had on the rate of recovered mutant flies, but at least there was no evidence of any qualitative difference in mutants observed after high and low doses. There was such a difference in the controls, which produced none of the *hes* mutants and a few of the types of OB observed in radiation series.

In view of the above considerations, it seems best to the author to calculate mutation rates in this study on the basis of the F_1 scoring.

TABLE 1

Rates of visible mutations after γ-radiation

Dosage in kr	No. flies	Number and percent of major classes*								Increase over control
		hes	%	*M*	%	OB	%	Total	%	
0	50,970	0	.00	61	.12	25	.05	86	.17	. .
.5	34,241	8	.02	64	.19	39	.11	111	.33	.16
1	22,103	20	.09	68	.31	40	.18	128	.58	.41
2	15,420	19	.12	85	.55	45	.29	149	.97	.80
3	13,640	33	.24	117	.86	75	.55	225	1.65	1.48
4	10,338	35	.34	116	1.12	74	.72	225	2.18	2.01
5	4,438	18	.41	55	1.24	68	1.53	141	3.18	3.01
6	4,331	28	.65	103	2.38	64	1.48	195	4.50	4.33
7.5	3,519	26	.75	95	2.70	71	2.02	192	5.46	5.29
9	2,041	21	1.03	67	3.28	56	2.74	144	7.06	6.89
10	2,361	25	1.06	83	3.52	87	3.68	195	8.26	8.09

* Description of mutant classes:
hes = *h, th, st, p, cu, sr, e.*
M = Minutes.
OB = Other bilaterals.

Presentation of data

The frequencies and rates of F_1 mutant flies in these tests are given in Table 1. Included are the number of F_1 flies classed, the number and percent of each of the three major classes of mutations observed and the total number and percent of mutant flies. The last column shows the increase over the total control value in the total percent of mutant flies in each radiation dosage test. That represents the net increase due to the effects of radiation.

Two numerical criteria have been met at each radiation dosage test: at least 2000 flies classed and at least 100 mutants scored. The goal in the controls was a minimum of 50,000 flies from noncrowded cultures. About as many more control flies have been excluded because of overcrowded cultures in most of the control series in the early part of the work. Overcrowding is indicated when a large portion of the F_1 emerge between days 14 and 18. In optimal conditions the large majority of flies emerge in control and low dosage tests between days 10 and 14 after the beginning of development. When it became apparent that only under such optimal conditions were Minutes obtained at the indicated frequency (it decreases sharply under overcrowded conditions), all cultures indicating overcrowding were excluded from control and low dosage series. Overcrowding was not observed in tests of doses above 1 kr.

Had *hes* mutants appeared in the control tests in the same proportion as in the 1705 radiation series mutants, there would have been some ten of them among the 85 control mutants. That they do occur, however, without known radiation treatment is reasonably certain. The author has found alleles of most of these mutant loci in chromosomes extracted from natural populations, and has observed *de novo* mutations for *h* and *th* in other wild type stocks which had a long history of inbreeding. The control data in Table 1 indicate only that 50,000 chromosome tests is an insufficient sample to measure the mutation rate for the *hes* loci, even as a group.

As data accumulated in the control tests it became apparent that a few abnormal phenotypes which are not individually hereditary occur repeatedly in flies produced by the particular cross of Oregon-R to *hes*, probably as developmental anomalies. Flies of these specific types have been excluded from the OB column in all of the entries in Table 1. Most of the control OB, in contrast to those in radiation series, proved to be nonhereditary, and could have been less frequently occurring development anomalies than the types which were arbitrarily excluded from all series. Three of the hereditary control OB, involving different phenotypes, were found as clusters of mutant flies and were traced to the *hes* stock. Several similar cases appeared in the radiation series. In each instance the mutation was scored as only one mutant, in the culture of flies first showing it, and only *hes* cultures free of the mutant were used thereafter.

Tests of some of the control Minutes indicated that about half of those were cases of haplo-4, the loss of one fourth chromosome. Each of the other autosomes contributed some Minutes to the control group, including the *hes* marker chromo-

some itself. The haplo-4 phenotype was much less common among the radiation Minutes.

