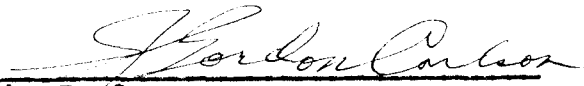


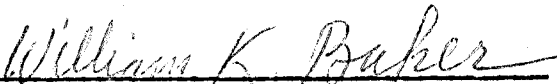
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To the Graduate Council:

I am submitting herewith a thesis written by Charles Wendell Edington entitled "The Constitution of Recessive Lethals Induced by Radiations of Different Ion Density." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Zoology.

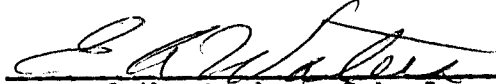

Major Professor

We have read this thesis and
recommend its acceptance:






Accepted for the Council:


Dean of the Graduate School

THE CONSTITUTION OF RECESSIVE LETHALS INDUCED BY
RADIATIONS OF DIFFERENT ION DENSITY

A THESIS

Submitted to
The Graduate Council
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Doctor of Philosophy

by

Charles Wendell Edington

August 1955

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CHAPTER I

INTRODUCTION

One of the fundamental problems confronting geneticists today is the determination of the mechanism by which spontaneous and induced mutations arise. In order to obtain quantitative information on the induction of mutations by radiation in *Drosophila*, sex-linked recessive lethals have been utilized by many investigators because of the high frequency of their occurrence and the relative ease with which they can be detected.

It has been demonstrated that recessive lethals are not composed of intragenic changes (point mutations) alone, but arise partly as the result of chromosome deficiencies (Slizynski, 1938) and in association with gross structural changes (Oliver, 1932; Demerec, 1937; Dubinin, Khvostova, and Mansurova, 1941; Fano, 1944; Herskowitz, 1946; and Ives, Levine, and Yost, 1954). It has also been shown that, in the majority of cases in which the lethal is associated with a chromosome rearrangement, the lethal cannot be separated from the aberration by crossing over (Demerec, 1937), which suggests that such lethals either are located at, or near, the breakage points, or are position effects. Although the frequency of lethals associated with gross rearrangements increases with increasing dose (Oliver, 1932; Demerec, 1937; Dubinin, Khvostova, and Mansurova, 1941; Herskowitz, 1946; and Ives, Levine, and Yost, 1954), that proportion of the total lethals which are associated with rearrangements remains constant (Ives, Levine, and Yost, 1954).

Since the frequency of induced chromosome aberrations such as

translocations and inversions increases as a power of the dose greater than one, it would seem logical to assume that the total frequency of recessive lethals associated with such aberrations might be expected to increase exponentially with respect to dose. Since it has been demonstrated that primary chromosome breaks increase linearly with dose (Sax, 1940, 1941; Carlson, 1941; and others), lethals which are associated with chromosome breaks will also increase linearly with respect to dose. If the lethals are the result of position effect, they should increase exponentially with respect to dose. This, however, is not the case, since it has been adequately demonstrated by Oliver, 1930, 1932; Demerec, 1933; Timoféeff-Ressovsky, 1939, and others that the total frequency of recessive lethals increases linearly.

On the basis of the available frequency-dose data for the various cytological and genetical effects studied, Lea and Catcheside (1945) and Herskowitz (1946) independently postulated that the induction of recessive lethals in *Drosophila* was associated with chromosome breakage per se with few, if any, lethals arising as the result of chromosome rearrangement (position effect) or point mutation. Muller (1940, 1950, 1954), however, argues that some recessive lethals are position effects and that the remainder are point mutations and deficiencies. Since Muller (1950, 1954), Catcheside (1948), Fano (1947), and Herskowitz (1946) discuss these hypotheses in more detail, the reader is referred to their papers.

With the above-mentioned hypotheses in mind, it should be possible to obtain evidence on the composition of recessive lethals by use of radiations of different ion densities. For example, early reports in the literature concerning the relative biological effectiveness (RBE) of

radiations of different ion densities indicated that fast neutrons were 1.5 to 2.0 times more effective in producing dominant lethals and viable rearrangements (Dempster, 1941, and Fano, 1943, 1944) than less densely ionizing X rays but less effective (0.7) in producing recessive lethals (Timoféeff-Ressovsky and Zimmer, 1938; Zimmer and Timoféeff-Ressovsky, 1938, 1942; Dempster, 1941; Giles, 1943; and Fano, 1944).

Recently, however, it has become established that fast neutrons from both the cyclotron and nuclear detonations are 5 to 6 times more efficient than X rays in producing translocations (Lewis, 1954; Stone, Alexander, Clayton, and Dudgeon, 1954) and 4 to 7 times as effective in producing dominant lethals (Baker and Von Halle, 1954) in *Drosophila*. In contrast to the high RBE values reported for the above effects, the RBE for sex-linked recessive lethals induced by fast neutrons, as compared to X rays, has been reported to be about 2 to 3.5 (Mickey, 1954; Ives, Levine, and Yost, 1954), instead of 0.7 as found earlier.

The striking difference between the RBE for symmetrical exchanges and dominant lethals on the one hand and for recessive lethals on the other is unexpected if one assumes that a large number of these recessive lethals are associated with rearrangements. Since neutron dosimetry at the present is much more reliable than in the past, it was considered appropriate to make a detailed comparison between the effects of X rays, gamma rays, and fast neutrons on the induction of recessive lethals in *Drosophila* in an effort to gain further information concerning the mechanism involved in the induction of mutations.

Another method which will yield information concerning the composition of recessive lethals is one in which the frequency of induced lethals in

a ring-X chromosome is compared to the frequency induced in a rod-X chromosome. Since a ring chromosome can be lost after chromosome breakage as a result of torsional restitution or sister union of the broken chromosome ends and a rod chromosome can be lost only after sister union, one would expect to observe a decrease in the frequency of recessive lethals induced in a ring-X as compared to a rod-X chromosome, if one assumes that lethals occur as the result of chromosome breakage. The occurrence of interchromosomal rearrangements involving a ring-X will tend to accentuate this effect since the probability that such a rearrangement is viable is much less for the ring than the rod.

