EARLY in radiation genetic investigations it was reported that X-ray-induced sex-linked recessive lethals were not homogeneous in composition. Instead they included: (1) apparent intragenic changes without observable cytological abnormalities (point mutations); (2) deficiencies; and (3) changes associated with and, for the most part, inseparable from chromosome rearrangements (Oliver 1932; Demerec 1937). It was also reported that recessive lethals induced by X rays increased linearly with dose (Oliver 1932; Timoféeff-Ressovsky 1939). More recently Edington (1956) reported that X-ray-induced sex-linked recessive lethals increased more rapidly than expected on the basis of linearity, but Oster (1958) reported no departure from linearity.

Although intragenic changes and small deficiencies increase linearly with dose, gross deficiencies and chromosome aberrations have been shown to increase as the 1.5 power of the dose (Bauer 1939; Timoféeff-Ressovsky 1939; Muller 1940). If the aberration-associated lethals were dependent on the chromosome aberration for their expression (position effects) as Muller advocates (Muller and Prokofyeva 1935; Muller 1940, 1950, 1954), X-ray-induced recessive lethals should increase faster than expected on the basis of linearity. Since this was not observed, Lea and Catcheside (1945) and Herskowitz (1946) independently postulated that recessive lethals arise as the result of chromosome breakage, with few, if any, originating as point mutations or position effects. This hypothesis was based on the observation that the frequency of X-ray-induced chromosome breaks increased linearly with dose (Sax 1940; Carlson 1941). Intragenic changes were considered therefore, to have arisen as the direct result of breakage with subsequent restitution of the broken ends, and aberration-associated lethals owed their expression to the breaks that by chance became involved in a chromosome rearrangement. Under these conditions the frequency of lethals induced by X rays would be expected to increase linearly with dose in the same manner as chromosome breaks.

Fano (1947) and Muller (1950) pointed out, however, that the recessive lethal data are at variance with results expected on the breakage hypothesis of lethal origin. It was explained that breaks increase linearly with dose, but the incidence with which these breaks recombine in inviable combinations (domi-
nant lethals) increases more rapidly than the first power of the dose. As a result the incidence of breaks in the surviving cells must increase less rapidly than expected on the basis of linearity. To reconcile this fact with the observed linear frequency-dose relation, MULLER stated that an additional component of lethals that increases more rapidly than the first power of the dose (position effects due to chromosomal rearrangement) must be postulated. This component, when added to the linear component, would cause the total recessive lethal frequency-dose relation to approach linearity. Following these interpretations, HERSKOWITZ (1951) discarded the single origin hypothesis of recessive lethals in favor of a multiple origin hypothesis that provided for breakage lethals as well as point mutational and position-effect lethals.

Variegated (V-type) position effects are known to occur. Some of these are found to be lethal in X/Y males and nonlethal in X/Y/Y males. Others have been shown to be viable in X/Y males but lethal in X/0 males (see LEWIS 1950; SCHULTZ 1941). From experiments designed to recover sex-linked recessive lethals covered by the Y chromosome, LINDSLEY, EDINGTON and VON HALLE (1958, 1960) showed that approximately 20 percent of all lethals induced at 3–4kr are Y-suppressed V-type position-effect lethals. The majority of these, however, were lethal as X/0 males but survived as X/Y males, and consequently would have been missed by lethal-detecting procedures currently in use, in which the absence of X/Y males is the criterion of lethality. While demonstrating that Y-suppressible V-type position effects make up a considerable fraction of lethals (although not detected by the Muller-5 test), these experiments provide no indication of the contribution of position-effect lethals that are not made viable by the addition of one or two Y chromosomes or of S-type (stable) position effects.

The purposes of the experiments reported in this paper were to determine the frequency-dose relation of X-ray-induced Y-suppressed lethals, which would be expected to increase faster than expected on the basis of linearity, and to ascertain the frequency-dose response of all lethals detectable by the S-5 mating technique. It will be shown that the frequency of Y-suppressed lethals increases more rapidly than the first power of the dose and that the addition of these lethals to those normally recovered causes the total lethal frequency to deviate significantly from linearity.

MATERIALS AND METHODS

To detect orthodox lethals (lethals recovered by the usual recessive lethal tests) as well as Y-suppressed lethals (X/Y viable, X/0 lethal) in the same experiment, the S-5 test already described in detail by LINDSLEY and EDINGTON (1957) and LINDSLEY et al. (1960) was used.