The data of Table 1 show that the rate of each of the three classes of mutations increased with dose. The rate of increase, given in the final column, is more than linear when the total mutation rate is considered. This can be seen by comparing the mutant percentages in the various multiples of dose represented in the ten dosages. (It can also be seen in the direct plotting of the data in Figure 2 in the discussion section.) The data seem to indicate that the rate-dose relationship is exponential for these classes of visible mutants.

To test for a possible exponential relationship the data were plotted on two-cycle double-log graph paper. Examination of the plot suggested that the relationship is not only exponential but two phased, with a change in phase occurring in the middle of the dosage range at about the 4 kr level. With this in mind a best-fitting sight line was drawn for each phase. The results are shown in Figure 1.

The lines in Figure 1 indicate that the mutation rate increased approximately as the 1.2 power of the dose in the 0.5 kr to 4 kr range and as the 1.5 power of the dose in the 5 kr to 10 kr range. The transition from one phase to the other may be more gradual than the lines suggest, and could conceivably be a continuous change from approximately linear at very low doses to the 1.5 exponential phase in the 5 kr to 10 kr range. For convenience it will be treated as essentially two phased as indicated by the lines.

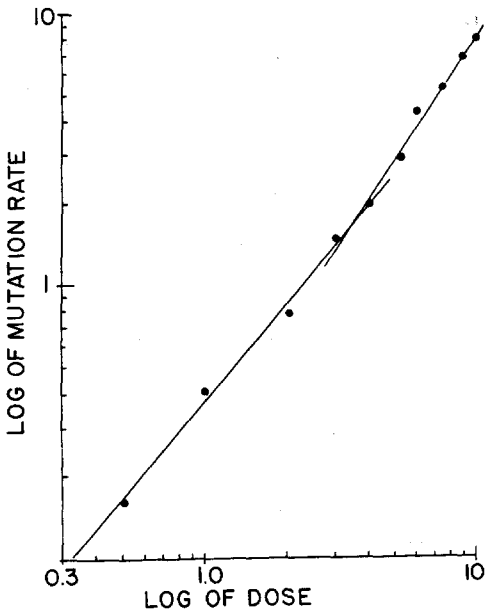

FIGURE 1.—The rate-dose relationship on a log scale. Vertical axis: percentile rate of visible mutations on a log scale. Horizontal axis: kr dose of γ radiation on a log scale. The points are the percentages given in the last column of Table 1. The lines were fitted by sight.

The data for Minutes and OB are not large enough to avoid the confusion of increased scatter when one plots them separately in a manner similar to Figure 1. But a glance at the individual percentile mutant rates at different dosage levels in Table 1 indicates that they are in general agreement in showing a faster rate of increase at doses above 5 kr than at doses below 4 kr. Thus the lines in Figure 1 can be reasonably interpreted as applying to both major classes of mutants. The *hes* data are too small to warrant such an analysis in detail, but at least they are not strikingly different from the other two classes in apparent rate-dose relationship.

Since there appears to be two phases to the rate-dose relationship it is of interest to compare the relative proportions of the different classes of mutants found in each phase range. The numbers of mutant flies are not large enough to warrant doing this on a dose-by-dose basis within each phase range. But they are large enough to compare the two ranges, group by group. This is done in Table 2.

The data of Table 2 show that the proportion of *hes* mutants was similar in the two dose ranges, and that Minutes were a little less frequent and OB a little more frequent, proportionately, in the 5 kr to 10 kr range. The change in proportion of Minutes and OB, though relatively small, is significant at the 0.1 percent level, indicating that the difference may be considered as established.

The relative proportions of the various *hes* phenotypes among the mutants may be compared in a similar manner. The data are given in Table 3. In this tabulation five cases of double phenotype, *th st*, were considered as five mutants of each single phenotype. Those two loci are closely linked and can be included in the same viable deficiency. But if one were testing the two loci separately, such a deficiency would appear as a mutant for whichever of the two markers one was using.