The results of this study show that, whereas the recessive lethals induced by X rays are predominantly point mutations, a portion of those produced by fast neutrons are associated with chromosome breakage.

CHAPTER II

MATERIALS AND METHODS

Since the interpretation of the experimental results reported in this paper depends upon accurate neutron dosimetry, a rather detailed description of the physical operations for neutron exposure will be given.

All neutron exposures in these investigations were carried out in the biological facility (Hurst, Mills, Conte, and Upton, Radiation Research, in press) of the Oak Ridge National Laboratory's 86-inch cyclotron (Livingston, 1952). Neutrons were produced by the bombardment of a beryllium target with 22-mev protons. The neutrons which were emitted had an energy spectrum which ranged from that of thermals to approximately 3.5 mev (with only occasional neutrons up to 10 mev) and a peak energy of 1.5 mev. The radiation produced by a cyclotron not only contains fast neutrons but also thermal neutrons and gamma radiation. If the true neutron dose is to be known, it is necessary to determine the extent to which each of these contaminating components is contributing to the total dose. The problem of determining the neutron dose in this facility has been undertaken by members of the Health Physics and Biology Divisions of the Oak Ridge National Laboratory, and the procedures used have been reported by them in detail (Hurst, Mills, Conte, and Upton, Radiation Research, in press; and Sheppard, Darden, Slater, Kimball, and Atta, Unpublished Manuscript, 1955, to be circulated). It was necessary for these investigators to employ various methods of measurement so that the amount of contaminating radiations could be determined as accurately as possible. The total radiation dose was measured by the use of tissue-equivalent ionization chambers which have been

described by Rossi and Failla (1950), whereas the gamma-ray component was determined by the use of a chamber constructed of metallic bismuth and by the more reliable photographic film technique (Ehrlich and Fitch, 1951). Although the number of thermal neutrons present was quite large (measured by neutron-induced gold or indium activation), the contribution of these to the total dose was negligible (using the cadmium-difference technique). Since this is true, the dose of fast neutrons can be determined by subtracting the portion of the dose caused by gamma radiation from the total radiation dose. An ethylene-polyethylene proportional counter was also used as an additional check in the determination of the neutron dose and has been described by Hurst, Mills, Conte, and Upton (Radiation Research, in press). The gamma-ray dose at the position of exposure in the biological facility was found to be 7 per cent of the total.

Since the operation of the cyclotron was not as steady as one would desire for day-to-day exposures, owing to the variation in the beam intensity, a paraffin-enclosed boron trifluoride (BF_3) monitor was located in the cyclotron maze. This was connected through a preamplifier and linear amplifier to a scaler located at the utilities control panel. In this manner, all neutron exposures were measured on a relative basis as counts on the counter. The counter was tested from time to time by a standard neutron source and calibrated periodically against the various dosimeters. After all biological treatments were made, statistical tests were carried out and corrections made for several sources of uncontrolled variation. Then the best rep/count for a given day of exposure was determined, and the neutron dose delivered by the cyclotron on any given day could be evaluated. In addition, it should be mentioned that, at the time during which all of

these dosage measurements were being made, biological material of various types was being exposed and the biological response to the cyclotron radiation observed. Since the agreement between the results of similar experiments which were carried out at different times by different or the same investigator is good, considerable reliance can be placed on the dosimetry which has been employed in determining the neutron dose in this cyclotron.

All biological exposures in these investigations were made in thin (4-mm) lucite radiation chambers which were placed in the center of the biological facility at the site of all dosage measurements. Prior to these runs, however, a dominant-lethal test was made in an effort to determine whether or not proton equilibrium could be obtained. The radiation chamber mentioned above was constructed in such a manner that it was possible to compress the flies which were to be exposed into a thin layer or to expand the chamber so that no crowding was achieved. Regardless of the manner in which the flies were exposed, no difference in the frequency of dominant lethals was observed. Therefore, one can assume that proton equilibrium existed during all cyclotron exposures.

The hard-gamma radiation used in these experiments was obtained from a 300-curie Co^{60} source which emits gamma rays of 1.17- and 1.34-mev energy in cascade (this distributed source is similar to that already described by Ghormley and Hochanadel, 1951). The radiation intensity in the cobalt source was approximately 1625 r/minute. The flies were placed in a gauze capsule which was inserted in a lucite radiation chamber that could be centered in the field of the source. The cobalt was then lowered around the material to be exposed, producing a uniform radiation field.

All X-ray treatments were administered by a 250-kvp General Electric Maxitron X-ray machine operating at 250 kv and 30 ma. The X-ray machine had an inherent filtration which was equivalent to 1 mm of aluminum and an external filter of 3 mm of aluminum was added. The approximate half-value layer for the filtered beam under these conditions has been found experimentally to be 0.55 mm of copper. This value corresponds to an effective wavelength for the X-ray photons of $0.20 \text{ \AA}$ and mean energy of approximately 60 kev (Kirby-Smith and Daniels, 1953). All dosage measurements and biological exposures were made inside lucite tubes at a distance of 49 cm from the anode. The intensity of the radiation was approximately 255 r/minute as measured by an 100-r Victoreen ionization chamber.

In those tests in which the relative biological effectiveness of fast neutrons are compared to gamma rays in inducing sex-linked recessive lethals, Oregon-R males were exposed to either 278, 556, 1112, and 1668 rep of fast neutrons or to 1000, 2000, 3000, and 4000 r of gamma rays. It is of importance to note here that all neutron doses have been corrected for 7 per cent gamma contamination. Control experiments were carried out at the same time that the irradiation series were made. The frequency of sex-linked recessive lethals was determined by the Muller-5 technique (Spencer and Stern, 1948), or a modification of this method which allows balancing of the lethal in the F-2 generation (Edington and Parker, 1954). The criteria used for classifying sex-linked recessive lethals is that described by Baker and Edington (1952).

