A GENETICAL AND CYTOLOGICAL STUDY OF THE DOSE-DEPENDENCE OF MUSTARD GAS INDUCED LETHALS AND CHROMOSOME REARRANGEMENTS IN DROSOPHILA MELANOGASTER.

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Thesis Presented to the University of Edinburgh for the Degree of Doctor of Philosophy.

May, 1953.
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INTRODUCTION

During the last few years, many chemical substances have been used to induce mutations in various organisms. Mustard gas, the mutagenic agent of this study, is one of the most effective of the chemical mutagens. Its mutagenic action was discovered by Auerbach and Robson (1942, 1947) but there has been no attempt to study mustard gas or other chemicals on a quantitative basis similar to the work carried out on X-rays. The main object of the present work is to study the relationship between the frequency of chromosome rearrangements and the mutagenic agent applied on a quantitative basis. This study will provide, in addition, a method for studying the mechanism of chromosomal rearrangements. An attempt is also made to study quantitatively the relation between dominant lethals and the dose of the mutagenic agent applied.

In the case of X-radiation, one of the main points which had been studied thoroughly on a quantitative basis was the relationship between the frequency of mutations produced and the dosage used. Such a relationship has been securely established through the independent researches of many authors in the field of genetics. Muller (1928) in his first C1B experiments found that there is a direct proportionality between the dosage of X-rays applied and the percentage of mutations induced. Oliver (1930, 1931, 1932), by varying the time of exposure and keeping the dosage per unit time constant, found a direct proportionality between the number of lethals
induced and the duration or the amount of treatment. The extensive investigations of Timofeeff-Ressovsky further confirmed these findings. Studying the effect of X-rays upon mature sperms of *Drosophila melanogaster*, he showed, in 1933, that between 1200 r and 14800 r the frequency of visible and lethal mutations varied linearly with the dose. Schechtman (1930), Efroimson (1931) using dosage from 1125 r to 9000 r, and Zimmer (1934) independently arrived at similar conclusions. Haskins (1935) in his study on the frequency distribution of the mutation to white eye colour in *Drosophila* under X-irradiation found that the points for mutations are best fitted by a straight line. Heptner & Demidova (1936) studying the mutability of the normal allelomorphs of yellow, achete, scute, white and forked under the action of X-rays found, for each of them, a direct dependence of the frequency of mutation upon the X-ray dose. The fundamental work in plant material was done by Stadler who, in a series of studies, showed that in dormant and germinating seeds the mutation rate varies directly with the radiation dose (1928, 1930, 1939, 1931). The consistency of results thus independently derived has resulted in the formulation of the "target theory" of radiation-produced mutations. According to this theory, point mutations are produced as a result of "single hits" upon the hereditary material during the passage of ionizing radiation through the nucleus. The fact that the mutation rate produced by a given ionization dose is independent of the wave length, the time over which the
dose is given, continuous or fractionated dose, fertilization immediately or after a period as long as one month from treatment, and also temperature between 5°C and 37°C leads to the conclusion that the single hit which causes mutation is an ionization.

Some of the apparent point mutations are in fact minute rearrangements which involve two breaks. In *Drosophila*, the discovery of the giant chromosomes in the salivary glands made it easy to detect these small deficiencies and differentiate them from point mutations. Sliżynska & Slizynski (1947) in their cytological analysis of X-ray-induced lethals, found about 20% of them are due to small rearrangements.

As regards the mechanism(s) responsible for the production of gross structural changes, Painter & Muller (1928) pointed out two opposite views; either the breakage of the chromonemata occurred first, to be followed by fusion; or the process of breakage was dependent on the prior approximation and perhaps fusion of the chromonemata. In the first case they presumed that the breaks occur independently of each other, while in the latter case the different breaks concerned are the results of a common initiating process. These two views have been elaborated by Serebrovsky (1929) and Stadler (1932).

Serebrovsky's "Contact Hypothesis" assumes that when chromosomes or parts of chromosomes come into contact, they may in one way or another become attached to each other. If
later a mutagenic event makes these attached chromosomes break away from each other; then the breaks associated with one rearrangement are due to the same cause. This hypothesis was further modified by Muller (1932), who suggested that one break acts to induce another one or, perhaps more likely, both or all breaks associated with one translocation are related to a common cause. This process acts as a sort of illegitimate crossing-over at points of contact between two chromosomes or between parts of the same chromosome.

According to Stadler's "Breakage-first Hypothesis", the mutagenic events break the chromosomes at various points, and then the fragments tend to attach themselves to one another by their broken ends. The re-fusion may occur between the broken ends that were originally parted; in this case no structural change results. Or the broken ends may fuse in new ways resulting in a structural change. Thus all the known chromosomal derangements may be viewed as secondary effects of one primary process, chromosome breakage.

The chief distinction between the two hypotheses lies in the assumption that the breaks taking part in a structural rearrangement are simultaneously induced by a single mutagenic event, or are independent of each other, being induced separately. In the first case we should expect a linear proportionality between the dosage and the frequency of structural rearrangements; while in the second case, if the breaks are separately induced by separate acts, we should
expect the frequency of rearrangements to be proportional to the square of the dose.

Initially, the early data seemed to support the "Contact Hypothesis". Muller & Altenburg (1928) found that the frequency of translocations between non-homologous chromosomes produced by X-rays, rose approximately as the frequency of detectable gene mutations. Oliver (1931, 1932), using five different dosages, found that a simple and direct proportionality existed between induced rearrangements and the amount of irradiation, when mature germ cells of Drosophila males were exposed. Dubinin & Khvostova (1935) studying the mechanism of the occurrence of complex chromosome rearrangements concluded that the interpretation of data, based upon the contact hypothesis fitted the results of their experiments with Drosophila. A straight line passing through the origin was also obtained by Marshak (1935), who plotted the frequency of chromosome abnormalities induced in buds of Gasteria irradiated in several stages of the chromosome cycle. In 1939, in a preliminary note to Drosophila Information Service, Buzzati-Traverso reported a direct proportionality between the frequency of translocations between the second and the third chromosomes of Drosophila melanogaster and the quantity of irradiation.

All the above-mentioned work seemed to indicate that gross chromosome rearrangements vary directly with the dose of radiation. However, more recent work has been done which strongly supports the idea of separate independent breaks as the
basis for large rearrangements, and already in the early work there occurred observations which seemed to favour the "breakage-first hypothesis". Although Muller in 1936 stated that most investigations confirmed Oliver's results, he found in his own experiments that the frequency of rearrangements increased more rapidly than the dose. In 1938 he reported on experiments carried out in Berlin during 1933 which showed that two-break deletions vary in frequency at a rate greater than the first power of the dosage, and yet less than the square. The same was found for inversions by Berg & Panshin and Borrishoff in Leningrad (Muller 1938).

Belgovsky (1937) studying the frequency of translocations at two different dosages, one four times as great as the other, showed that the increase was greater than expected on the basis of a direct proportionality between the frequency of induced rearrangements and dosage, but less than the square of the dosage, which would be expected if the breaks represented independent events. He concluded that chromosome breaks are, most likely, caused by a direct action of the X-rays representing independent events, whereas the capacity of the broken pieces to reunite decreases as the dosage increases.

Khvostova & Gavrilova (1938), using the position effect method of the cubitus interruptus gene as a means of discovering translocations, found a direct proportionality between the dose of X-rays and the increase of aberrations, whereas the linkage method showed a disproportionate increase of aberrations. Bauer, Demerec and Kaufman (1938) accepted the
reported proportionality relationship for cases like "cubitus interruptus" arguing that this is only what might be expected, even on the breakage-first theory, for structural changes of such a nature that one of the breaks is required to occur in a region of very limited extent (here the fourth chromosome), while the other break is allowed much latitude. For such cases there might always be enough breaks of the second type available, and so the frequency of the combination would directly depend on the frequency of the breakages in the limited region alone, and hence would be proportional to the dose. Sax (1938) found that the percentage of gross chromosomal rearrangements in Tradescantia increases geometrically with increased X-ray dosage, and concluded that this indicates that most of the chromosome fusions are dependent upon two independent hits. Marshak (1939) showed that the chromosomal abnormalities produced by X-rays in pachytene of Vicia faba pollen-mother cells is an exponential function of the dose. Using a different technique, Fabergé (1939), in his experiments on chromosome fragmentations produced by X-rays in Tradescantia, found that the contact hypothesis is only with difficulty reconciled with his data.

The above findings led to the abandonment of the contact hypothesis by most authors. In addition, evidence of a different nature was brought forward against it. Dubinin and his co-workers found that in rearrangements derived from more than three breakages, the type of exchange
is likely to be such as would require the meeting of four or more threads at exactly the same point. As this is very improbable, they rejected the contact hypothesis (Muller 1938). For the same reason, Catcheside (1938), working with Drosophila, rejected the contact hypothesis, although his data showed a direct proportionality with the dose between 1000 r and 4000 r. His data obtained with high dosages made it necessary to assume that three or more chromosome threads were in contact at the time of breakage. This idea was discarded as it would require that the chromosomes be more tightly packed into the volume of the nucleus than is actually the case. However, in 1939, working with plants, Catcheside concluded that both contact and separate breakage mechanisms may be responsible, but that the former mechanism operates much more frequently.

Although, in 1939, most of the data supported the breakage-first hypothesis, there were still some doubts concerning this question. Therefore, two large-scale investigations to solve this problem were started independently. Muller and his co-workers carried out a series of genetical experiments while Bauer, Demerec and Kaufmann tackled the problem cytologically. Muller, Makki and Sidky (1939); Muller (1940), found that the frequency of gross rearrangements produced in Drosophila spermatozoa varies approximately as the $3/2$ power of the dosage of $X$-radiation when the latter is of the order of one to four thousands of r-units. In 1973 fertile cultures from $\Sigma$
whose fathers had been treated with 4000 r Muller found 114 translocations between the second and the third chromosomes, i.e., a frequency of 8.3%, while at 1000 r 118 translocations occurred among 10,196 fertile cultures, giving a frequency of 1.16%. The expected value for 1000 r, based upon the 3/2 power, was found by calculation to be 1.04%. This finding was interpreted to mean that individual ionizations produce the breakage, and that union of pieces occurs subsequently. The fact that the frequency of translocations increased at a lower rate than the square of the dose, which would be expected if independent breaks are the primary events, was explained on two grounds: (1) the so-called saturation effect, cases in which one break has been produced by more than one ionization; (2) the inviability of multiple combinations. The deviation from the square of the dose holds for inversions and deletions as well as translocations (Muller 1940). Also Bauer, Demerec and Kaufmann (1938) in their cytological analysis of induced rearrangements in Drosophila showed that the frequency of chromosomal aberrations increases as the 3/2 power of the dose. 