TABLE 2

Proportions of the major classes of mutations

kr range	Total mutants	Number and percent of major classes*					
		hes	%	*M*	%	OB	%
0.5 to 4	838	115	13.7	450	53.7	273	32.6
5 to 10	867	118	13.6	403	46.5	346	39.9
Total	1705	233	13.7	853	50.0	619	36.3

* For description of major classes see Table 1.

TABLE 3

Mutation frequencies at the hes *loci*

kr range	Number of mutations									
	h	*th*	*(th st)*	*st*	*p*	*cu*	*sr*	*e*	Total	Av.
0.5 to 4	7	7	(2)	17	20	7	31	28	117	16.7
5 to 10	6	14	(3)	19	18	18	30	16	121	17.3
Total	13	21	(5)	36	38	25	61	44	238	34.0

Since the total number of *hes* mutants observed in one dosage range is approximately the same as that observed in the other, one can compare the frequencies of the several loci directly, without recourse to percentages. Compared in this way, using the information in Table 3, the seven phenotypes can be seen to vary considerably in frequency. Considering the totals for the entire range of doses, there are too many *sr* and too few *h* mutants, each difference significant at the 0.1 percent level. Broken down into the two dose ranges, however, the numbers become too small to reveal clearly even two-fold differences in relative frequencies of mutants at the several loci. It can only be suggested that *th* and *cu* mutants seemed to be relatively more frequent and *e* mutants less frequent in the 5 kr to 10 kr range.

DISCUSSION

This study is concerned with the production of visible mutations by ionizing radiation in the mature sperm of *Drosophila melanogaster*. Each of the three classes of mutations observed here has long been known to be associated with various kinds of chromosomal mutations. (See MULLER 1954 for an extended discussion of the points which will be considered here and for earlier literature references.)

Mutations at the *hes* loci specifically have been studied by WARD and ALEXANDER (1957) who found them associated half of the time with cytologically demonstrable chromosome aberrations when occurring in flies treated with 3 kr of X-rays. Minutes, commonly associated with deficiencies and deletions when occurring after radiation treatment, have recently been studied by GLASS (1955) in a manner similar to the present study. Many of the visibles classed here as OB have frequently been found by others during the past 30 years as position effects or as mutations closely associated with chromosomal aberrations.

It is to be expected then that the rate-dosage relationship obtained in this study will be closely related to that established for chromosomal mutations. Mutational changes requiring only a single ionizing track (one hit) increase in rate in mature sperm of Drosophila normally as the first power of the dose, that is, linearly. Chromosomal rearrangements requiring two independent ionizing tracks (two hits) increase, under similar conditions, as the 1.5 power of the dose, the remainder of the amount of rearrangements represented in the 2.0 power (or square) of the dose being either lost or restituted.

In the present study, visible mutations increased in rate approximately as the 1.2 power of the dose in the 0.5 kr to 4 kr range of γ radiation and as the 1.5 power of the dose in the 5 kr to 10 kr range. Apparently there was a mixture of one-hit and two-hit mutants recovered after low doses (under 4 kr) and predominantly two-hit mutants after high doses (above 5 kr).

On this basis it appears likely that most of the visible mutants recovered in the 5 kr to 10 kr tests were associated with gross chromosomal rearrangements, probably as position effect mutants. Two direct observations in the present experiments support this interpretation. First, OB, the common kind of position effect

visible mutant, increased in rate a little more rapidly in the high dosage tests than did the rate of Minutes. Second, nearly all of the high dosage OB which were adequately tested showed the reduced crossing over which is characteristic of gross chromosomal rearrangements.

The question arises: would one expect enough recoverable chromosome aberrations to be produced in the 5 kr to 10 kr range to allow for so many position effect mutations? In concurrent tests of lethal X chromosomes recovered after 12.5 kr treatment (IVES 1959) it was found that some 40 percent of the lethal chromosomes were apparently associated with gross chromosomal rearrangements requiring two or more hits for their origin. Such chromosomal mutations should occur in all the chromosomes carried by radiated sperm. In the *hes* tests there was the chance to detect any of them irrespective of their specific chromosomal linkage, whenever a position effect visible mutation existed as a result of the aberration. A rough calculation on this basis indicates that recoverable chromosomal mutations probably outnumbered the observed visible mutants by a factor of at least two in the 5 kr to 10 kr range.