Experiments were also undertaken to compare the frequency of recessive lethals induced in a ring-X chromosome ($X^{cl}, \underline{y}/sc^8.Y$) and an acrocentric-X

(rod-X) chromosome (In(1)EN, y/Y; the origin of this chromosome is described by Novitski, 1949) of equivalent length. Males, two to four days old, were exposed simultaneously to either 278, 556, and 1112 rep of fast neutrons or to 250, 500, 750, 1000, 2000, 3000, and 4000 r of X rays. Control experiments were also run for both chromosomes at the same time as the radiation series. In the neutron experiments, the Muller-5 technique was utilized for the detection of recessive lethals; but it was discovered that crossing over was occurring between the Muller-5 and In(1)EN chromosomes. Thereafter, in the X-ray series, the X^{cl} and In(1)EN males were mated to In(1)delta-49, y w B virgin females, and the frequency of recessive lethals determined as in the Muller-5 technique.

In all radiation and control experiments, the parents were transferred to fresh cultures every two days for six days and then discarded.

CHAPTER III

RESULTS

In order to measure accurately the relative biological effectiveness of radiations of different ion densities in inducing recessive lethals, the frequency of recessive lethals induced by each radiation at several dose levels was determined. The results obtained from the individual experiments at a given dose level were tested for homogeneity by the chi-square method and the variation observed in all tested cases was found to be that expected from random sampling of a binomial population. Since no heterogeneity was observed at any dose level for any radiation, the values reported at all dose levels are the average values of the pooled data. However, in the experiment in which the frequency of sex-linked recessive lethals and the sex-ratio distortion induced by fast neutrons in the ring- and rod-X chromosome were compared, only one experiment was done at each dose level.

When one compares the frequency of recessive lethals induced by gamma radiation and fast neutrons in males of the wild, Oregon-R strain of Drosophila melanogaster (Table I), it is apparent that the densely ionizing recoil protons produced in tissue by the neutron irradiation are more effective in producing recessive lethals than the less densely ionizing gamma rays. A quantitative comparison can best be made by determining the weighted linear regression which best fits the experimental points by the least-squares method. A comparison of the slopes of the regressions of recessive lethals on dose for fast neutrons, 3.25×10^{-3} , and gamma rays, 1.93×10^{-3} (Figure 1), shows that the RBE for neutrons, as compared to

TABLE I

THE FREQUENCY OF RECESSIVE LETHALS INDUCED BY FAST NEUTRONS AND
GAMMA RAYS IN OREGON-R MALES OF DROSOPHILA MELANOGASTER

Fast Neutrons				Gamma Rays			
Dose (rep)	No. of Chromosomes Tested	No. of Lethals	Percentage of Lethals	Dose (r)	No. of Chromosomes Tested	No. of Lethals	Percentage of Lethals
0	2225	3	0.135 ± 0.08	0	1875	1	0.053
278	2164	30	1.39 ± 0.25	1000	2045	43	2.10 ± 0.32
556	1873	34	1.82 ± 0.31	2000	2046	87	4.25 ± 0.45
1112	2124	74	3.48 ± 0.40	3000	1502	83	5.53 ± 0.59
1668	1595	92	5.77 ± 0.58	4000	1228	92	7.49 ± 0.75