On this interpretation of the dosage-effect relationship for large rearrangements it would be expected that, at lower doses, when the frequency of multiple-break rearrangements decreases rapidly, and differential viability becomes negligible, the frequency of viable structural changes should increase more nearly as the square of the dose. Accordingly, Muller (1940) in a series of low-dosage experiments
in which X-rays and gamma rays were used found that the frequency of translocations increased, exponentially, the exponent in this case being equal to 1.8.

In addition to X-rays, other types of radiation have been applied to this kind of study. It has been shown that in Drosophila, neutrons are less efficient than X-rays in producing sex-linked recessive lethals, although their effects are qualitatively the same. The most significant difference between X-rays and neutrons lies in the relationship of the frequency of gross rearrangements to dosage. Giles (1940, 1943); Thoday (1942); and Giles and Conger (1950) showed that the frequency of gross chromosomal rearrangements in Tradescantia induced by fast neutrons varies linearly with the dose. This indicates that both breaks necessary to produce an interchange are caused by a single recoil proton. Muller and Valencia (1951) working with Drosophila, showed that the frequency of translocations induced by fast neutron-irradiation of spermatozoa varies linearly with the dose even at doses sufficient to produce multiple proton tracks per sperm. Although these results seem to support the contact hypothesis, they are what actually is expected on the basis of the breakage-first hypothesis, because every proton track is capable of inducing at least two breaks required for a rearrangement.

In studying the mutagenic properties of mustard gas
we are faced with the same problems as have just been discussed in connection with X-rays and the same methods are available for solving them. However, the chief difficulty as regards the use of mustard gas consists of finding a method of standardising the dose so that the results of different tests are comparable with one another. Even where it is possible to administer the same amount of gas under strictly comparable conditions, the actual amount which can penetrate to the chromosomes under test, whether they are in the germ cells or in somatic cells, depends on anatomical and physiological factors. These vary between different lines, between individuals of the same line, between different stages of development and, moreover, bodily activity during exposure can influence the amount of mutagenic agent reaching the nucleus (Auerbach & Robson, 1947). With other chemicals and methods of application standardisation may be feasible. Rappoport (1947), working with Drosophila, found a linear relationship between the amount of formaldehyde in the food and the frequency of sex-linked recessive lethals. Gibson, Brink and Stahmann (1950), who treated maize pollen with nitrogen mustard gas vapours with the help of an apparatus giving accurately graded doses, found a linear relationship between dosage and number of partially sterile F1 plants. Fahmy & Bird (1953), after injection of Drosophila with triazine, obtained a linear proportionality between the dosage injected and the
frequency of sex-linked recessive lethals. Because of the
difficulties in standardising the dose of mustard gas in
our experiments, sex-linked recessive lethals were used to
measure the dose which had reached the germ cells.
Objection may be raised against this method which assumes
that sex-linked recessive lethals are based upon single hits.
From X-ray work it is known that about 30% of sex-linked
recessive lethals are large rearrangements (Demerec, 1937).
However, since in the case of mustard gas the frequency of
large rearrangements among lethals is only 7% (Sliżyinska &
Sliżyński, 1947), the method may be regarded as a valid one.

A second problem raised by the previous work on
mustard gas is the marked shortage of gross rearrangements
as compared with that induced by X-rays giving a similar
frequency of sex-linked recessive lethals (Auerbach &
Robson, 1947). This discrepancy was brought to light in
experiments based on genetic techniques. Mehtab (1951, 1953)
came to the same conclusion by cytological means.

In Drosophila work the number of breaks observed
does not represent the actual number induced by the
treatment, since some of them occur in the heterochromatic
regions of the chromosomes. Also, many of them probably
heal without loss or change of the chromosomal material and,
still, many of the induced changes yield chromosomes which
are mechanically unfit to survive several cell generations,
and act as dominant lethals. Therefore, it is impossible to
say which of the following two alternatives underlies the observed shortage of large rearrangements after mustard gas treatment: (1) mustard gas produces fewer breaks than X-rays, (2) fewer rearrangements are formed from the induced breaks.

As regards the first possibility, there is no evidence, genetic or otherwise, which throws any light upon this question.

According to the second possibility, the shortage of large rearrangements may be due to a tendency of the broken ends to restitute in the original configuration rather than in new ones. Alternatively, they may also be caused by some secondary effect, or effects, interfering with both restitutions and new reunions, or with reunions only. One such mechanism which certainly must, to some extent, interfere with the formation of new reunions may be the delayed action effect of mustard gas (Auerbach 1948). This situation would result in few breaks being open at any one time. A further possibility may be the property of mustard gas to interfere with reunions of any kind, resulting in a large number of open breaks. This matter will be discussed below. Lastly, it may be that the scarcity of large rearrangements may be a reflection of a combination of all these factors.

The solution of the problem concerning the shortage of large rearrangements can be approached by a study of dominant lethals. After irradiation these are due to two
principal types of structural chromosome changes. The first of these is a chromosome break or isochromatid break followed by a sister-union at the breakage point. Pontecorvo (1942) assumes that single chromosomal breaks are produced by radiation at a rate proportional to the radiation dose and that those that do not undergo restitution nor participate with other breaks in a rearrangement undergo fusion of the sister chromatid broken ends. This fusion starts a breakage-fusion-bridge cycle such as described for maize by McClintock (1914). Through this process the orderly distribution of a balanced chromosomal complement in successive nuclear division is disturbed and this eventually results in genetic unbalance and death. The second cause of dominant lethality is asymmetrical exchange between two chromosomes, resulting in the production of a dicentric chromosome and an acentric fragment. This type of dominant lethal probably occurs with considerable frequency at higher dosages (Pontecorvo 1942). Aneucentric translocations and sister chromatid-reunions owe their lethality to deficiencies and, in lesser degree, to duplications produced when the affected chromosomes go through mitosis (Pontecorvo 1942).

Therefore, by measuring the frequency of dominant lethals, it may be possible to get some insight into the mechanism underlying the shortage of large rearrangements. If mustard gas is incapable of producing as high a frequency of chromosome breaks as an equivalent dose of X-rays, there
will be, relatively, a shortage of dominant lethals as well as of rearrangements. However, if it is the process of chromosome reunions which is interfered with, there will be as high a frequency of dominant lethals as in X-rays, but an accompanying shortage of large rearrangements.

Rearrangements obtained in the main study were grouped according to type and distribution of breaks in order to decide whether there are any chromosomal or regional specificities to the action of mustard gas. Previous work with X-rays indicates that there is little or no evidence for specificity apart from euchromatic versus heterochromatic regions. Muller (1941) showed, genetically, that although breaks were distributed throughout all chromosome regions, there was a higher frequency among heterochromatin than would be if the breaks were randomly distributed. Prokofyeva-Belgovskaya & Khvostova (1939) and Kaufmann (1939), in independent cytological studies, showed that breaks in Drosophila tended to occur preferentially in intercalary heterochromatic regions. However, using different chemicals, one obtains a gradation of specificity. In the first place Darlington and McLeish (1951) showed that by treating Vicia faba roots with maleic hydrazide the breaks were confined entirely to heterochromatic regions. At the other extreme, Oehlkers & Linnert (1949); Oehlkers, Linnert and Stange (1951) found no specificity of action of urethane upon the chromosomes of Oenothera hybrids. Lastly, Ford (1949) found that nitrogen mustard induced
breaks throughout all the chromosome regions of *Vicia* root tip cells, with certain regions being affected more frequently than others. Loveless & Revell (1949) with the same material and chemical found that these more susceptible regions lie near or in heterochromatin.

In the case of *Drosophila* there are, as yet, no overall data concerning the ability of chemical mutagens to induce breaks in specific regions of the chromosome. The work of Slizynska & Slizynski (1947), showed that in sex-linked recessive lethals induced by mustard gas the breaks tended to occur preferentially at the distal end of the X chromosome, with some indication of clustering in the mid-portion. Therefore, despite the smallness of the sample in this study, the data are of some interest inasmuch as it is the first attempt to map all cytologically observed breaks.

Some additional light may be thrown on the mechanism of the reunion of broken ends by comparing the frequency of intra-chromosomal breaks relative to that of inter-chromosomal breaks. If, in comparing the observed frequencies of the several types of rearrangements with the calculated expected relative frequencies, it is found that there is a preponderance of one or another type, it may be concluded that reunions are not at random but are biased in certain specific directions. With this end in view, two types of comparisons were made from data derived in this study. The genetically determined frequencies of translocations and deletions were compared and, similarly, the cytologically determined frequencies of translocations
and inversions.

In calculating the theoretically expected relative frequencies of inter- and intra-chromosomal rearrangements the long arms of the autosomes are regarded as separate chromosomes and the fourth chromosome is disregarded. There are thus five chromosomes, with the X, of assumed equal length. Accordingly, if the chromosome ends resulting from a break in one of the five chromosomes have an equal opportunity to combine with ends resulting from a second break in the same or in any other limb, inversions should occur with one-fourth the frequency of reciprocal translocations. By regarding the two limbs of one chromosome as separate entities, pericentric inversions are counted as translocations, but can be detected as such, only cytologically. In previous X-ray studies, Bauer, Demerec and Kaufmann (1938); Catcheside (1938); Bauer (1939); Kaufmann (1941) found that the observed ratio between translocations and inversions is actually 2:1 instead of the 1:1 expected ratio. This indicates that exchanges within the same arm are twice as frequent as those between different arms. This may be interpreted by assuming that more inversions survive because selection operates more heavily against the translocations. But it may also be assumed that when a break occurs in a chromosome limb, it has a greater opportunity of combining with other broken ends if they are in the same limb than if they are in other limbs. Bauer (1939) interpreted this to imply a restricted combination
zone as might be imposed by spatial limitations.

Auerbach & Robson (1947); Auerbach (1949, 1950) found a shortage of translocations and deletions after mustard gas treatment, and that the shortage of translocations is much greater than for deletions. After mustard gas, therefore, the discrimination seems to be against inter-chromosomal rather than against intra-chromosomal rearrangements. Data from the present study confirm these previous findings.

Some subsidiary observations made during the course of this study concern evidences of mosaicism and the occurrence of a reverse-repeat duplication.
MATERIAL AND METHODS

STOCKS.

The stocks used in this study were:

(1) **The OrK Stock:**

This is a wild type stock which, during many years, has been used for mutation work in the laboratories. It has been tested repeatedly over the last 6 years and has consistently given a spontaneous mutation rate of 0.3%. Another test was carried out and it was found to yield the same rate. OrT and OrN were derived from OrK by the method to be mentioned below.