GLASS (1955) has also reported a two-phase rate-dose relationship in his study of Minutes, using X-rays and a more limited dose range. In his case the phase change occurred at a lower dosage level, about 2 kr, but at about the same mutation rate level, roughly one percent Minutes, allowing for small differences in the scoring of Minutes by different observers.

GLASS was able to continue scoring mutants in flies emerging well beyond the 18 day limit which was necessary in the present tests. He found a higher proportion of Minutes, particularly of extreme Minute phenotypes such as are normally associated with more extreme chromosomal changes, in late emerging flies. This could account for the difference in the dosage level of the phase change in these two independent studies.

The similarity of the Minute mutation rate level at which the phase change occurred in these two studies is especially interesting. It may be that, in a general way, for those combinations of irradiation doses and mutant eclosion time limits which together result in mutation rates of less than about one percent Minutes, the rate-dose relationship is linear or nearly so, while for combinations resulting in substantially more than one percent Minutes the rate increases as the 1.5 power of the dose, reflecting the predominating influence of two-hit rearrangements. This is a modification of GLASS's suggestion in connection with a difference between his results and those of IVES, LEVINE and YOST (1954). The latter study was not extensive enough at doses above 3 kr to test the present interpretation.

One of the major purposes of this report is to compare the results obtained in the study of mutants which are detected completely on the basis of phenotypic change with those obtained in the study of mutants which are detected completely on the basis of viability effects, in this case a lethal effect produced by the X chromosome as reported previously (IVES 1959). This comparison can best be made by the simple rate-dose plotting of the two sets of data which is given in

Figure 2. There is a marked contrast between the exponential relationship found for the visibles and the linear relationship found for the lethals.

It has already been indicated that the available evidence suggests that most of the visibles are probably associated with two-hit chromosomal aberrations, probably gross chromosomal rearrangements, in the 5 kr to 10 kr range. Some 60 percent of the lethal X chromosomes, however, appear to be free of gross chromosomal rearrangements. This in itself should decrease the influence of the rate of such chromosomal mutations on the rate-dose relationship for X chromosome lethal mutations as compared to its influence on the relationship for visibles. It may be expected, however, that some influence should be seen in the X-lethal rate-dose curve from the effects of the chromosome rearrangements which do occur, as represented in 40 percent of the 12.5 kr lethals.

There are at least five possibilities to be considered here. First, X-lethal mutations may possibly occur mostly as simple point mutations, independently of chromosome rearrangements. In such a case the resulting rate-dose relationship would be linear, as was actually observed in these experiments. Second, chromosomal rearrangements involving the X chromosome may very often maintain the

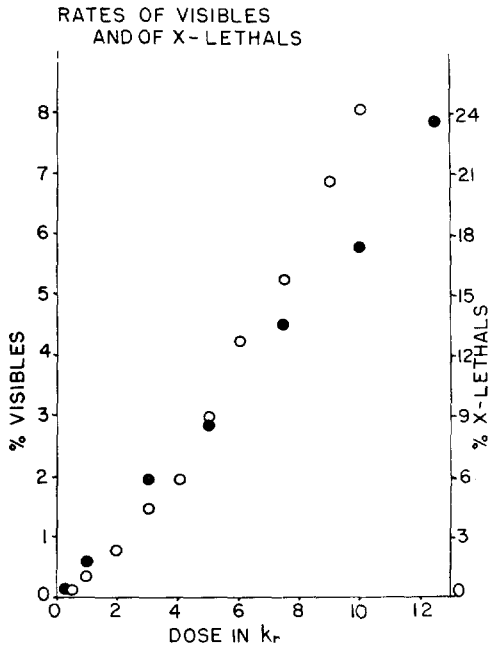

FIGURE 2.—Rates of visibles and of X-lethals in mature sperm of *Drosophila melanogaster* after cobalt-60 γ radiation. Open circles represent percentages of total visible mutations from Table 1, last column. Solid circles represent percentages of lethal X chromosomes similarly from Table 1 of IVES 1959. The percentage scales are adjusted to facilitate comparison of the two rate-dose relationships.