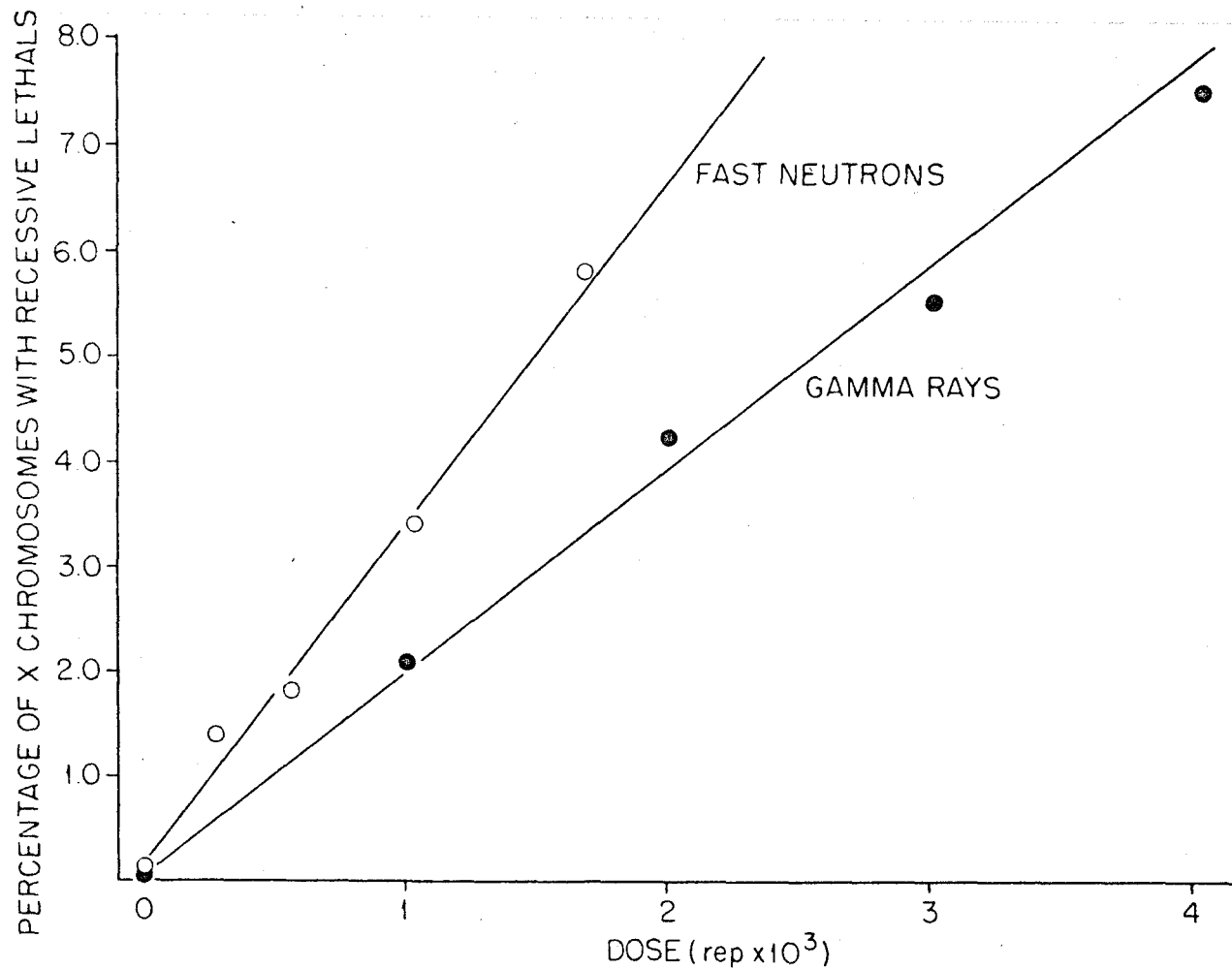


Figure 1. The frequency of recessive lethals induced by fast neutrons and gamma rays in *Drosophila melanogaster*.

gamma rays in inducing recessive lethals, is 1.68. The slopes of these two regressions have been found to be significantly different when tested by the "t" method ($t = 5.87$, d.f. = 6, $p = < 0.01$).

The greater effectiveness of fast neutrons as compared to less densely ionizing radiations in producing this genetic effect is also demonstrated when the slopes of the regressions for recessive lethals induced in the In(1)EN chromosome on dose for fast neutrons, 4.41×10^{-3} (Figure 2) and X rays, 2.46×10^{-3} (Figure 3) are compared. The RBE in this case is 1.79. When these slopes are tested for significance by the "t" test, the following values are obtained: $t = 2.25$, d.f. = 8, $p = 0.055$. This difference is considered a significant one because the slope of the regression for the neutron dose action curve is a minimum one, for the reasons that will be discussed below.

In order to gain further information concerning the relationship of recessive lethals with chromosome breaks, a rod-X, In(1)EN, and a ring-X chromosome, X^{cl} , were exposed simultaneously to either fast neutrons or X rays. The results of these experiments are tabulated in Table II for the neutron exposures and in Table III for the X irradiations. Upon examination of these tables, two things are obvious immediately. First, the frequency of recessive lethals induced by fast neutrons in the ring-X chromosome is much lower than the frequency of recessive lethals induced in the rod-X chromosome. When the slopes of the two regression lines (Figure 2) are tested to determine whether the difference between them is real, it is found that they are significantly different ($t = 3.35$, d.f. = 4, $p = 0.03$). This difference is also considered a minimum one since exchange which is known to occur between the In(1)EN chromosome and the Muller-5 test chromosome must