(2) **The Muller-5 Stock:**

The complete formula for this stock is sc\(^{SI}\)Bin-Sw\(^{a}\)sc\(^{8}\). It carries in the X-chromosome the long sc\(^{8}\) inversion and, within this inversion, a shorter one In-S is included so that crossing-over with a normal chromosome is extremely rare. It carries a recessive marker, apricot, and a dominant one, Bar. This stock is used for the detection of sex-linked recessive lethals.

(3) **The M168 Stock:**

The females of this stock have attached-X with the markers yellow, vermilion and forked; dumpy on the second chromosome and ebony on the third. The males are M-5; dumpy on the second, and ebony on the third chromosome. This stock is used for the detection of translocations and
large deletions. The complete formula for this stock is:

\[ \begin{align*}
\text{dp} & \quad \text{M-5} \\
\text{dp} & \quad \text{e} \\
\text{dp} & \quad \text{e}
\end{align*} \]

(4) The ywsc Stock:

This stock carries on the sex-linked recessive markers yellow, white and singed. It is used for the experimental cytological work and was first tested cytologically for the presence of large rearrangements.

(5) The vg Stock:

Carries on the second chromosome recessive marker vestigial. It was used for the detection of dominant lethals because the flies are easily handled. A sub-line was selected for high fecundity and fertility.

Purification of the OrK Stock:

Before beginning the study of the frequency of large rearrangements in relation to dosage, it was necessary to test the OrK stock for any already existing chromosomal rearrangement. Two methods were used for purifying the OrK stock:

(a) Genetical. Test for Translocation: OrT Stock.

1. OrK males were mated in mass matings or separately to virgin yvf; dp; e females (M16B stock).

2. The F1 males containing the original X-chromosome and also one of each of the second and the third chromosomes of the OrK stock, were backcrossed to virgin yvf; dp; e females (M16B stock) in individual cultures so that the
distribution of the markers among the progeny of each of them could be determined.

3. The F\textsubscript{2} cultures were examined for the presence of a translocation.

If there are no translocations the character differences dependent upon different pairs of chromosomes will be distributed at random according to Mendel's law of independent assortment.

However, if there is a translocation, the markers in the chromosomes involved will appear, among the F\textsubscript{2} generation, as though they were linked. This is due to the formation of gametes which contain duplications or deficiencies for the translocation regions of the chromosomes. These gametes will be inviable. The viable types of gametes in which the translocation is present will establish a new linkage relationship for genes located within the translocated regions.

The following scheme applies to the method used here:

\[
\begin{align*}
\text{P}\textsubscript{1} & : \delta^{3} \text{ DrK} \times \text{ yvf; dp; e } \quad \text{ F}\textsubscript{2} \\
\text{F}\textsubscript{1} & : \delta^{3} \times \text{ yvf; dp; e } \quad \text{ F}\textsubscript{2} \\
\text{F}\textsubscript{2} & : \text{ All the females are yvf, whether or not there is a translocation present. However, the distribution of dp and e in males and females depends upon the presence or absence of a translocation.}
\end{align*}
\]

(1) No translocation: One fourth of females are dp; one fourth are e; one fourth are dp; e and one fourth are neither. The males will also be
of the four types in equal numbers.

(2) Translocation present:

a. Flies are of two types; dp and e, or neither dp nor e. In this case it may be concluded that there has been a translocation between the second and the third chromosomes. (See Figure 1)

b. Females are dp and dp; e and the males are normal and e. We may conclude that there is a translocation between X- and the second chromosomes. This possibility is illustrated in Figure 1.

c. Females are e and dp; e and the males are normal and dp. It may be concluded that there is a translocation between X- and the third chromosomes.

d. All the females are dp and e, and all the males are normal. This signifies that there is a translocation between X-, second and the third chromosomes.

It is possible that simple translocations have occurred. If so, they may be detected in a similar fashion.

In establishing the OrT stock, several lines were started by mating a virgin female to a male from the same OrK stock. 16 F1 males from each line were tested by one son only according to the following scheme:
Fig. 1. Scheme showing the method of detecting a translocation between II & III.

DF = deficiency
Dp = duplication
Fig. 1. Scheme showing the method of detecting a translocation between X & II.
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\[ F_1 \quad \delta \text{OrK} \times \varphi \text{OrK (virgin)} \quad \text{(pair mating)} \]

\[ F_1 \quad 16 \delta \text{OrK} \times y^{v} f d p e \text{ (M16B)} \quad \text{(pair mating)} \]

\[ F_2 \quad 1 \text{son, } \frac{+}{d p} \frac{+}{e} \text{ from each } x y^{v} f d p e \quad \text{(pair mating)} \]

\[ F_3 \quad \text{segregation as described in the previously outlined scheme.} \]

If one of the parents in \( F_1 \) was heterozygous for a translocation, the chance that for one son to be heterozygous is \( \frac{1}{2} \). The chance that one \( F_2 \delta \) is heterozygous for the translocation is \( \frac{1}{4} \). The chance that it does not carry a translocation that was present in \( F_1 \) is \( \frac{3}{4} \).

Thus, the probability that all 16 \( F_2 \) males tested do not carry a translocation which was present in the \( F_1 \) generation is \( \left(\frac{3}{4}\right)^{16} \) or 0.01.

The originally established lines were kept going during this process in mass cultures. Of these, seven were tested and found to be free from translocations by the criterion used. In order to avoid inbreeding, the seven lines were mixed into a new stock called OrT. This mixing increases the probability of having missed a translocation. In each line the probability of missing a translocation if it were present in the \( F_1 \) is \( \frac{1}{2} \), in the long run 1 in 100 trials will give a negative result (no translocations detected), even when the translocation is present in the \( F_1 \). Therefore, if 7 lines are tested, the probability that any one or more test will give a negative result when there is a translocation in the \( F_1 \) is

\[ 1 - \frac{(99)^7}{(100)} = 0.068 \]
(b) Cytological. OrN Stock.

An experiment was done with the established OrT stock. In the cytological study, an inversion in 3R was detected frequently (ten inversions in 176 larvae). It is pictured in Figure 2. This inversion must have been present in either OrT or in the ywsn stocks. This inversion was not scored in the results. It was considered desirable, therefore, to set up by cytological methods an OrN and a ywsn stock which would be free of rearrangements.

Three lines of OrT and two lines of ywsn were established by pair matings. From the F1 of these five lines reciprocal crosses between OrT and ywsn were made by pair matings; 16 crosses of each of the six possible types. In all 96 pair matings between OrT and ywsn were set up. In this fashion, both stocks could be tested simultaneously. One female third instar larva from each of the 96 cultures of the F2 was dissected and permanent salivary gland preparations were made. One line from the OrT stock, and another from the ywsn, were discarded because of the presence of the previously discovered inversion. The other three lines were free, and from them the new OrN and ywsn stocks were established. As noted above, the probability of missing an inversion in each line, if it were present in the F1, is 1% since 16 F1 cultures were tested each by one individual. This OrN stock has been used for all later experiments.
Fig. 2. Inversion present in the OrK stock.
Rearing of P₀:

Parent flies from the OrN stock were allowed to lay eggs over-night, after which they were discarded. When the flies started hatching the bottles were shaken, and the males for treatment were collected on the second day. Usually all the males for treatment were collected on the same day in order that experimental flies have as uniform a developmental time as possible since Strommaes (1949) found a variation in dominant lethality in early and late emerging males treated with the same dose of X-rays when the parents were allowed to lay eggs over a period of ten hours. The flies were reared on maize meal, molasses and agar culture medium and kept at 25°C.

Treatment:

The apparatus used was designed by Dr. H. Moser (1951). It is illustrated in Fig. 3. Only one alteration to it was made. This consisted in causing the air to bubble through the liquid mustard instead of passing over its surface. Thus a higher concentration of gas was obtained. Its essential feature is the exposure of the flies at constant temperature to a regulated flow of air which is carrying mustard gas vapours.

The apparatus is designed to allow a current of air carrying mustard gas vapour, to flow through a glass vial containing flies. A two-way stopcock controls the path of the air current so that it may either by-pass the mustard
Mustard-gas exposure apparatus.
container, or bubble through the liquid mustard. The mustard gas is destroyed after leaving the fly-containing vial, by passing it through a flask containing concentrated nitric acid. The temperature of the water bath and the air of the room in which treatment is carried out was kept constant at 16° C. After treatment with the gas, a flow of fresh dry air was allowed to pass through the vial containing the flies for about 10 minutes to revive and decontaminate them.

**Measurements of the Dose.**

The quantity of gas delivered may be controlled by keeping the rate of flow constant and changing the time of exposure, or vice versa, but in these experiments the rate of flow was kept constant, and the time of exposure was changed.

The size of the vial containing the flies was kept the same in the first and the second experiments but was changed to a larger size in the third and the fourth experiments. The number of flies treated simultaneously was about 800 in the first two experiments, while in the third and the fourth experiments it was increased to 1200 flies. It was found that increasing the size of the vial necessitated increasing the time of exposure in order to achieve a given frequency of sex-linked lethals.

After the last experiment the liquid mustard gas was found to give inaccurate results in other work being done because of the sample being used for almost two years.
and had, no doubt, started to break down chemically. It was, therefore, replaced by a fresh sample.

Table 1 gives the details of the exposure for mustard gas treatment and shows the influence of the size of the vial.

**TABLE 1.**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Size of Vial</th>
<th>Dose as measured by time of exposure</th>
<th>% of sex-linked recessive lethals</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNI</td>
<td>small</td>
<td>25 minutes</td>
<td>12.4 ± 1.43</td>
</tr>
<tr>
<td>HNII</td>
<td>small</td>
<td>17 minutes</td>
<td>8.2 ± 0.95</td>
</tr>
<tr>
<td>HNIII</td>
<td>large</td>
<td>25 minutes</td>
<td>7.05 ± 0.59</td>
</tr>
<tr>
<td>HNIV</td>
<td>large</td>
<td>17 minutes</td>
<td>4.75 ± 0.51</td>
</tr>
</tbody>
</table>

Rearing of $P_1$:

The $OrN$ male flies were treated at the age of 3-4 days, after which they were kept on food for one day before being mated. Mass matings were made for all tests except dominant lethals. Flies were reared in half-pint bottles on the same standard medium.

The number of flies per bottle differed according to the dose. The more effective the dose, the larger the number of bottles set up and the greater the number of flies per bottle. For the high dose, the mass matings consisted of 25-30 pairs, while for the medium and low doses, the number was 20 and 15 pairs respectively. For the cytological work, the numbers were usually lower than those
for the genetical tests.

The cultures were kept at $25^\circ$ C. and, after three days, the male parents were discarded while the female parents were transferred to fresh food bottles to continue laying eggs. The cultures for cytological work were kept at $25^\circ$ C. for two days, after which the parents were discarded and the bottles were transferred to room temperature and more dry yeast was added to each culture. This temperature and feeding method allows better growth of the larvae.