In these experiments two doubly inverted X chromosomes, \( \text{Ins}(1) \) sc\(^{L,S1R} \), dl-49, \( \gamma^{17d} \)sc\(^{B} \)B (Biny) and \( \text{Ins}(1) \) sc\(^{L,S1R} \), S, \( \gamma \) sc\(^{B} \)u\(^{w} \) (S-5), and two X chromosomes with normal sequence, \( \gamma l(1)J1\)sc\(^{L,S1R} \), \( \gamma f \)f and \( \gamma v \)car, were used. In addition to the normal Y chromosome, the \( \gamma^{+}Y \) (sc\(^{B} \cdot Y \) chromosome that carries the distal end of \( \text{In}(1) \)sc\(^{B} \) containing the normal allele of yellow, \( \gamma^{+} \); MULLER 1948) was also used. The mutants represented by the gene symbols above are: \( \gamma^{17d} \) and
\( y \) = yellow; \( sc^- \) = scute deficiency; \( v \) = vermilion; \( f \) = forked; \( B \) = bar; \( sc^4 \) = scute-4; \( w^a \) = apricot; \( l(1)J1^{ss} \) = lethal J1; \( w \) = white; \( m \) = miniature; \( car \) = carnation. The inversion symbols used are: dl-49 = Inversion delta-49; and \( S \) = Inversion S.

Yellow vermilion carnation males, 2–4 days old, were exposed to varying doses of X rays and then mass mated to an equal number of virgin Biny/\( y l(1)J1^{ss} w m f \) females. Twenty-four hours later the males were separated from the females and discarded. The females were allowed to lay eggs for nine days (transferred every three days to fresh media). No F, sons are produced from this cross because of the scute deficiency in the Biny chromosome and \( l(1)J1 \) in its homolog; therefore virgin F, yellow vermilion heterozygous Bar females, Biny/\( y v \) \( car^* \) (* denotes the irradiated chromosome), were selected and pair mated to S-5/\( y^+Y \) males. Since the S-5 chromosome is deficient for most of the proximal heterochromatin (see Lindesley et al. 1960), pairing between the X and Y chromosomes is impaired and primary nondisjunction occurs frequently producing X/Y, and nullo X-nullo Y gametes in addition to the regular X- and Y-bearing gametes. In the F, therefore, males that are \( y v \) \( car^*/y^+Y \) (vermilion carnation) and \( y v \) \( car^*/0 \) (yellow vermilion carnation) are expected.

F, cultures can be scored, therefore, as nonlethals (vermilion carnation and yellow vermilion carnation males present); orthodox lethals (both males absent); Y-suppressed lethals (vermilion carnation males present and yellow vermilion carnation males absent); and Y-enhanced lethals (vermilion carnation males absent and yellow vermilion carnation males present). In addition primary nondisjunction occurring in the F, female can also be detected in the F, by the presence of yellow vermilion heterozygous Bar (\( y v \) \( car^*/Biny \)) and vermilion heterozygous Bar (\( y v \) \( car^*/Biny/y^+Y \)) females. It is also possible to detect Y-suppressed lethals at loci in heterochromatin (e.g. bobbed lethals rather than position-effect lethals). Such lethals are inviable when heterozygous with the S-5 chromosome; therefore yellow heterozygous Bar females (\( y v \) \( car^*/S-5 \)) are absent from the F-2 of heterochromatic lethal-bearing cultures.

All suspected lethals, regardless of type, were remated for confirmation (\( y v \) \( car^*/S-5 \) females \( \times \) S-5/\( y^+Y \) males). An orthodox lethal was assumed to have been induced if no males were detected in the F, and F, generations. Y-suppressed lethals were recorded if vermilion carnation but not yellow vermilion carnation males appeared in the F-2 and F-3. In addition the Y-suppressed lethals were further tested to determine whether the males were fertile or sterile by mating them to \( XX/y^+Y \) and \( XX/0 \) (\( XX \) = attached-X) females. In these experiments, no Y-enhanced lethals were observed; therefore, no further mention of such lethals will be made.

Five different doses were run in each experimental series (0, 1082, 2164, 3246, and 4328r). All X-ray exposures were administered by a Philips 250 kv constant potential X-ray machine operated at 250 kv, 15 ma, with 2 mm Al filter, producing an average intensity of 192r/min at the target distance of 50 cm. All dose measurements were made with a 100r Victoreen thimble chamber before each exposure.
RESULTS

To measure as accurately as possible the frequency-dose relation of X-ray-induced orthodox and Y-suppressed lethals as well as total sex-linked recessive lethals, replicates of a five point dose curve were carried out in each experimental series. The average value for each dose and each type of lethal with its standard error is tabulated in Table 1.