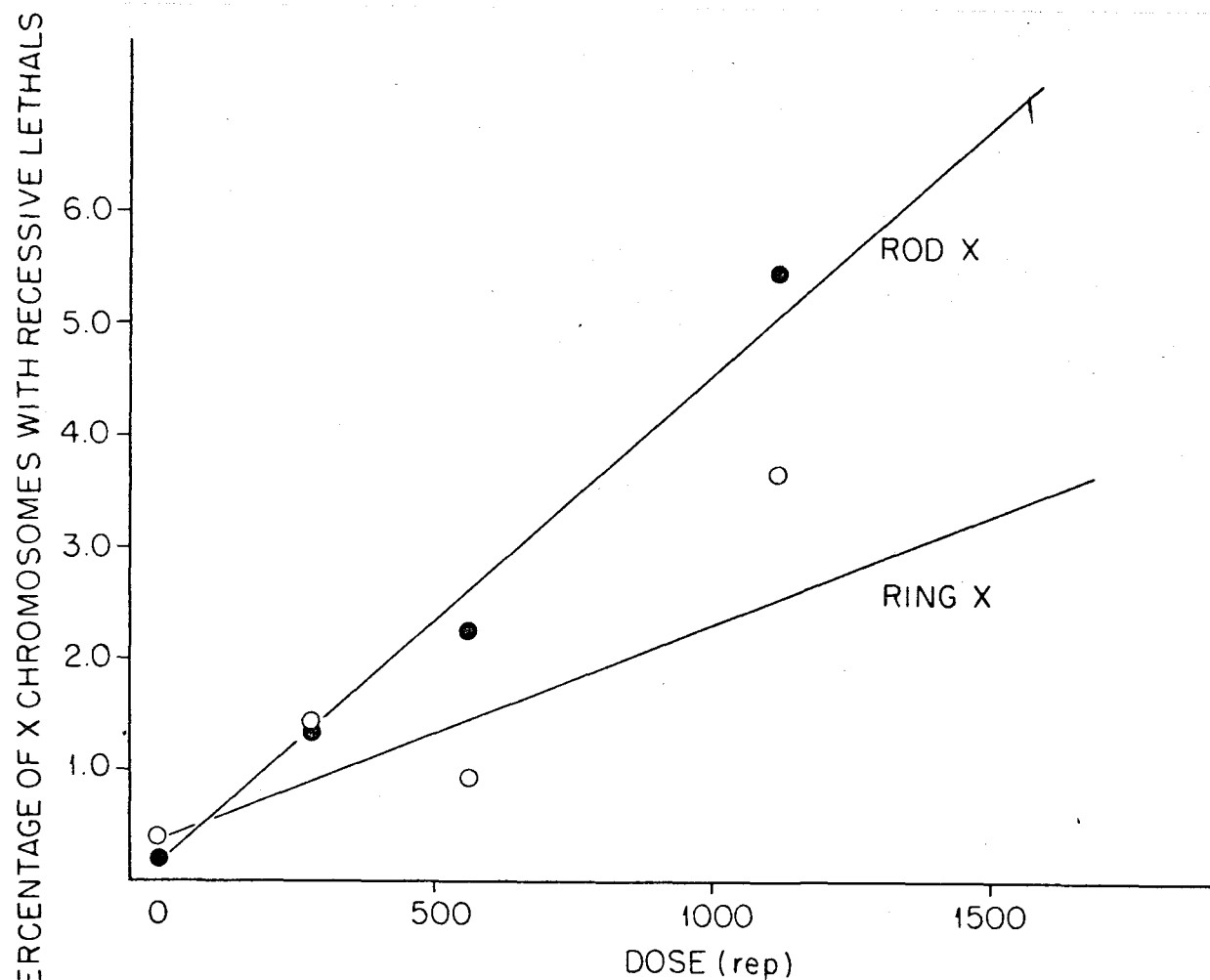


Figure 2. The frequency of recessive lethals induced by fast neutrons in rod-X and ring-X chromosomes.

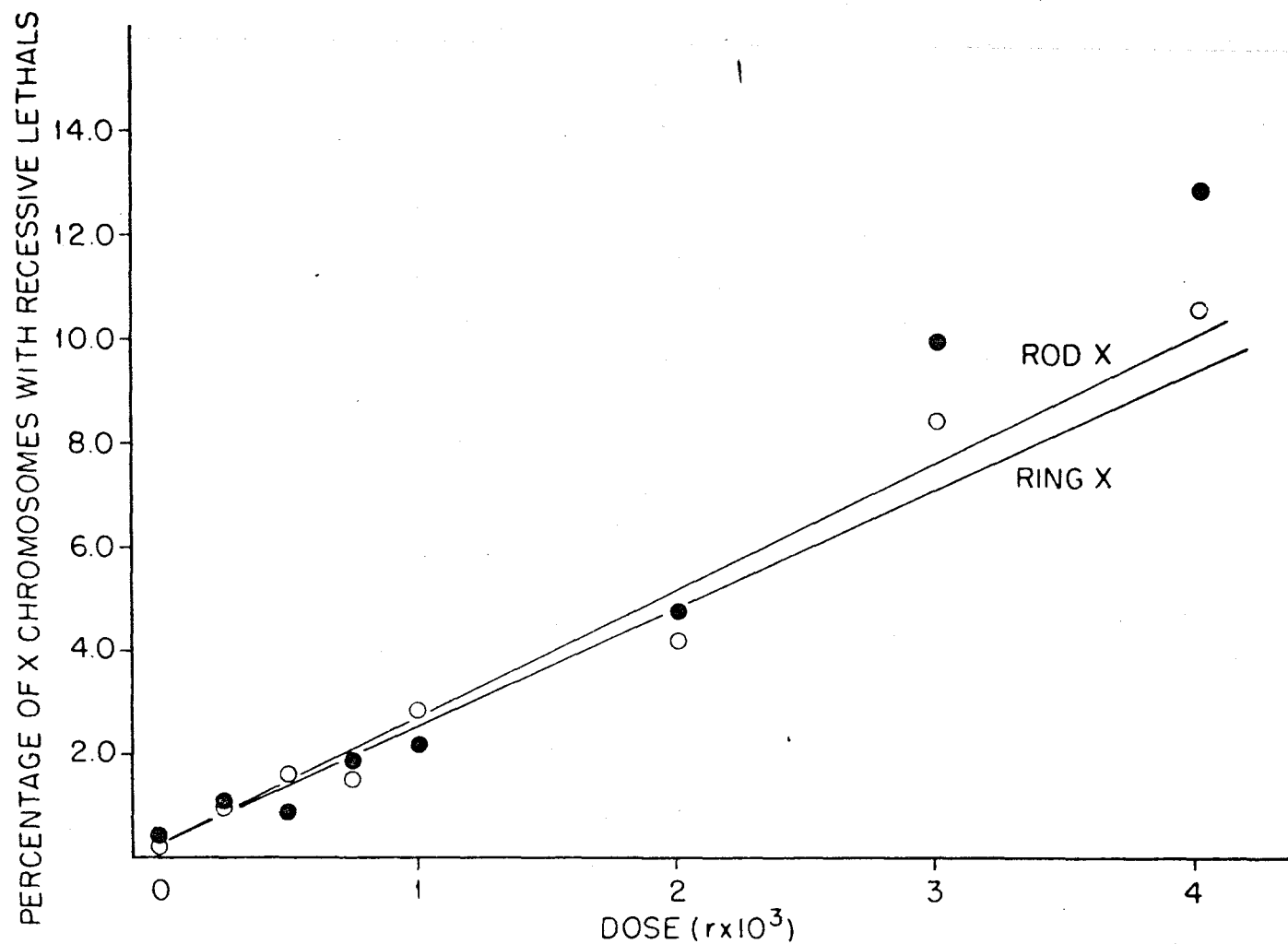


Figure 3. The frequency of recessive lethals induced by X rays in rod-X and ring-X chromosomes.