inheritance of position effect X-lethals which would be nonlethal in a normal gene sequence. If this were the predominant influence then the X-lethal rate-dose relationship should show exponential characteristics similar to those in the case of visibles, which it obviously does not. Third, if in their known destruction of cell lineages by abnormal division and separation, chromosomal rearrangements should also eliminate associated X-lethals which might be retained and recovered as single-break restitution lethals in lower dosage tests, then the X-lethal curve should increase less than linearly at doses above 5 kr, which it obviously does not do in the present instance. Fourth, if the effects mentioned as second and third here should both occur more or less equally, the net result could be a linear rate-dose relationship similar to what is actually observed. The present data can not be used critically in differentiating between the first and the fourth of these possibilities, in the opinion of the author. A fifth possibility, and the one which is most attractive to the author, is that all three types of effects occur (point mutation lethals, position effect lethals, and loss of potential restitution lethals) and that the net result happens to be approximately linear in the .3 kr to 12.5 kr range of Cobalt-60 γ radiation.

A final interesting point in the present observations concerns the chromosome region in which was located the loci of the most frequently occurring non-Minute mutations. The loci of the two most frequent *hes* mutants, *sr* and *e* alleles, and of the three most frequent OB, alleles of *Dl*, *H* and ss^a, are all linked together in a region of chromosome three which is only thirteen crossover units long. This region represents less than five percent of the total radiated euchromatin. It was the source of at least 20 percent of the OB, and its two *hes* loci produced mutations twice as frequently as did the five loci located elsewhere in this chromosome. While one is tempted to interpret this striking observation as indicating a chromosome region possessing exceptional sensitivity to γ radiation, several other possibilities can not be ignored, and there is no way to estimate their relative merits. First, these may be phenotypes to which the author is particularly sensitive as an observer. Second, this may be a region of the chromosome which is exceptionally well endowed, randomly, with genes which are more responsive phenotypically to position effects than is any similar number of genes in another comparably short chromosome region. Third, mutations induced by radiation in this group of genes may be less frequently dominant lethals than they are in other groups of genes.

The Stubble locus lies on the very edge of this unusual chromosome region. Among the many bristle mutations observed, none closely resembled Stubble, or second chromosome Bristle, and none when tested produced a population containing individuals resembling Stubble or Bristle. This suggests that probably not all the genes having the capacity for visible mutation and lying in this particular region happen to have the same responsiveness to the radiation treatment.

One can only conclude that this region of the third chromosome did contribute far more than its share to the observed non-Minute mutations. The reason for its doing so remains obscure at present.

SUMMARY AND CONCLUSIONS

1. The mature sperm of *Drosophila melanogaster* Oregon-R males were irradiated with Cobalt-60 γ rays and were tested for *hes*, Minutes (*M*) and other bilaterals (OB), the relative proportions being 14 percent *hes*, 50 percent *M* and 36 percent OB in the totals from ten dosage levels ranging from .5 kr to 10 kr.

2. Dominant mimic mutations of the *hes* loci are rare, and frequencies of the seven *hes* types are not equal, with too few *h* and too many *sr* (0.1 percent level of significance).

3. The total mutation rate increased as the 1.2 power of the dose between 0.5 kr and 4 kr and as the 1.5 power at higher doses, suggesting a mixture of one-hit and two-hit mutations at the lower doses and chiefly position effect rearrangements in the 5 kr to 10 kr range.

4. Both here and in GLASS (1955) the rate of Minutes shifted to the 1.5 power of the dose at about the one percent mutation rate point, but under different scoring conditions and at different dosage levels. It is suggested that the mutation rate rather than the radiation dose may be the important indicator of the change in phase.

5. The two-phase exponential rate-dose relationship observed here for visibles is compared to the linear relationship observed for X-lethals and is attributed in part to the apparently much lower proportion of chromosomal rearrangements in lethal X chromosomes and in part to a combination of other factors which are discussed.

6. The *ss–e* region of chromosome 3 contributed much more than its share to the total non-*M* visibles observed, a disproportion which may be due to any one or more of several indicated factors.

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