<table>
<thead>
<tr>
<th>Dose (r)</th>
<th>Chromosomes tested</th>
<th>Orthodox lethals</th>
<th>Percentage ±1 SE</th>
<th>Y-suppressed lethals</th>
<th>Percentage ±1 SE</th>
<th>Total lethals</th>
<th>Percentage ±1 SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4358</td>
<td>1</td>
<td>0.02</td>
<td>0</td>
<td>0.00</td>
<td>1</td>
<td>0.02</td>
</tr>
<tr>
<td>1082</td>
<td>3852</td>
<td>111</td>
<td>2.88±0.27</td>
<td>10</td>
<td>0.26±0.08</td>
<td>121</td>
<td>3.14±0.03</td>
</tr>
<tr>
<td>2164</td>
<td>3605</td>
<td>232</td>
<td>6.44±0.41</td>
<td>30</td>
<td>0.83±0.15</td>
<td>262</td>
<td>7.27±0.43</td>
</tr>
<tr>
<td>3246</td>
<td>2813</td>
<td>259</td>
<td>9.21±0.55</td>
<td>44</td>
<td>1.56±0.23</td>
<td>303</td>
<td>10.77±0.59</td>
</tr>
<tr>
<td>4328</td>
<td>2206</td>
<td>292</td>
<td>13.24±0.72</td>
<td>61</td>
<td>2.77±0.35</td>
<td>353</td>
<td>16.00±0.78</td>
</tr>
</tbody>
</table>

Since experimental techniques normally used in recessive lethal studies allow detection of chromosomes on which recessive lethals are located, those chromosomes on which two or more lethals have been induced cannot be distinguished from those with only one lethal. Therefore, the frequency of chromosomes with lethals will not increase linearly with dose but will increase in proportion to the exponential equation, \( y = 1 - e^{-kD} \) or \( y = 1 - \exp (-kD) \), where \( e \) or \( \exp \) = the base of natural logarithms; \( y \) = the predicted proportion of chromosomes with recessive lethals at any dose, \( D \); and \( k \) = the slope of the curve (CATCHESIDE 1948; MULLER 1954). If a recessive lethal is dependent on more than one independent primary event (chromosome break) for its expression, as would be expected for position-effect lethals, the frequency of such lethals would be proportional to the multihit equation, \( y = (1 - e^{-kD})^n \) or \( y = [1 - \exp (-kD)]^n \), where \( n \) = the average number of independent events required to produce the effect studied.

For the above reasons, weighted exponential regressions that best fit the experimental points were calculated by the least squares method using the IBM 7090 computer at the Oak Ridge National Laboratory. The residual sum of squares, which is distributed approximately as chi-square, was used to test for departure from regression.

The weighted exponential regressions for Y-suppressed lethals on dose are plotted in Figure 1. It was found that Y-suppressed lethals deviated significantly (chi-square = 13.878; df = 3; P = < 0.01) from the exponential function, \( y = 1 - \exp (-0.4299 \times 10^{-2}D) \) where \( y \) is the predicted proportion of lethals and \( D \) is the dose in kr. The data, however, fit closely the function, \( y = [1 - \exp (-0.3641 \times 10^{-2}D)]^{1.573} \), (chi-square = 0.003; df = 2; P = > 0.99). This would be expected if Y-suppressed lethals are, for the most part, position-effect lethals that depend on multihit rearrangements for their expression.
Y-SUPPRESSED LETHALS

Although some of the Y-suppressed lethals at each of the three highest doses can be demonstrated to be heterochromatic lethals (2164r, 2/30; 3264r, 5/44; 4328r, 4/61), these were not subtracted from the total Y-suppressed lethals prior to our statistical treatment.

The percentage of orthodox lethals induced by X rays was found to fit the regression, \[ y = 1 - \exp (-0.422 \times 10^{-4}D) \] (chi-square = 0.083; df = 2; \( P = 0.95-0.98 \)), better than the function, \[ y = 1 - \exp (-0.304 \times 10^{-4}D) \]. The experimental data did not deviate significantly, however, from the latter regression (chi-square = 3.447; df = 3; \( P = 0.30-0.50 \)). When orthodox and Y-suppressed lethals were combined, the percentage of total lethals deviated significantly from the exponential function, \[ y = 1 - \exp (-0.353 \times 10^{-4}D) \], (chi-square = 0.109; df = 3; \( P = 0.02-0.05 \)) but fit closely the function, \[ y = [1 - \exp (-0.608 \times 10^{-4}D)]^{1.266} \], (chi-square = 0.148; df = 2; \( P = 0.90-0.95 \)). The regressions and experimental points for orthodox and total lethals are plotted in Figure 2.