In the second experiment the males were mated to fresh virgin females after the first three days, and left with these for another three days. Tests for sex-linked recessive lethals and translocations were carried out separately for the first and the second broods.

Methods of Test.

(a) GENETICAL

(1) The Muller-5 Test.

   a. This test is employed for the detection of sex-linked recessive lethals.

   b. Treated male flies of the OrN stock were mated to virgin M-5 females in mass matings.

   c. After the F1 had emerged, the flies were transferred for ageing to fresh food bottles for two days. All the males were M-5, and the females were red eyed and heterozygous for Bar.

   d. The flies were pair-mated to their brothers, and in the case of shortage of males, M-5 stock males
were used. The effectiveness of the treatment determined the number of cultures which should be raised. The higher the dose, the lesser the number of cultures set up.

e. The F2 were examined for males with red eyes like the wild type used in the F1, absence of which showed that a lethal had been induced by the treatment.

f. After examining the F2, the lethal and doubtful cultures were kept for another two days to observe the later-hatching flies. Those cultures which contained more than 10-15 M-5 males only were scored as lethals. The others which contained less than 10 M-5 males were retested for a third generation.

\[
\begin{align*}
F_1 & \quad \begin{array}{c} \text{\textcolor{red}{+}} \end{array} \quad x \quad \begin{array}{c} M-5 \end{array} \\
     & \quad \begin{array}{c} M-5 \end{array} \quad = \quad \text{treated X} \\
F_1 & \quad \begin{array}{c} \text{\textcolor{red}{+}} \end{array} \quad x \quad \begin{array}{c} M-5 \end{array} \\
     & \quad \begin{array}{c} \text{M-5} \end{array} \\
F_2 & \quad \begin{array}{c} + \end{array} \quad \begin{array}{c} M-5 \end{array} \quad \begin{array}{c} M-5 \end{array} \quad + \\
     & \quad \begin{array}{c} \text{M-5} \end{array} \quad \begin{array}{c} \text{M-5} \end{array} \quad \begin{array}{c} \text{dies if any lethal induced} \end{array}
\end{align*}
\]

(2) **Dominant Lethals Test.**

a. About 30-35 treated males, and the same number of untreated controls, taken from the same cultures and at the same time, were mated individually to virgin vg females (3-4 days old) for two days in food vials.
b. The pairs were transferred to new vials containing slides covered partly with agar media brushed with acetic acid to encourage the laying of eggs.

c. The next morning the eggs were counted and set out for hatchability tests for at least 24 hours.

d. When not enough eggs had been laid on the first day, the pairs were transferred to fresh food vials for the day, and in the afternoon were returned for a second hatchability test.

e. After 24 hours the hatching eggs were counted. In mass matings, some females may not have been mated. Eggs laid by these females will not hatch and will be scored as dominant lethals. To avoid this, pair-mating was used in preference to mass matings. When none of the eggs laid by a particular female hatched, the female usually was dissected to see whether the seminal receptacle contained sperms or not. If it did not contain any sperm, these eggs were not included in the count.

f. The relative dominant lethality was calculated by dividing the hatchability in the treated series by that in the controls and multiplying by 100. This relative hatchability was subtracted from 100 to get the percentage of relative dominant lethality. This method
is essentially the same as that used by Wallace (1951) for calculating the frequency of dominant lethals in which the difference between the frequencies of unhatched eggs in the treated series and in the control is divided by the frequency of viable eggs in the control.

Example:

(a) Hatchability in Controls = 94.9%
   "   " Treated = 25.4%
   Relative hatchability = \(\frac{25.4}{94.9} \times 100\)
   = 26.7%

Relative lethality = 73.3%

(b) Applying Wallace’s (1951) method to the same data:

Frequency of unhatched eggs in control exp. = 0.051
Frequency of unhatched eggs in treated exp. = 0.746
Frequency of viable eggs in control exp. = 0.949

Frequency of dominant lethals = \(\frac{0.746 - 0.051}{0.949}\) = 0.695
                           = 0.949
                           = 0.732

(3) Test for Large Deletions and Translocations:

a. Treated males were mated to virgin yvf; dp; e females (M16B).

b. The F1 males were used for translocation tests as explained before.

c. The F1 females were examined for large deletions in the X-chromosome.
Large deletions in the X-chromosome of a treated sperm result in hyperploid females in which one or two of the three marker genes are covered up by the presence of their normal allelomorphs in the deleted fragment, as seen in Fig. 1.

Cytological Technique.

Treated males were mated to yw sn virgin females in mass matings for two days. The salivary glands of the F1 third instar female larvae were dissected and fixed in aceto-carmine for a few minutes. The technique used was as described by Slizynski (1949, 1952). The glands were squashed on an albuminised slide after covering them with cellophane paper, transferred to water for about 5 minutes until the cellophane separated, leaving the squashed material fixed to the albuminised slide; hydrolysed in normal hydrochloric acid for 5 minutes at 55-60°C, stained in hot basic fuchsin (not decolourised) for 13-14 minutes at 55-60°C, washed in water, bleached in SO2 water for 5 minutes, washed again with water, dehydrated in an increasing alcohol series and mounted in Euparal.

For cytological observations Bridges' reference system (1935) was used.
Fig. 4. A scheme showing detection of a deletion.

TREATED

DELETION

SPERM WITH FRAGMENT

OVUM WITH ATTACHED—X's

RESULTS IN HYPERPLOID ♀

PHAENOTYPE: NORMAL BODY COLOUR, VERMILION, FORKED.
GENETICAL RESULTS

1. Dosage-Effect Relationship:

The following tables show the results obtained on the relationship between the frequency of translocations and the dose as measured by sex-linked recessive lethals.

Table 2 contains the original results in the different experiments.

In HNVI, where the percentage of sex-linked recessive lethals was 12.1%, 2.1% translocations were obtained.

In HNII, the first brood HNIIa contained 8.2% of sex-linked recessive lethals and 0.7% of translocations, while the second brood, HNIIb, contained 11.2% sex-linked recessive lethals and 1.75% translocations.

In HNIII the percentages of sex-linked recessive lethals and of translocations were 7.05 and 0.68 respectively.

In HNIV, while the frequency of sex-linked recessive lethals was 4.75%, that for translocations was 0.34%.

It will be seen that in HNII, where, after three days, the treated males were mated to new virgin females for another three days, the frequency of both sex-linked recessive lethals and of translocations increased markedly in the second brood. Auerbach (1950) has already shown that there is a difference in susceptibility of the various stages of spermatogenesis to the mutagenic action of the mustard gas. In her experiments, the mutation rate was about doubled in the third brood between the 6th and the 9th
day after treatment, while in the second brood between the 3rd and the 6th day after treatment it rose slightly over the first brood.

It is therefore doubtful whether the results of HNIIb may be combined with the other data, all of which were obtained from the first broods. The question arises whether the increase in sensitivity is the same for lethals and translocations. As table 2 and fig. 5 show, HNI and HNIIb are so similar in respect of both lethals and translocations that it seemed legitimate to count HNIIb as an additional experiment with a dose nearly as high as HNI. But, to be safe, the statistical analysis was made both with and without HNIIb.

The graph representing all the points obviously cannot be fitted by a straight line; the curve looks more like a second order curve. However, this matter will be dealt with in the statistical treatment.

For the purpose of statistical analysis the data were grouped into three degrees of treatment; high, medium and low as shown in Tables 3 and 4.

In Table 3 the high dose is represented by the pooled data from HNI and HNIIb, as it was found that there is no significant difference between the frequencies of sex-linked recessive lethals and translocations in the two experiments.

\[ X^2 = 0.626 \text{ for sex-linked recessive lethals} \]
\[ = 0.257 \text{ for translocations;} \]

each for 1 degree of freedom.

Thus, the mean high dose yielded 11.5% sex-linked recessive
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Sex-Linked Recessive Lethals</th>
<th>Translocations X &amp; II &amp; III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Sperms tested</td>
<td>No. of Lethals</td>
</tr>
<tr>
<td>HNI</td>
<td>532</td>
<td>66</td>
</tr>
<tr>
<td>HNIIa</td>
<td>828</td>
<td>68</td>
</tr>
<tr>
<td>HNIIb</td>
<td>907</td>
<td>100</td>
</tr>
<tr>
<td>HNIII</td>
<td>1845</td>
<td>130</td>
</tr>
<tr>
<td>HNIV</td>
<td>1726</td>
<td>82</td>
</tr>
</tbody>
</table>

HNIIa; first brood
HNIIb; second brood
### TABLE 3.
Frequency of Translocations and Sex-Linked Recessive Lethals After Grouping the Experiments According to Dose.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Experiment</th>
<th>Sex-Linked Recessive Lethals</th>
<th>Translocations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of Sperms tested</td>
<td>No. of Lethals</td>
</tr>
<tr>
<td>High</td>
<td>HNI + IIb</td>
<td>1439</td>
<td>166</td>
</tr>
<tr>
<td>Medium</td>
<td>HNIIa + III</td>
<td>2673</td>
<td>198</td>
</tr>
<tr>
<td>Low</td>
<td>HNIV</td>
<td>1726</td>
<td>82</td>
</tr>
</tbody>
</table>
### TABLE 4.

Frequency of Translocations and Sex-Linked Recessive Lethals after Grouping according to Dose, but without HNI\textsubscript{IIb}.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Experiment</th>
<th>Sex-Linked Recessive Lethals</th>
<th>Translocations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of Sperms tested</td>
<td>No. of Lethals</td>
</tr>
<tr>
<td>High</td>
<td>HNI</td>
<td>532</td>
<td>66</td>
</tr>
<tr>
<td>Medium</td>
<td>HNIII\textsubscript{a} + III</td>
<td>2673</td>
<td>198</td>
</tr>
<tr>
<td>Low</td>
<td>HNIV</td>
<td>1726</td>
<td>82</td>
</tr>
</tbody>
</table>
INCREASE IN FREQUENCY OF TRANSLOCATIONS WITH INCREASING THE DOSE AS MEASURED BY SEX—LINKED RECESSIVE LETHALS.

SOLID CIRCLE REPRESENTS THE MEAN OF THE TWO MEDIUM DOSE POINTS USED IN FITTING CURVE SHOWN IN FIG. 6

Fig. 5.
lethals and 1.87% translocations.

The medium dose is represented by the pooled data from HNIIa and HNIII, both of them first broods, as there is no significant difference between them.

\[ \chi^2 = 1.13 \text{ for sex-linked recessive lethals} \]
\[ = 0.368 \text{ for translocations,} \]
each for 1 degree of freedom.