TABLE II

THE FREQUENCY OF RECESSIVE LETHALS INDUCED BY FAST
NEUTRONS IN RING-X AND ROD-X CHROMOSOMES

Dose (rep)	Rod-X			Ring-X		
	No. of Chromosomes Tested	No. of Lethals	Percentage of Lethals	No. of Chromosomes Tested	No. of Lethals	Percentage of Lethals
0	1116	2	0.18 ± 0.13	1088	4	0.37 ± 0.18
278	1113	15	1.35 ± 0.35	1117	16	1.43 ± 0.36
556	1117	25	2.24 ± 0.44	1092	10	0.92 ± 0.29
1112	1115	61	5.47 ± 0.68	684	25	3.66 ± 0.72

TABLE III

THE FREQUENCY OF RECESSIVE LETHALS INDUCED BY X RAYS
IN RING-X AND ROD-X CHROMOSOMES

Dose (r)	Rod-X			Ring-X		
	No. of Chromosomes Tested	No. of Lethals	Percentage of Lethals	No. of Chromosomes Tested	No. of Lethals	Percentage of Lethals
0	2643	11	0.42 ± 0.13	2335	6	0.26 ± 0.096
250	2461	27	1.10 ± 0.21	2111	20	0.95 ± 0.21
500	1898	17	0.90 ± 0.22	1619	26	1.61 ± 0.31
750	2179	41	1.88 ± 0.29	2054	31	1.51 ± 0.27
1000	1236	27	2.19 ± 0.42	1120	32	2.86 ± 0.50
2000	1113	53	4.76 ± 0.64	974	41	4.21 ± 0.64
3000	743	74	9.96 ± 1.10	723	61	8.44 ± 1.03
4000	654	84	12.84 ± 1.31	283	30	10.60 ± 1.83

have caused some F-2 lethal cultures to be classified as non-lethals. The second thing which is apparent after an examination of these data is the fact that the frequencies of recessive lethals induced by X rays in the rod-X chromosome and the ring-X chromosome are the same. This is in agreement with results which have been reported earlier by Muller (1940) and Novitski (1952). The reason for this difference in response to the two radiations will be discussed later.

The data tabulated in Tables IV and V show that a definite shift in the sex-ratio occurs after irradiation with either fast neutrons or X rays in the F-1 offspring of males bearing the ring-X chromosomes. If one calculates an unweighted regression line for the neutron and X-ray frequency-dose relationships (Figure 4), it is apparent that the shift in the sex-ratio is much greater in the offspring of the ring-X males after neutron exposure than after X-ray exposure and this difference is definitely a significant one ($t = 2.944$, d.f. = 8, $p = 0.014$). The RBE for this genetic effect is found to be about 2. It is surprising to find that very little, if any, deviation in the sex-ratio from the control ratio occurs in the offspring of the rod-X bearing males after neutron or X-ray exposure. This indicates that the frequency of sister union is lower than has been previously supposed (see review of Catcheside, 1948).

TABLE IV

THE FREQUENCY OF F-1 MALES AFTER IRRADIATION WITH FAST
NEUTRONS OF RING-X AND ROD-X BEARING MALES

Dose (rep)	Rod-X		Ring-X	
	No. of F-1 Offspring	Percentage of F-1 Males	No. of F-1 Offspring	Percentage of F-1 Males
0	5256	47.77	5138	46.56
278	4775	48.38	3215	50.39
556	3821	48.08	3101	54.76
1112	3636	47.41	1962	56.83

TABLE V

THE FREQUENCY OF F-1 MALES AFTER IRRADIATION WITH
X-RAYS OF RING-X AND ROD-X BEARING MALES

Dose (r)	Rod-X		Ring-X	
	No. of F-1 Offspring	Percentage of F-1 Males	No. of F-1 Offspring	Percentage of F-1 Males
0	5406	48.96	4840	51.36
250	4316	49.10	4252	53.29
500	3507	47.59	3256	54.12
750	5625	49.44	4883	54.07
1000	3543	48.86	3449	56.80
2000	2850	49.33	2785	62.80
3000	1525	46.69	1760	65.00
4000	1236	47.74	886	70.20

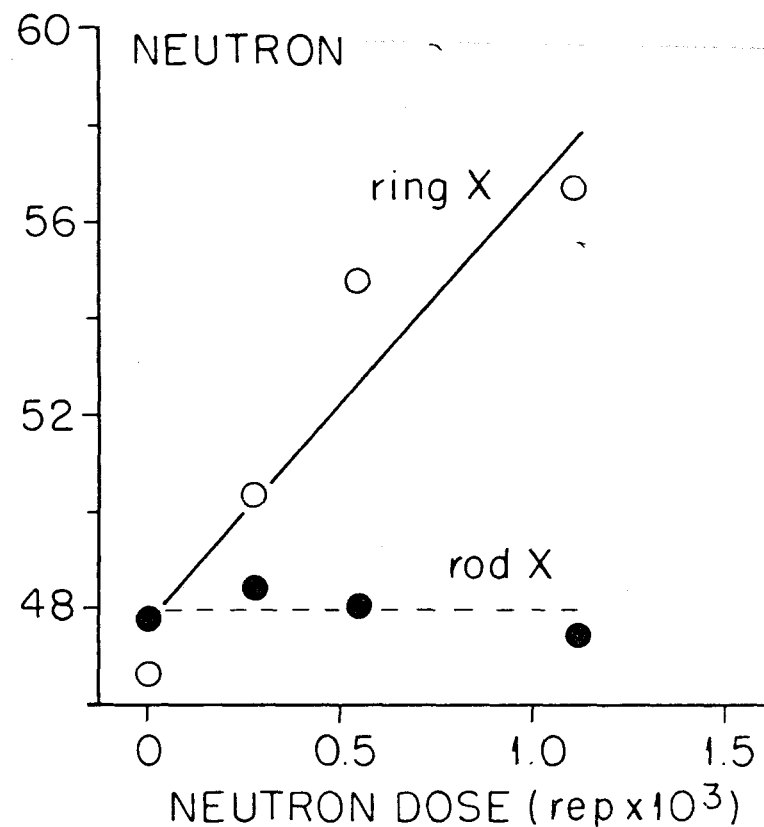
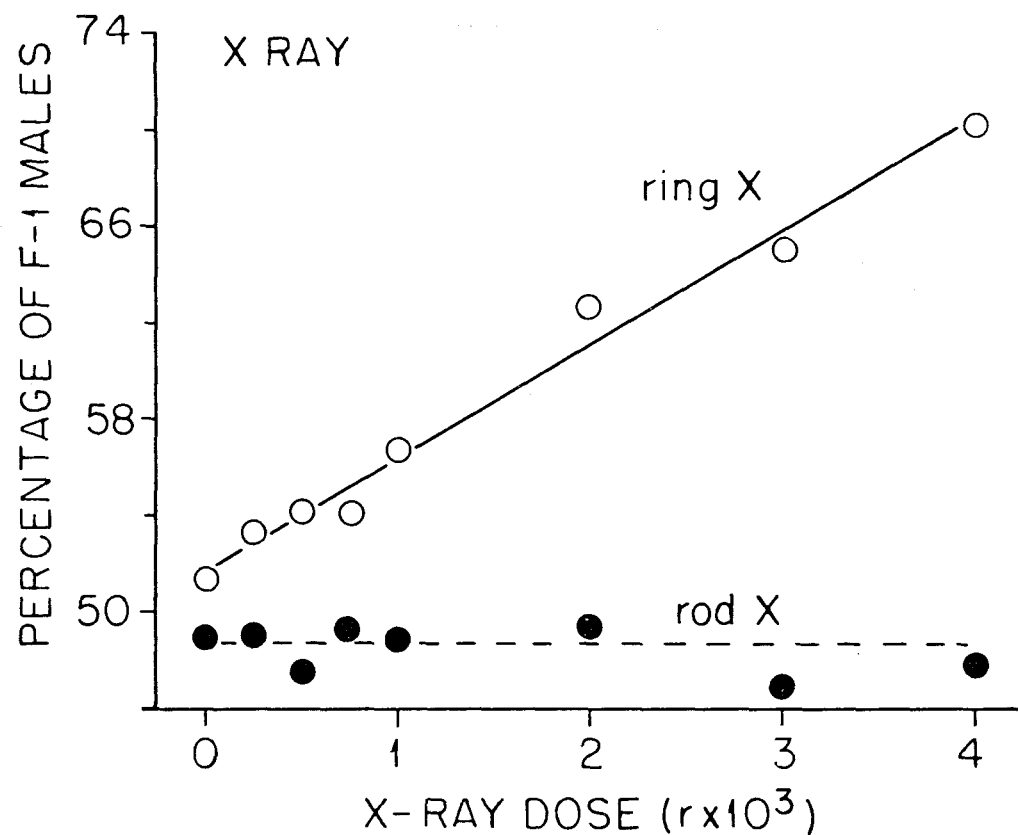