LINDSLEY, EDINGTON and VON HALLE (1958, 1960) initially classified their Y-suppressed lethals into three categories. They were (1) heterochromatic lethals, presumably bobbed lethals; (2) nonheterochromatic, male sterile lethals; and (3) nonheterochromatic, male fertile lethals. Their subsequent cytogenetic analysis of 32 lethals showed that in general the heterochromatic lethals were deficiencies in the proximal heterochromatin with normal disjunction in females.
but not in males. The nonheterochromatic male sterile lethals were associated with reciprocal translocations with high primary nondisjunction (20–40%) in females. Nonheterochromatic male fertile lethals were associated with inversions and insertional translocations with normal disjunction in both males and females.

No cytological analysis of Y-suppressed lethals recovered in the present study has been carried out; however, it can be seen from an examination of Table 2

TABLE 2

<table>
<thead>
<tr>
<th>Dose (r)</th>
<th>Total lethals</th>
<th>Orthodox lethals</th>
<th>Heterochromatic</th>
<th>Male sterile</th>
<th>Male fertile</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With N.D.*</td>
<td>With N.D.</td>
<td>With N.D.</td>
<td>With N.D.</td>
<td>With N.D.</td>
<td>With N.D.</td>
</tr>
<tr>
<td>0</td>
<td>1 0</td>
<td>1 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 0</td>
</tr>
<tr>
<td>1082</td>
<td>121 18</td>
<td>111 13</td>
<td>10 0</td>
<td>8</td>
<td>5</td>
<td>2 0</td>
</tr>
<tr>
<td>2164</td>
<td>262 52</td>
<td>231 33</td>
<td>31 2</td>
<td>20</td>
<td>18</td>
<td>8 1</td>
</tr>
<tr>
<td>3246</td>
<td>303 68</td>
<td>259 45</td>
<td>44 7†</td>
<td>24</td>
<td>19</td>
<td>11 2</td>
</tr>
<tr>
<td>4328</td>
<td>353 107</td>
<td>292 69</td>
<td>61 48†</td>
<td>34</td>
<td>29</td>
<td>17 3</td>
</tr>
</tbody>
</table>

* N.D. = Primary nondisjunction.
† Lethals that were classified as Y suppressed but lost before testing.
‡ Includes three questionable heterochromatic lethals. No F-2 y v car*/*yw* B females but males lost before testing.
§ Includes one questionable bobbed lethal.
that these lethals can also be divided genetically into the same three distinct categories. The majority of lethals that are male sterile are associated with primary nondisjunction (one percent or more exceptional \(F_2\) females) in the females heterozygous for the lethal whereas nondisjunction is much less frequent in the male fertile lethals. Since these results are consistent with those reported by Lindley et al. (1960), it is valid to assume that a cytological examination of the Y-suppressed lethals recovered in these experiments would show that the majority of male sterile lethals were associated with translocations, which is in agreement with the earlier results reported by Schultz (1947), and that the male fertile lethals owe their expression primarily to inversions and insertional translocations of the X chromosome.

Since primary nondisjunction can be positively correlated with the presence of chromosome aberrations, especially translocations, this genetic event can be used to estimate the frequency of orthodox lethals that are associated with chromosome aberrations. Such an estimate, however, underestimates the total frequency of such lethals induced. It is evident from an examination of the data in Table 2 that the proportion of orthodox lethals associated with nondisjunction increases with increasing dose (11.71\% at 1082r, 14.29\% at 2164r, 17.37\% at 3246r, and 23.63\% at 4328r). These results are in agreement with the observations reported by Oliver (1932), Demerec (1937), and Herskowitz (1946).

**DISCUSSION**

The results reported in this paper clearly indicate that at each dose of radiation studied a considerable fraction of induced sex-linked recessive lethals is suppressed by the presence of a single Y chromosome in the male. Although a small percentage of these lethals, especially at the higher doses, are heterochromatic lethals, the majority are nonheterochromatic, Y-suppressed recessive lethals. Lindley et al. (1960) have shown conclusively by cytogenetic analysis that Y-suppressed lethals comprise deletions of proximal heterochromatin and V-type position effects that are dependent on gross rearrangements for their expression. The demonstration that Y-suppressed lethals increase more rapidly than the first power of the dose indicates that these lethals are dependent on gross rearrangements for their expression. Thus these results provide additional evidence that the nonheterochromatic Y-suppressed lethals arise as the result of the V-type position effects.