Thus, with the medium dose, 7.4% sex-linked recessive lethals and 0.7% translocations were found.

The low dose is represented by HNIV, in which 1.75% sex-linked recessive lethals and 0.34% translocations were obtained.

The same grouping has been used in Table 4, where HNIIb was not included.

**Statistical Analysis:**

The aim is to decide whether the frequency of gross chromosomal rearrangements varies linearly with the dose, or as the square of the dose, or whether the value is intermediate between 1 and 2. Thus the formulae to be tested will be:

\[ t = a l^1 \]
\[ t = a l^2 \]

where \( t \) = frequency of translocations

\( l \) = frequency of sex-linked recessive lethals.

One method of dealing with this problem was suggested by Dr. E.C.R. Reeve, of the Institute of Animal Genetics. This method consists of plotting the logs. of the
percentages of lethals and translocations against each other, and then testing for linearity and for the slope of the line. For fitting a line we have to transform these probabilities to the log scale. Natural logs were used in order to simplify the formulae. In its general form, the following equation is obtained:

\[ \log t = \log a + b \log l \]

The two main hypotheses will be given by the two values of \( b = 1 \) or \( 2 \), and we can test whether the calculated value of \( b \) differs from either of them.

For calculating the regression coefficient \( b \), the weighting coefficients must be calculated which, in turn, require calculation of the variances.

For lethals and translocations, one may calculate the variance of the fraction obtained.

If \( p = \) fraction of lethals (or translocations) in sample of \( N \) and \( q = 1 - p \), the variance of \( p = \frac{pq}{N} \).

Variance of \( \log (p) \) is approximately the square of the coefficient of variation of \( p = \frac{p(1-p)}{N(p^2)} = \frac{1-p}{Np} = \frac{q}{Np} \).

It is the same for any other of the six probabilities in the three groups. The actual values are as follows:

<table>
<thead>
<tr>
<th></th>
<th>lethals</th>
<th>translocations</th>
</tr>
</thead>
<tbody>
<tr>
<td>High dose</td>
<td>0.005</td>
<td>0.03</td>
</tr>
<tr>
<td>Medium dose</td>
<td>0.005</td>
<td>0.036</td>
</tr>
<tr>
<td>Low dose</td>
<td>0.011</td>
<td>0.11</td>
</tr>
</tbody>
</table>
Since the variances of the lethals are so very much smaller than those of the frequency of translocations, it can be taken as the fixed variable.

Thus only the variances of the log fractions of translocations need be taken for calculating the weighted regression of log t on log l.

The regression coefficient is calculated as:

\[ b = \frac{\sum w x y - \left( \sum w x \right) \left( \sum w y \right)}{\sum w x^2 - \left( \sum w x \right)^2} \]

where \( x = \log l \)
\( y = \log t \)
\( w = \text{weight of } \log t = \frac{1}{\text{variance of } \log t} \)

The deviation of the regression coefficient \( b \) from the two theoretical values (1 or 2) can then be calculated.

The standard error of \( b \) is:

\[ \text{S.E.} = \frac{1}{\sqrt{\sum w x^2 - \left( \sum w x \right)^2}} \]

Any theoretical value lying within the range of \( b \pm 2 \text{S.E.} \) is within twice the standard error of the actual value, and any value lying outside the range can be rejected with confidence.

The calculated regression coefficient of the data presented in Table 3 (with HNIIb), and its standard error is:

\[ b = 2.00 \pm 0.39 \]

The data in Table 4 (without HNIIb) were also calculated, and the regression coefficient and its standard error are:

\[ b = 1.94 \pm 0.46 \]

There is no significant difference between the two results.
Both estimates are very close to the value of $b = 2$ and deviate by 2.576 and 2.036 times their standard error respectively from the value of $b = 1$. Since this is a one-sided test the probability of the true value of $b$ being equal to 1 is 0.005 and 0.02, respectively, and so one can reject the linear relationship between the frequency of translocations and the dose as measured by recessive sex-linked lethals.

Since $3/2$ is the value determined for X-rays, it was calculated whether $3/2$ would also fit in this case. The value for $b$ is not significantly different from the value $b = 3/2$. However, it fits the value, $b = 2$ better.

Having derived the value of $b$, the value of $a$ may be calculated by using the weighted means of $t$ and $l$.

Having this information the actual regression curves may be obtained.

The weighted mean for $t = -4.60$

The weighted mean for $l = -2.46$

The value for $b$ is not significantly different from the value $b = 3/2$.

Having derived the value of $b$, the value of $a$ may be calculated by using the weighted means of $t$ and $l$.

The equation for the regression curve is, therefore:

$t = 1.38 (l)^{2.00}$ (includes HNIIb)

This equation is illustrated in Figure 6.

The weighted mean for $t = -4.34$

The weighted mean for $l = -2.57$

The equation for the regression curve is, therefore:

$t = 1.14 (l)^{1.94}$ (HNIIb not included)

Thus, the analysis of the data makes it possible to reject the idea of linearity and makes highly probable
RELATIONSHIP BETWEEN FREQUENCY OF TRANSLOCATIONS AND THE DOSE AS MEASURED BY SEX—LINKED RECESSIVE LETHALS

THE CURVE $t = 1.381 \cdot 10^{-2}$, THE CONSTANTS BEING ESTIMATED BY LEAST SQUARES

Fig. 6.
the increase of translocations as the square of the dose. However, the 3/2 power relationship cannot be excluded.

II. The Frequency of Dominant Lethals.

Table 5 represents the relation between the frequencies of sex-linked recessive lethals and the percentage of relative dominant lethality induced in the different experiments. Both frequencies are obtained from the first brood only.

The results are arranged in a descending order of dose. It can be seen that the percentage of sex-linked recessive lethals increases from 4.75 to 12.4 and that of relative dominant lethality increases from 20.6 to 73.3.

In Figure 7 a straight line was drawn by hand to fit the experimental points. For a more accurate description of the relationship between dominant and recessive lethals the regression coefficient must be calculated. For this, the weighting coefficients of the results have to be calculated which needs, in turn, the determination of the variances.

The percentage of the relative dominant lethality is expressed as:

\[
1 - \frac{P_2}{P_1}
\]

where \( P_1 \) = hatchability in controls
\( P_2 \) = hatchability in treated

and since the variance of \( P_1 \) is small, this factor can be neglected.
TABLE 5.

Frequencies of Sex-Linked Recessive and Dominant Lethals.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Sex-Linked Recessive Lethals</th>
<th>% Controls</th>
<th>% Hatchability</th>
<th>Treated</th>
<th>% Hatchability</th>
<th>Relative Lethality</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNI</td>
<td>12.4</td>
<td>1177</td>
<td>1118</td>
<td>94.9</td>
<td>1438</td>
<td>365</td>
</tr>
<tr>
<td>HNII</td>
<td>8.2</td>
<td>1123</td>
<td>1073</td>
<td>95.5</td>
<td>1185</td>
<td>423</td>
</tr>
<tr>
<td>HNIV</td>
<td>7.17</td>
<td>985</td>
<td>908</td>
<td>92.2</td>
<td>940</td>
<td>434</td>
</tr>
<tr>
<td>HNI, V</td>
<td>7.05</td>
<td>695</td>
<td>652</td>
<td>93.8</td>
<td>1205</td>
<td>726</td>
</tr>
<tr>
<td>HNIV</td>
<td>4.75</td>
<td>267</td>
<td>247</td>
<td>92.5</td>
<td>1041</td>
<td>765</td>
</tr>
</tbody>
</table>

HNII: first brood only.
RELATIONSHIP BETWEEN PERCENTAGE OF RELATIVE DOMINANT LETHALITY AND SEX—LINKED RECESSIVE LETHALS.

SOLID CIRCLE REPRESENTS THE MEAN

Fig. 7.
The weighting coefficient approximately is
\[ \frac{n_2 p_1^2}{p_2 q_2} \]

where \( n_2 \) = the number of eggs laid in the treated and
\( q_2 = (1 - p_2) \)

However, since \( n_2 \) is fairly constant and \( p_2 q_2 \) is also fairly constant, it can be seen that the weighting will make little or no difference to the regression coefficient of the percentage of the relative dominant lethality on the percentage of sex-linked lethals.

Thus, the following equation can be used for calculating the regression coefficient:
\[ b = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sum (x - \bar{x})^2} \]

where \( \sum (x - \bar{x})(y - \bar{y}) = \sum xy - \frac{\sum x \sum y}{n} \)
and \( \sum (x - \bar{x})^2 = \sum x^2 - \left( \frac{\sum x}{n} \right)^2 \)

\( x = \) frequency of sex-linked recessive lethals
\( y = \) relative dominant lethality.

The value for the regression coefficient \( (b) \) is
\[ b = 6.76 \]

The equation for the regression line is
\[ y = a + b x \]
\( a = \) constant
\( b = 6.76 \).

The actual line must be drawn through \( x \) and \( y \).

These mean values have been determined as
The value for the regression coefficient, \( b \), indicates that there is an increase of 6.76\% relative dominant lethality for each 1\% sex-linked recessive lethals. The line based upon these calculations is shown in Figure 7.

If extrapolation is permissible, it would be of interest to find the point where the regression line intersects the abcissa. This represents the point where the treatment does not produce any dominant lethals and would theoretically correspond to the spontaneous rate of recessive sex-linked lethals. This point may be calculated by determining the value of \( a \) in the equation \( y = a + bx \).

\[
4.85 = a + 6.76(7.9)
\]

\[
a = -4.9
\]

Thus setting \( y = 0 \) the point where the regression line intersects the abcissa may be found.

\[
0 = -4.9 + 6.76x
\]

\[
x = 0.7
\]

This value is not significantly different from the point 0.3\% which represents the spontaneous sex-linked recessive lethal rate for this stock.
### TABLE 6.
Distribution of Translocations on the Chromosomes.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of Spermatozoa Tested</th>
<th>Number of Translocations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>X &amp; 2</td>
</tr>
<tr>
<td>HNI</td>
<td>519</td>
<td>3</td>
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<tr>
<td>HNIIa</td>
<td>1361</td>
<td>2</td>
</tr>
<tr>
<td>HNIIb</td>
<td>1139</td>
<td>4</td>
</tr>
<tr>
<td>HNIII</td>
<td>2497</td>
<td>4</td>
</tr>
<tr>
<td>HNIV</td>
<td>2655</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>8171</strong></td>
<td><strong>15</strong></td>
</tr>
</tbody>
</table>
III. Distribution of Translocations on the Chromosomes.

The frequency of translocations among the three major chromosomes in the different experiments is shown in Table 6.