Figure 4. The frequency of F-1 males from irradiated rod-X and ring-X bearing males.

CHAPTER IV

DISCUSSION

In an effort to gain further information concerning the mechanism of origin of induced mutations, it was deemed profitable to determine as accurately as possible the RBE of fast neutrons as compared to less densely ionizing radiations in producing sex-linked recessive lethals. It is evident from the results reported in this paper that the effectiveness of different radiations in producing recessive lethals in *Drosophila* increases with increasing ion-density. This finding is in agreement with results previously reported for the induction of recessive lethals in *Drosophila* by Nagai and Locher (1935); Mickey (1954); and Ives, Levine, and Yost (1954). Since the investigations with fast neutrons reported in this paper and by Mickey (1954) were carried out separately in the ORNL 86-inch cyclotron, it is of interest to see how well the two sets of data agree. After a comprehensive statistical analysis was carried out on all the physical measurements involved in the neutron dosimetry and the best rep/count for a given day of exposures was applied to each set of data, it was found that the neutron dosimetry used by Mickey was in error. Dr. George H. Mickey has kindly consented to the incorporation in this paper of the corrected results for his recessive lethal data. After this dosage correction is made, it is found that the slope of the regression of recessive lethals induced by fast neutrons in the experiments of Mickey is 3.11×10^{-3} instead of 6.0×10^{-3} as reported. This is in excellent agreement with the slope of 3.25×10^{-3} found in our experiments. When the slopes of the regressions for each of these experiments is compared to the slope of the regression

for X rays (Timofeeff-Ressovsky, 1939), it is found that the RBE of fast neutrons, as compared to X rays, is 1.07, instead of 2.0 as reported by Mickey, and 1.12 for our experiments. It is evident from these comparisons that there is good agreement between the two sets of data.

Although the RBE for chromosome aberrations and dominant lethals is high and although Fano (1944); and Ives, Levine, and Yost (1954) have reported that the frequency of recessive lethals induced by fast neutrons which are associated with rearrangements is about 40 per cent, the RBE for recessive lethals has been shown in these experiments and the investigations of others to be quite low. This is in contrast to what one would expect if lethals were dependent upon chromosome rearrangements (position effect) for their expression. Therefore, it seems reasonable to conclude that the position effect hypothesis of recessive lethal origin is not a valid one.

It is also evident that sex-linked recessive lethals are induced by fast neutrons and X rays in different ways when one considers the results of the ring-rod experiments. Both radiations induce chromosome breakage which, in some cases, subsequently results in the loss of the chromosome. When one considers the ring-X chromosome, torsional restitution or sister union will cause loss of this chromosome. That loss of the X-chromosome actually occurs is unambiguously shown when one observes the shift in the sex-ratio of the F-1 offspring of ring-X bearing males which were exposed to either fast neutrons or X rays. This shift in the sex-ratio, however, is not observed in the offspring of rod-X bearing males after irradiation. The reasons for this will now be discussed.

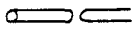
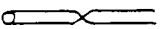
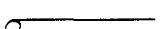
After a single break event, if one lets $(1-n)$ equal the probability that the broken chromosome ends will form a sister union and n equal the

probability that the ends will either undergo torsional restitution or normal restitution (Catcheside and Lea, 1945, show that no healed ends remain), and if one assumes that the last two events occur with equal frequency, then $n/2$ is the probability that the broken ends will undergo torsional restitution and it is also the probability that the ends will reconstitute normally. If a single break occurs in the ring-X chromosome followed by either torsional restitution or sister union of the broken ends, the probability that the ring will be recovered would be equal to the probability that normal restitution occurs ($n/2$). In the rod-X chromosome, only sister union would result in loss of the chromosome, and the probability that the rod-X chromosome will be recovered would be n ; therefore, it can be seen that, in this case, the recovery of the rod-X would be expected to occur twice as frequently as the recovery of the ring-X chromosome (see Figure 5).