The failure to recover such lethals in earlier experiments is now understandable. The presence of males carrying the irradiated chromosome in \(F_2\) cultures was considered indicative of a nonlethal and the culture was discarded. For this reason position-effect lethals that depended on chromosome aberrations for their expression and at the same time had their expression suppressed by a single Y chromosome were selectively eliminated from the total induced lethals. This caused the frequency of induced lethals recovered at higher doses to fall below that actually produced. Therefore, the observed frequency of recessive lethals (our orthodox lethals) induced by X rays appeared to increase linearly with dose.

When Y-suppressed lethals are added to orthodox lethals, it is found that the
frequency-dose relation of total lethals deviates significantly from linearity when
the saturation effect (more than one lethal induced on a chromosome) is con-
sidered. These results and those of LINDSLEY et al. (1960), therefore, clearly indi-
cate that a considerable proportion of induced recessive lethals are rearrange-
ment dependent, the majority of which behave as V-type position effects. It is
clear, therefore, that the breakage hypothesis of recessive lethal origin as ad-
vanced by LEA and CATCHESIDE (1945) and HERSKOWITZ (1946) is untenable
when total lethals, as detected by the S-5 test, are considered. The triple-origin
hypothesis advanced by HERSKOWITZ (1951), however, provides a more adequate
explanation for the origin of recessive lethals that are recovered by the experi-
mental techniques used in the present experiment. Since LINDSLEY et al. (1960)
have reported that approximately four percent of all orthodox lethals induced
at 3-4kr behave as V-type position effects (X/Y and X/0 lethal; X/Y/Y viable),
it is apparent that the triple-origin hypothesis is also a reasonable explanation for
the origin of orthodox lethals.

Another point of interest arising from these experiments concerns the shape of
the curve observed for X-ray-induced orthodox lethals. EDINGTON (1956) re-
ported that the frequency-dose relation for X-ray-induced orthodox lethals devi-
ated significantly from linearity. The results obtained in the present experiments,
however, show no such deviation. This discrepancy could be explained by any
one or a combination of several differences in experimental techniques. The
basic differences in experimental design between the two experiments were:
(1) quality of radiation, (2) duration of mating period, (3) screening tech-
niques, and (4) different X chromosomes were irradiated. The last two factors
should have little to do with the results; however, the first two could conceivably
alter the results.

In the present experiments the energy of the radiation beam was very close to
100 kv whereas the former experiments were performed using a heterogeneous
X-ray beam with an average energy of approximately 60 kv. It is now well es-
established that the relative biological effectiveness (RBE) of radiations of different
linear energy transfer (LET) on the induction of recessive and dominant lethals
in Drosophila increases with increasing LET (EDINGTON 1956; EDINGTON and
RANDOLPH 1958). Although it has been generally accepted that there is no
difference in RBE of X rays with wave lengths below 1.5 A, Kirby-Smith and
DANIELS (1953) have shown that 200 kv X rays are less effective than 60 kv
X rays for induction of chromome breaks in Tradescantia. In addition SWAN-
son (1955) has reported similar results in Tradescantia when 50, 100, and 250
kv X rays are used and exposure of inflorescences is carried out in air. If high
energy X rays, in the range discussed, are less effective in producing chromosome
breaks, one would expect fewer rearrangement dependent recessive lethals pro-
duced with 100 kv X rays than with 60 kv X rays. Since the reduction in X-ray-
induced rearrangements would be most pronounced at higher doses, it is con-
ceivable that the curvilinear effect obtained with 60 kv X rays would not be
observed when X rays of higher energy are used.

The difference in the length of the mating period used in the two experiments

could result also in an alteration of the shape of the frequency-dose curve. It is known that sperm which are immature at the time of radiation are more sensitive than mature sperm (Auerbach 1954; Lüning 1952; and others). It would be expected, therefore, that the frequency of induced rearrangements would be increased and consequently more rearrangement dependent recessive lethals would be observed. Inclusion of sperm that were immature at the time of radiation in the sperm sampled in the earlier experiments (six day mating period) could cause the frequency-dose relation to deviate from linearity. When a more homogeneous sperm sample is tested, as in the present experiments, it is apparent that the induction of orthodox lethals is linearly related to dose.

SUMMARY

An investigation has been made to determine the frequency-dose relations of total sex-linked recessive lethals as measured by the S-5 technique and the two major classes of lethals, orthodox and Y-suppressed, of which they are composed. It has been found that orthodox lethals increase linearly with increasing dose. Y-suppressed lethals, which behave for the most part as V-type position effects and constitute a large fraction (8.3% at 1082r–17.3% at 43281r) of total lethals at all doses studied, increase more rapidly than expected on the basis of linearity. When orthodox and Y-suppressed lethals were combined, it was shown that the frequency-dose relation of total lethals deviated significantly from linearity. The significance of these dose-response relationships to recessive lethal origin has been discussed.

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