Among 8171 cultures tested there were 15 translocations between X- and the second chromosomes, 18 translocations between X- and the third, and 34 translocations between the second and the third chromosomes.

By assuming the second and the third chromosomes to be approximately of the same length, each in turn about double the length of the X-chromosome, one should expect that the frequency of translocations between X- and the second, and between X- and the third would be about the same, and that between the second and the third chromosomes would be approximately equal to the sum of the two.

There is a slight difference, but not significant, between the frequencies of translocations between X- and the second and between X- and the third. This should be expected because the third chromosome is slightly longer than the second. The translocation frequency between the second and the third chromosome is about the sum of the frequencies between X- and the second, and between X- and the third chromosomes.

Inter- and Intra-Chromosomal Rearrangements Relationship.

Table 7 shows the relationship between inter- and intra-chromosomal rearrangements induced by mustard gas.
### TABLE 7.

Comparison Between the Observed Frequencies of Rearrangements Induced by Mustard Gas and those Expected for X-Ray Experiments Yielding the Same Amount of Sex-Linked Lethals.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Experiment</th>
<th>% Sex-Linked Lethals</th>
<th>No. of Spermatozoa Tested</th>
<th>No. Rearrangements</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Observed</td>
<td>Expected</td>
</tr>
<tr>
<td>High</td>
<td>HNI + Iib</td>
<td>11.5</td>
<td>1658</td>
<td>31</td>
<td>274</td>
</tr>
<tr>
<td>Medium</td>
<td>HNIIa + III</td>
<td>7.4</td>
<td>3858</td>
<td>27</td>
<td>328</td>
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<tr>
<td>Total</td>
<td></td>
<td></td>
<td>5516</td>
<td>58</td>
<td>602</td>
</tr>
</tbody>
</table>

**DELETIONS**

<table>
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<tr>
<th>Dose</th>
<th>Experiment</th>
<th>% Sex-Linked Lethals</th>
<th>No. of Spermatozoa Tested</th>
<th>No. Rearrangements</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>HNI + Iib</td>
<td>11.5</td>
<td>2659</td>
<td>9</td>
<td>25</td>
</tr>
<tr>
<td>Medium</td>
<td>HNIIa + III</td>
<td>7.4</td>
<td>7469</td>
<td>6</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>10128</td>
<td>15</td>
<td>61</td>
</tr>
</tbody>
</table>
The inter-chromosomal changes are translocations, while the intra-chromosomal rearrangements are deletions.

In order to compare the relative frequencies of inter- and intra-chromosomal rearrangements with those found for X-rays, the expected numbers of translocations and deletions were calculated from data obtained by X-radiation. The basis for calculating expectations for X-rays is the validity of the 3/2 power relationship between the frequency of translocations and the dose as proved by Muller and his co-workers (1939, 1940), and confirmed later by Auerbach and Robson (1947) for large deletions. Thus, the calculations of the expected numbers of translocations and deletions were carried out on the assumption that the frequency of large rearrangements varies as the 3/2 power of the dose.

The expected numbers of translocations were calculated on the basis of four sets of data obtained by Muller, Makki and Sidky (1939) and Muller (1940). Muller, Makki and Sidky (ibid) obtained at a dose of 4000 r 67 translocations between the second and the third chromosomes among 790 cultures tested. The expected number was calculated as follows.

4000 r produce 67 translocations in 790 sperms.

Applying the 3/2 power rule - 1000 r should produce

\[67 \left(\frac{1}{2}\right)^3 \times \frac{1000}{790} = 10.6 \text{ translocations per 1000 r per 1000 sperm.}\]
At a lower dose, 1000 r, the same authors obtained 72 translocations between the second and the third chromosomes among 5900 cultures tested. Application of the same method results in an expectation of 12.2 translocations among 1000 cultures for 1000 r. For Muller's results (1940), expectations of 10.4 and 11.5 translocations were obtained. The average of these four determinations was found to be about 11 translocations between the second and the third chromosomes among 1000 spermatocytes tested for 1000 r. This number was taken as a starting point. However, since the work herein reported translocations involving the three major chromosomes were studied, a correction will be introduced. It was shown that the number of translocations between the second and the third is about the same as the sum of the frequency between X- and the second plus the frequency between the X- and the third.

Therefore, the number, 11, is doubled to yield 22 translocations between X-, the second and the third chromosomes among 1000 cultures for 1000 r for X-rays.

The expected numbers of deletions have been calculated on the data that an X-ray dose of 14000 r produces 1% deletions of the types detectable in this study (Bishop 1938; Pontecorvo 1940).

4000 r produces 10 deletions in 1000 sperms
1000 r produces $10 \sqrt{\left(\frac{1}{2}\right)^3} = 10 \times \frac{1}{2} = 1.25$ deletions in 1000 sperms.
Thus, we may take an expectation of 1.25 deletions among 1000 individuals for 1000 r as a starting point for further calculations.

For comparison with mustard gas experiments 1000 r are assumed to be equivalent to 3% sex-linked recessive lethals.

Two doses have been compared; the high dose which comprises the pooled data from HNI and HNIIB, and the medium dose which comprises those of HNIIA and HNII. The frequency of translocations and deletions for the low dose was so small and, consequently, had such a large error attached, that these data have not been included in the present comparison.

In the high dose, where the percentage of sex-linked recessive lethals was 11.5%, 31 translocations between X-, second and third chromosomes among 1658 fertile cultures tested, and 9 deletions among 2659 individuals were examined.

In the medium dose, where 7.14% sex-linked recessive lethals were obtained; 27 translocations between the three major chromosomes among 3858 cultures tested, and 6 deletions among 7469 individuals examined, were found.

For the high dose, the expected number of translocations is 271/4, while the observed one is 31 among 1658 fertile cultures tested; a ratio of 9:1.

For the medium dose, 328 translocations were expected, while the observed number was 27; a ratio of 12:1.

For the total of 5516 cultures, the expected number
of translocations is 602, and the observed one is 58. The ratio of expected to observed is \textit{10:1}.

In the case of deletions, the expected number for the high dose is 25, while the observed one is 9 among 2659 individuals examined, with a ratio of \textit{3:1} for the high dose. For the medium dose it is 36 deletions expected and 6 observed among 7469 individuals examined, with a ratio of \textit{6:1}. By adding them together, the expected number of deletions is 61 and the observed one is 15 among 10128 individuals, and the ratio is \textit{4:1}.

Cytological Results.

1. Frequency of Large Rearrangements.

Table 8 gives a summary of the frequencies of the various classes of chromosomal rearrangements observed. Inversions and translocations with both breaks within the heterochromatic regions could not be observed. Also, translocations involving entire arms of the chromosomes could not be observed. Only the following structural changes were observed as shown in Table 8.

1. Reciprocal Translocations; in which two chromosomes had exchanged segments. In Table 8 they are written as \textit{X-2R, 2R-3R}, etc.

2. Pericentric Inversions; the two breaks were on the opposite sides of the centromere. These are written in Table 8 as \textit{2L-2R}.

3. Paracentric Inversions; both breaks in the same arm of the chromosome. These are
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Individuals Analysed</th>
<th>Translocations</th>
<th>Pericentric Inversions</th>
<th>Paracentric Inversions</th>
<th>Reversed Repeats</th>
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<tr>
<td>HNI</td>
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<td>176</td>
<td>2R - IV</td>
<td></td>
<td></td>
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</tr>
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<td>2L - 3R</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>HNIIA + HNIII</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>581</td>
<td>X - 2R</td>
<td>2L - 2R</td>
<td>X</td>
<td></td>
</tr>
<tr>
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<td></td>
<td>X - 3L</td>
<td>2L - 2R</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2L - 3R</td>
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<td>3R</td>
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<td>Total</td>
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<td>2</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>
referred to in Table 8 as X, 2R, 3R, etc.

4. Reversed Repeats; probably resulted from chromatid breaks. These are referred to in Table 8 as X.

The frequencies of the different types of rearrangement observed cytologically in the different experiments are shown in Table 9.

The high dose comprises all the preparations of HNI, while the medium dose shows the results obtained from the pooling together of HNIIia and HNIII. All the preparations were made from the first brood.

In the high dose, where the percentage of sex-linked recessive lethals obtained was 12.4%, five translocations and two paracentric inversions were observed among 176 female individuals analysed. The frequency of translocations is 2.8% and for the total it is 3.98%.

In the medium dose, where 7.4% sex-linked recessive lethals were obtained; three translocations, two pericentric inversions, three paracentric inversions and one reversed repeat were observed in a total of 581 female individuals analysed. The percentage for translocations is 0.5 and for the total 1.5.

The data are insufficient for carrying out a statistical analysis to test the relationship between the frequency of gross chromosomal rearrangements and the dose measured by lethals. But one can see that there is an
TABLE 9.

Cytological Types and Numbers of Gross Chromosomal Rearrangements Compared with Percentage of Sex-Linked Recessive Lethals.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Experiment</th>
<th>% Sex-Linked Recessive Lethals</th>
<th>No. of Individuals Examined</th>
<th>Inter-chromosomal Rearrangements</th>
<th>Intra-chromosomal Rearrangements</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Translocations</td>
<td>Inversions</td>
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</tr>
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<td></td>
<td></td>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
</tr>
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<td>HNI</td>
<td>12.4</td>
<td>176 99</td>
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<tr>
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<td>HNIIa</td>
<td>7.4</td>
<td>581 99</td>
<td>3</td>
<td>0.5</td>
<td>2</td>
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<tr>
<td></td>
<td>HNIII</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

HNIIa; first brood only
increase in the total number of chromosomal changes (given in the two last columns of the Table) as the dose increases ($x^2 = 3.9$ for 1 degree of freedom). It is not significant, but for translocations alone the increase, with increasing dose, is significant ($x^2 = 6.9$ for 1 degree of freedom).

Adding together the reciprocal translocations and pericentric inversions, there are 10 inter-chromosome-arm changes (8 translocations and 2 pericentric inversions) as against 6 intra-chromosome-arm rearrangements (5 paracentric inversions and 1 reversed repeat), a ratio of 1.7:1.

2. Distribution of Breaks among the Chromosomes.

Table 10 shows the distribution of breaks along the chromosomes in the different experiments. They are distributed as follows: 11 on X-, 5 on 2L, 8 on 2R, 2 on 3L, 6 on 3R and 1 on chromosome IV. The number of breaks is not large enough for a statistical analysis to show whether the breaks are randomly distributed or not. However, there seems to be some sort of clustering on the X-chromosome in the region from segment 5 to segment 10, and also on the distal end of 2R.

Incidental Observations.

Some incidental observations were made in the course of the genetical and cytological investigations. These will be briefly reported.
### TABLE 10.