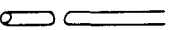
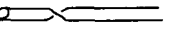
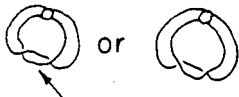
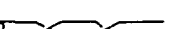
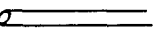
It is to be noted that the assumption is made that chromosomes in the sperm are split. The validity of this assumption is established, for at least a portion of the sperm, from direct observation (Cooper, 1949), from mosaic salivary chromosomes (Kaufmann, 1954), and from the pattern of induced mosaic offspring (Patterson, 1930). Although it has not been established that the chromosomes are split in all sperm, this simplifying assumption is made.

When two breaks are produced very close together in the X-chromosome by the same ionizing particle, two things can happen to the interstitial chromosome fragment produced. The fragment may or may not be lost. If the fragment is not deleted, the same events outlined above can occur at each break point. If one applies the same probability values in these cases as

ONE BREAK EVENTS

TYPE OF REUNION	PROBABILITY	RING X		ROD X	
		STRUCTURE	FATE	STRUCTURE	FATE
SU	$(1-n)$		L		L
TR	$n/2$		L		V
R	$n/2$		V		V

TWO BREAK EVENTS WITHOUT DELETION

(SU)(SU)	$(1-n)^2$		L		L
(SU)(TR)	$n(1-n)$		L		L
(SU)(R)	$n(1-n)$		L		L
(TR)(R)	$\frac{n^2}{2}$		L		V
(TR)(TR)	$\frac{n^2}{4}$	 INTERLOCKED	L or V		V
(R)(R)	$\frac{n^2}{4}$		V		V

SU - SISTER UNION

V - VIABLE

TR - TORSIONAL RESTITUTION

L - LOST

R - RESTITUTION

Figure 5. Types of rejoining in rod-X and ring-X chromosomes.

above, then the ring-X chromosome is recovered only when both broken ends reconstitute $(n/2)^2$, or when torsional restitution occurs at both breaks $(n/2)^2$ without interlocking of the rings. If interlocking occurs, however, many of the latter products presumably would not be recovered. Therefore, the probability that the ring will be recovered is $2(n/2)^2$ or less, depending upon the frequency with which interlocking of the rings occurs after two torsional restitutions. In the rod-X chromosome, however, torsional restitution at either break or both breaks will never cause loss of the chromosome; therefore, the rod would be expected to be recovered with a frequency of $4(n/2)^2$. Again it should be noted that the rod will be recovered twice as often as the ring. If the interstitial fragment is deleted, then one can apply the one break argument which has been discussed.

Therefore, if one assumes that recessive lethals originate as the result of chromosome breakage, production of minute deletions, or both, it is reasonable to expect that a marked decrease in the number of induced recessive lethals would be observed in the ring-X chromosome in comparison to the number induced in the rod. Breakage lethals or minute lethals would be expected to occur with approximately one-half the frequency in the ring as in the rod because the ring is lost at least twice as often. It is apparent that this is the case for the neutron experiments. The corollary to this conclusion is that one may rule out the possibility that most recessive lethals induced by fast neutrons are the result of point mutations. It also seems valid to rule out the hypothesis that some recessive lethals induced by fast neutrons are the result of point mutations which have been induced in the ring by a recoil proton which has also produced a break in the same chromosome at another locus. The reason for this conclusion is that one would expect two or more point mutation lethals to occur on the

same chromosome in a large number of cases and Fano (1944) has shown experimentally that association of more than one lethal on an X-chromosome is random with fast neutron irradiation.

On the other hand, interpretation of the X-ray data for the ring and rod experiments necessitates the adoption of a point mutation hypothesis in order to explain the experimental results. Although loss of the ring-X chromosome occurs after X-ray exposure, there is no decrease in the frequency of recessive lethals induced in the ring as compared to the rod chromosome. Therefore, it appears that the majority of recessive lethals induced by X rays are point mutations; i.e., no breakage of the chromosome is necessary for their induction. Although it has been reported by Oliver (1932); Demerec (1937); Dubinin, Khvostova, and Mansurova (1941); and others that X-ray induced recessive lethals are associated with chromosome aberrations, it is possible to consider that this is a chance association caused by the difficulty of separating, by crossing over, the lethal from the point of breakage of the rearrangement.

These data suggest the possibility that the composition of recessive lethals is a function of the ion density of the radiation. The present experiments indicate that with X rays, the majority of the induced lethals appear to be point mutations; whereas, with fast neutrons a portion of them are associated with chromosome breakage. It was not possible to determine if these breakage lethals were associated with single breaks or with minute deletions.

CHAPTER V

SUMMARY

The relative biological effectiveness of fast neutrons as compared to gamma rays for the induction of sex-linked recessive lethals has been found to be 1.68. When compared to X rays (data of Timofeeff-Ressovsky, 1939), neutrons are 1.12 times more effective. These low RBE values provide good evidence that the position effect hypothesis for recessive lethal origin is invalid.

The recessive lethals induced by fast neutrons are approximately half as frequent in a ring-X chromosome as in a rod-X chromosome of equivalent length. On the other hand, with X rays, the frequencies of recessive lethals induced in a ring-X chromosome (X^{cl}) and a rod-X chromosome ($In(1)EN$) are the same.

A pronounced shift with dose in the sex-ratio of the F-1 offspring of ring-X bearing males which have been exposed to either fast neutrons or to X rays is observed, but only a slight shift, if any, is observed in the offspring of rod-X bearing males after exposure to the same radiation.

It is concluded that sex-linked recessive lethals are produced in qualitatively different ways by fast neutrons and X rays, fast neutrons producing them as a result of chromosome breakage, minute deletions, or both, but X rays inducing them independently of chromosome breakage as point mutations.

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