**Distribution of Breaks on the Chromosomes.**

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<tr>
<th></th>
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</table>
Genetical Observations.

Gonosomal Mosaicism.

In the course of the study of the frequency of translocations, some F2 cultures were observed which were maleless. There were 21 maleless cultures among 7032 examined, and they are distributed among the different experiments as shown in Table 11. This is unexpected inasmuch as the males of the F1 and F2 cultures carry the same treated X chromosome. A lethal, present on the X, should be expected to act in both generations.

In all these maleless cultures, the females showed a regular 1:1:1:1 segregation of dp and e.

Cytological Observations.

1. Inversion Mosaic.

In the course of studying salivary glands of the F1 larvae, a very remarkable aberrant set of glands was observed. Instead of having the customary single type of tissue, either completely normal, or having all cells with the same aberration, this set of glands was mosaic for two inversions, one on the X-chromosome and the other on the 3R chromosome. The most striking thing was that the two inversions always appeared together in the same nucleus, or were both absent. No nucleus containing one, but not the other was observed. Thirty-eight nuclei were analysed in which the two inversions together were found in 17 of them, while the rest of the nuclei were normal.
### TABLE 11.

The Frequency of Maleless Cultures Within the Individual Experiments.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of Fertile Cultures</th>
<th>No. of Maleless Cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNI</td>
<td>519</td>
<td>2</td>
</tr>
<tr>
<td>HNII</td>
<td>1361</td>
<td>6</td>
</tr>
<tr>
<td>HNIII</td>
<td>2497</td>
<td>7</td>
</tr>
<tr>
<td>HNIV</td>
<td>2655</td>
<td>6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>7032</strong></td>
<td><strong>21</strong></td>
</tr>
</tbody>
</table>
It is most unlikely that this result is due to contamination. This follows from the method of preparation in which the two glands of only one individual were used for each slide. Also, as would be expected, both glands were of the same sex.

2. Small Deficiencies.

No serious attempt was made to observe small deficiencies. Two minute deficiencies were observed incidentally, one of them near the tip of X-chromosome, and the other in 3L chromosome. The latter is shown in Figure 8.

3. Reversed Repeat.

One reversed repeat was observed in the X-chromosome including the region 7 to 8 as shown in Figure 9.
Fig. 8. A minute deficiency in 3L-chromosome.

A translocation between the tip of 2R and the 4th chromosomes.
Fig. 9. Reversed repeat. Inversion in the X-chromosome.
DISCUSSION

The evidence from the data presented here clearly shows that the frequency of gross rearrangements (translocations) increases as the square of the dose as measured by sex-linked recessive lethals. This value is to be expected if the individual mutagenic events produce single chromosome breaks which are then followed by union of the broken ends. Thus, every chromosome rearrangement may be viewed as a secondary event following upon two primary events, two independent chromosome breaks.

After X-radiation, the frequency of rearrangements was found to vary as the $3/2$ power of the dose rather than the expected square. As has been discussed in the introduction, this deviation from the square is due to a saturation effect plus a disproportionately increased chance of inviable products among the multiple combinations. But at lower doses, when the source of both saturation effect and differential viability is removed, all rearrangements become of the simplest type known as double combinations. Therefore, the frequency of viable structural changes must rapidly approach the proportionality of the power 2 of the dose. This is, then, the relation to be expected at low doses if all gross structural changes arise by combination occurring subsequently to breakage, between broken ends derived from independently produced breaks.

Although in the present experiments the doses are
high enough for the induction of considerable percentages of sex-linked recessive lethals, the frequency of large rearrangements is very low when compared with those induced by X-rays having the same frequency of sex-linked recessive lethals. For example, in the present study, 1.16% translocations between the second and the third chromosomes were produced in the experiment which gives 12.4% sex-linked recessive lethals. This equals about 14000 r if one assumes that every 1000 r produces 3% sex-linked recessive lethals. But Muller (1940) found that 4000 r produced about 8.3% translocations between the second and the third chromosomes, while 1000 r produced 1.16% translocations. Thus, it seems reasonable to compare the results obtained here with the lower doses of X-rays, where one should expect the relationship between the frequency of large rearrangements and the dose to be nearer to the square of the dose. In agreement with this, the data, although they do not exclude an exponent of 3/2 for increase of translocation frequency with dose, are fitted much better by an exponent of 2.

Mehtab (1953) in her cytological study of mustard-gas-induced rearrangements suggested a slow increase of rearrangements with increasing dose. This was disproved in the genetical studies herein reported and, although the cytological data are insufficient for accurate quantitative evaluation, there is an increase in the total number of large rearrangements with increasing dose. For
translocations alone, the increase is significant at the 5% level.

A comparison of the mutagenic effects of X-rays and mustard gas shows that they differ from each other most significantly in the proportion of large rearrangements to sex-linked recessive lethals. For any given dose, X-radiation produces a higher frequency of large rearrangements relative to recessive sex-linked lethals than is the case with mustard gas. Since the present data confirm the relative shortage of large rearrangements, one would expect that mustard gas produces either fewer breaks than X-rays or fewer new rearrangements from the same number of breaks. A solution to this problem was attempted by a study of dominant lethals. Those breaks which are produced by radiation, or mustard gas, and which do not restitute or participate in the formation of rearrangements, undergo fusion of the sister chromatid ends. This results in the establishment of a breakage-fusion-bridge cycle and the ultimate death of the cells possessing such chromosomal disturbances. This type of dominant lethal (dicentric) probably occurs with considerable frequency at lower doses, whereas at higher doses saturation enters and causes a downward curvature.

When the frequency of sex-linked recessive lethals is plotted against the relative dominant lethality the data is found to fit best a straight line, which means a linear proportionality between the frequency of single breaks
induced by mustard gas and the dose as measured by sex-linked recessive lethals. Since dominant lethals may be equated to single break events, they may be used for comparing the frequency of breakage as opposed to rearrangements, after X-rays and mustard gas.

From the regression line, drawn through the mean of the data, one can read off the relative dominant lethality for any amount of sex-linked recessive lethals. Fano & Demerec (1941), Demerec & Fano (1944) have shown that the percentage of dominant lethals produced by 1000 r is about 15-20%. The mustard gas data, presented here, show a similar relationship. Three percent sex-linked recessive lethals are equivalent to 15-16% relative dominant lethality and, as has already been pointed out, a mustard gas dose producing 3% sex-linked recessive lethals is equivalent to 1000 r-units of X-radiation.

A more accurate comparison is not possible, because the estimate of dominant lethality has many sources of error. Demerec & Fano (1944) attributed the variability between experiments to semi-sterility and other non-genetic or genetic factors. Bhattacharya (1949) observed in his experiments with ethylene glycol that, although treated sperms may be present with the normal density and motility, some of them fail to penetrate the egg or activate development. For chemical substances in general, Wallace (1951) assumed that changes in several components of the sperm may act as sources of dominant lethals and not
all of these changes need be genetic in nature. The same considerations apply in the case of mustard gas.

On the basis of these considerations the main possible mechanisms underlying the shortage of rearrangements following treatment with mustard gas will be discussed. If the shortage of rearrangements is due to a shortage of induced breaks one would expect also a low frequency of dominant lethals. On the other hand, if the induced breaks are as frequent as in the case of X-rays, the shortage of rearrangements may be explained in two ways: (1) a high frequency of restitutions, in which case one would expect a relatively low frequency of dominant lethals; (2) a secondary effect interfering with restitution and reunion or reunion only, in which case there will be no shortage of dominant lethals but of rearrangements. Since it has been found in the present study that mustard gas produces at least as many dominant lethals as X-rays, but fewer rearrangements, it may be concluded that the shortage of large rearrangements is due not to an initially low frequency of breaks, but to some sort of mechanism interfering with reunions. Since it had been shown that mustard gas has a delayed action (Auerbach, 1947, 1950), this delay may be considered as the mechanism. For, as a result of delayed action it may be unlikely that two or more breaks needed for a rearrangement are open at the same time.

Wallace (1951) concluded, in his discussion of this question, that nitrogen mustard was as effective in the production of
breaks as was X-rays.

It seems possible to say that mustard gas produces fewer breaks than X-rays and few of them undergo restitution or participate with other breaks in a rearrangement leaving the majority to undergo fusion of the sister-chromatid broken ends resulting in dominant lethals. These "lost" rearrangements augment the number of dominant lethals, thus bringing about as high a frequency of dominant lethals as would be induced by a more efficient break-producing mechanism of X-radiation. Finally, it also may be possible that mustard gas produces even more breaks than X-rays, but that more undergo restitution and few participate in rearrangements, leaving the rest to act as dominant lethals. However, this would seem to be the least likely.

The shortage of rearrangements after mustard gas brings it in line with the data on ultra-violet radiation. Mustard gas is less efficient than X-rays and more than ultra-violet radiation in the production of large rearrangements. Fabergé (1939) reported that ultra-violet radiation produces chromosome breaks at a very high frequency in Zea, but a high proportion of these breaks are restored and so remain undetected unless special means are used. Fabergé & Mohler (1952) showed that ultra-violet radiation produces numerous chromosome breaks in Drosophila, followed by restitution. It was found by Swanson (1940) for plants, Kaufmann & Hollaender (1946) for Drosophila, that ultra-violet treatment not only produces few breaks but also
prevents X-ray-induced breaks from becoming manifest. It may well be that the low frequency of mustard gas rearrangements is due to some secondary effect on the opening-up of latent breaks, upon their restitution or reunion into new combinations.

As regards the relative frequencies of inter- and intra-chromosomal rearrangements induced by mustard gas, it is found that both frequencies are significantly smaller than those induced by equivalent doses of X-rays. The divergence is not so marked for deletions as for translocations. These results are in agreement with those obtained by Auerbach & Robson (1947); Auerbach (1950) with mustard gas, and by Bird & Fahmy (1953) with diepoxybutane. The fact that the shortage is different for inter- and intra-chromosomal rearrangements supports the idea that there is some mechanism interfering with the formation of new rearrangements. As Table 7 shows, the ratio between expectation and observation for both types of chromosomal rearrangements becomes less at higher doses. This is due to the fact that the expected frequencies were calculated on the assumption that the numbers of translocations and deletions vary as the $3/2$ power of the X-ray dose, while it is proved in the present study that for mustard gas these frequencies vary as the square of the dose as measured by lethals.

The cytological data provide additional information concerning the relative frequencies of inter- and intra-chromosomal rearrangements. For X-rays, the observed ratio
between inter- and intra-chromosomal rearrangements is 2:1 instead of the expected ratio of 1:1 (Catcheside 1938; Bauer, Demerec & Kaufmann, 1938; Bauer, 1939). These values were calculated on the assumption that the four arms of the second and the third chromosomes and also the X-chromosome are counted as separate chromosomes. In the case of mustard gas, 10 inter-chromosome-arms (8 of them translocations and 2 pericentric inversions) against 6 intra-chromosome-arm rearrangements (5 paracentric inversions and 1 reversed repeat) were observed, with a ratio of 1.7:1. This ratio approaches the observed ratio of 2:1 for X-rays.

An unexpected finding was the increase in brood b of the HHII experiment. It was thought safe to use flies from a second brood because of Auerbach's (1950) earlier findings. In her experiments she found that the mutation rate is about doubled in the third brood produced between the 6th and the 9th day after treatment, while in the second brood, produced between the 3rd and the 6th day, the rate of mutation rises slightly from the first brood. This increase means that there is a difference in susceptibility of the various stages of spermatogenesis to the mutagenic action of mustard gas (Auerbach, 1950). In the present study the increase occurred in the second brood, which was earlier than usually obtained by Auerbach. This
may reflect a difference in the age of the males at time of treatment, or a difference in the breeding rate. It is worth noting that the increased sensitivity, at least in this one experiment, is parallel for sex-linked recessive lethals and rearrangements. This point, if confirmed, would be of some interest. Kaufmann, Gay & Rothberg (1949) found an increase of translocations in late broods, but increase in both lethals and translocations together had not been published before.

From the genetical data, the distribution of translocations on the three major chromosomes seems to be uniform. The frequency of translocations between X- and the second, and between X- and the third is about the same, while that between the second and the third is approximately equal to the sum of the two.

Whilst there are many studies on plants concerning the distribution of chemically-induced breaks along the chromosomes, there are very few on Drosophila and those that do exist were done on selected samples, usually among chemically-induced sex-linked recessive lethals (Slizynska & Slizynski, 1947, with mustard gas, and Bird & Fahmy (1953) with diepoxynbutane.) The present study, although small, is an unselected one, taking into account all breaks induced by the mustard gas treatment. It seems, from the data derived, that there is a clustering of breaks on the X-chromosome between the segments 5 to 10, and also near the distal end of 2R. More data would be needed to confirm this.
It is not surprising that cases of mosaicism were observed in the present study with both genetical and cytological methods. Twenty-one completely maleless cultures were found among about seven thousand F2 cultures tested, while after X-rays Sidky (1940) found only two such cases among about twelve thousand individuals tested. It seems that the frequency of this type of abnormal F2 is much higher after mustard gas than after X-rays. Also, Auerbach & Robson (1947) reported the occurrence of maleless F2 cultures after mustard gas treatment. Sidky (1940) concluded that such cases could arise in one of the following ways:

1. As a mutation, either dominant or recessive, on the original material of the X-chromosome.
2. As an autosomal dominant mutation, followed by translocation of the piece carrying the mutation, to the X-chromosome.
3. As a bobbed lethal mutation on the X-chromosome.

Since the females obtained in all cultures in this study were \( \hat{y}vf; \) and \( \frac{1}{2}dp; \frac{1}{2}e; \frac{2}{3}dp\ e; \) and \( \frac{1}{2} \) neither dp nor e, it follows that assortment was completely at random. This excludes the second possibility, and the third would require that the mother had no Y-chromosome or had a Y-chromosome not containing the normal allelomorph of bobbed. It seems most probable that the first possibility provides the explanation for this phenomenon. Since the females were \( \hat{X}XY; \) it is impossible to ascertain whether the lethal was dominant or
recessive. Since the F₁ males were normal and fertile, the lethal must have been present in each cell of the gonads, and the soma, in part at least, have been free from the lethal gene, or the males would not have survived. This type of mosaicism was called gonosomal mosaicism (Sidky, 1910). As mustard gas is known to produce an after-effect (Auerbach, 1916) the lethal may have arisen as a result of an after-effect of the mustard gas treatment during the ontogenesis of the egg fertilised by the treated sperm. The gonads, thereafter, developed from the derivatives of the cell in which the lethal arose. It is also possible that this lethal mutation occurred spontaneously in the Fi heterozygous male at an early stage of embryonic development, but this seems hardly likely because of the low frequency of spontaneous mutations.

A reversed repeat was observed on the X-chromosome, in the salivary gland chromosomes of a larva whose father's sperm had been treated with mustard gas. Mehtab (1951, 1953) found two reversed repeats in her cytological study with mustard gas. These two and the one obtained in the present study comprise a total of 3 reverse repeats among 28 rearrangements induced by mustard gas. This is a higher frequency than found for X-rays. Kaufmann & Bate (1938); Kaufmann (1939) found a few reversed repeats in the salivary gland chromosomes of Drosophila melanogaster whose father was treated with X-rays. The duplicated
sections had paired with the strand of maternal origin. This is due to the exact homology of the repeated sections and those in the maternal strand. These repeats may be formed as a result of either chromatid or chromosome breaks in the sperm. In the first case, a minimum of three breaks is necessary, two in one chromatid and one in the other, with one break in each at identical points. As a result, the section from the chromatid with two breaks may be inserted into the sister chromatid, giving one strand with a duplicated section in reverse and another strand deficient for that section. Kaufmann & Bate (1938) have suggested that the latter chromosome, as a result of the deficiency, may form a non-viable nucleus and may therefore be lost. Demerec, Kaufmann & Sutton (1939) described a case in which some of the nuclei of a pair of glands showed a duplicated section in reverse, while other nuclei of the same pair of glands were deficient for that section. It is also possible that the deficiency is present in other parts of the larva, but cannot be observed as only salivary gland chromosomes are examined. If the breaks may be induced in the unsplit chromosome, a reversed repeat may be formed after fertilisation, at the time of splitting of the chromosomes. One daughter cell may get the chromosome with the duplicated section, and the other the deficient chromosome.

A very remarkable aberrant set of glands was
observed in the salivary gland chromosomes of an F1 larva whose father was treated with mustard gas. This set of glands was mosaic for two inversions. The most striking feature was that the two inversions either appeared together, or were both absent. No nucleus containing one and not the other was observed among all the nuclei examined. Previous cases of mosaics for single rearrangements have been reported. Helfer (1940) found two sets of glands mosaic for translocations, and also another for an inversions, in the salivary glands of the F1 larvae whose normal fathers of Drosophila pseudoobscura were treated with X-rays, and he suggested the following mechanisms:

(1) That two sperms fertilised a single egg and that each sperm had one chromosome aberration in it, resulting in a mosaic individual. Such an individual might have two different types of salivary gland tissue.

(2) That the treatment had no effect on the sperm, but that during the course of development these aberrant tissues arose spontaneously. Since spontaneous chromosomal changes are relatively very rare, it is most unlikely that so rare an event has taken place twice during the development of a single individual.

(3) That one inversion took place due to the treatment, and the other occurred spontaneously after fertilisation in the modified tissue.

(4) That the breakage of the chromosomes due to the
treatment need not occur at the time of treatment, but may be delayed for one or more cell generation.

The fourth mechanism seems to be correct for the explanation of such an aberration. It may be assumed that the action of mustard gas has weakened or actually broken the chromosomes at $4$ different points and that a sperm with these potential breaks has fertilised a normal egg. During the process of chromosome duplication in the first two cleavage divisions, the weaknesses or potential breaks in the chromosomes may have persisted. It is possible that in one of the resulting cells the broken ends have become reunited to restore the original gene rearrangement, thus giving rise to cells with normal chromosomes. In the case of the other cell, however, restitution has not occurred and rearrangements are, thus, produced. It is not known in this case whether one gland was entirely normal and the other gland contained the inversions, as in the process of smearing the two glands were mixed. The process as outlined in this paragraph would result in such a situation. However, if both glands were mosaic the delay in expression of the breaks extended over a longer period of time and is, therefore, more remarkable.

Since it is a known fact that mustard gas has a delayed effect (Auerbach 1950), it seems likely that this mosaic for the two inversions can be explained according to the fourth suggestion.
SUMMARY

1. The percentages of sex-linked recessive lethals, dominant lethals, translocations and deletions were studied at various doses by genetical methods within a stock which previously had been freed from large chromosome rearrangements.

2. When the percentage of mustard-gas-induced translocations is plotted against the percentage of sex-linked recessive lethals produced by the same treatment, a second-degree curve is obtained. This is taken to show that the primary effect of the treatment consists of independently produced chromosome breaks which subsequently reunite to form rearrangements.

3. The frequency of dominant lethals increases in approximately linear proportion to that of recessive lethals, indicating that dominant lethals are mainly due to single breaks.

4. If a comparison is made between doses of X-rays and mustard gas which produce similar frequencies of recessive lethals, it is found that after mustard gas treatment the frequency of dominant lethals is comparable to that after irradiation, whilst that of large rearrangements is considerably lower. This shortage was more pronounced for translocations than for deletions. The possible mechanisms behind the problem of breaks has been discussed.
5. There was a marked increase in the frequencies of both sex-linked recessive lethals and translocations in the second brood as compared with the first brood, indicating a higher sensitivity of the younger stages of spermatogenesis to the mutagenic action of mustard gas.

6. The genetically obtained translocations were distributed uniformly on the three major chromosomes.

7. Twenty-one cases of gonosomal mosaicism for a sex-linked lethal were obtained. This type of mosaicism appears to be more frequent after mustard gas treatment than after X-ray treatment, and possibly is due to the delayed action of mustard gas.

8. In three experiments, samples of salivary glands from F\textsubscript{1} 99 were studied cytologically. The shortage of structural changes as compared with X-rays is apparent.

9. Because of this shortage, the data could not be used for a quantitative study of relationship between dose and effect. But with increasing dose there was an increase in the total number of rearrangements. For translocations, alone, the increase was significant at 5\% level.

10. The frequencies of inter-chromosome-arm changes and intra-chromosome-arm changes approached the ratio of 2:1 obtained with X-rays.
11. The distribution of breaks observed suggests a clustering on the X-chromosome between the segments 5 to 10, and another near the distal end of 2R.

12. Cytological observations of special interest were a reversed repeat on the X-chromosome, and a set of glands which was mosaic for two inversions, which were absent or present together in a given nucleus. Both these cases must be chromatid effects and may be due to delayed action.
ACKNOWLEDGEMENTS

I am very grateful to Professor C.H. Waddington, F.R.S.,
Director of the Institute of Animal Genetics, Edinburgh
University, for providing facilities to carry out this work
in his Department.

I also wish to thank Dr. C. Auerbach for suggesting
the problem and for her interest and encouragement during the
course of this study. My thanks are due to Dr. H. Slizynska
for her helpful instructions in the cytological methods;
and to Dr. E.C.R. Reeve for suggesting the statistical methods.

Finally, I wish to express my gratitude to the
Egyptian Government for financial support.
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