THE FREQUENCIES OF

DOUBLE- AND MULTIPLE-BREAK TRANSLOCATIONS

IN RELATION TO RADIATION DOSE,

AND OTHER GENETICAL STUDIES.

ABD EL RAZZAK SIDKY,

B.Sc. (Edin.), M.D.A. (Cairo).

(Institute of Animal Genetics, University of Edinburgh,
and the Ministry of Education, Cairo.)

Presented to the University of Edinburgh as a
Thesis for the Degree of Ph.D. May, 1939.

TABLE OF CONTENTS.

THE FREQUENCIES OF

DOUBLE- AND MULTIPLE-BREAK TRANSLOCATIONS

IN RELATION TO RADIATION DOSE.

A. R. Sidky.

TABLE OF CONTENTS.

THE FREQUENCIES OF DOUBLE- AND MULTIPLE-BREAK TRANSLOCATIONS IN RELATION TO RADIATION DOSE.

A. R. Sidky.

Introduction.

The Relation of Dosage of Radiation to the Frequency of Gene Mutations:

It was quite natural that one of the first points to be attacked in the study of the genetic effects of radiation was the relation between the dosage given and the effect produced (Muller 1927).

In 1928-29, Oliver, in the Texas laboratory, carried out experiments on Drosophila to find the relation between the dosage of X-rays and the frequencies of mutations produced. The results of these experiments were published in 1930 and 1932. They showed that the increase in the mutation rate was proportional to the increase in the dose given. Similar results in Drosophila/

Drosophila were obtained with X-rays and with radium by Hanson, Heys, and Stanton (1929, 1931, 1932), Serebrovsky (1930), Timofeeff-Ressovsky (1931 et seq.), Patterson (1931), and others. Stadler (1930 et seq.) found that this principle holds in plants.

Gene Rearrangements:

In addition to the gene mutations, Muller in his early experiments (1927) also obtained rearrangements in the linear order of the genes. They were proved to involve inversions, deficiencies, translocations, etcetera.

The first proved case of a rearrangement of genes was that of a translocation. This appeared as an isolated case, which was encountered in 1918 by Bridges (1923). It was demonstrated genetically, but the portion involved was too small to be demonstrated cytologically by the methods available at the time. It was not until 1926 that a second case which might be considered as a translocation was discovered by Stern (1926, 1927) and was/

was demonstrated both genetically and cytologically; this case, however, is now thought not to be a typical translocation, as it seems to involve a crossing-over of homologous parts of the inert regions of the X- and Y-chromosomes.

Typically, a translocation is the result of the disconnection of a section of a chromosome and its attachment to another, non-homologous chromosome. At one time it was believed that a translocation could result from a chromosome breaking at one point and the detached fragment becoming attached to the end or the side of another, non-homologous chromosome. Then Muller and Altenburg in 1930, reporting on their experiments concerning the frequency of translocations induced by X-rays, suggested "as a reason for the greater tendency for attachment to a long autosome it may be that a breakage occurring in one chromosome favours the attachment to it of a fragment from another chromosome and th at breakage is more likely to occur in a longer chromosome." They still thought, though, that the "breakage of a recipient chromosome is however not necessary for the occurrence of/

of attachment."

Analysis, genetical and cytological, of a number of spontaneous and induced translocations was carried on by many investigators. It confirmed the supposition of the breakage of both chromosomes involved. It was found that translocations are mostly mutual exchanges; when they are not, they at least involve mutual breakage, and the broken piece of the one chromosome in this case becomes inserted into a gap made by the breakage of the other chromosome.

Thus in the Genetics Congress of 1932 both Muller and Stadler put forward the principle of exchange as a general interpretation of structural changes in chromosomes. This was still followed by stronger and more definite confirmation, as the study of the "inert regions" explained the cases that were thought to be exceptions.

So we see that a minimum of two breaks - one in each of the hon-homologous chromosomes - is necessary before a translocation involving these two chromosomes can take place. Attachments take place only/

only at the broken ends. No broken end is left naked if the chromosome is to survive.

The same rule of double breaks was found to apply both in inversions and deletions. An inversion or a deletion takes place in one chromosome, while a translocation involves a minimum of two chromosomes. Nearly every case of inversion or deletion could be shown to involve two breaks (in the same chromosome) and reunion at the breakage points. The very few apparent exceptions were no more than the expected number of cases in which one break was too near the end of the chromosome to be detectable. In inversions the detached chromosomal fragment lying between the two breaks may be considered as rotating through 180° and so the arrangement of the genes is changed. In deletions the detached acentric section of the chromosome between the two breaks is lost.

A translocation involving two chromosomes may be one of the following types:-

(1) Mutual: Two breaks, one in each of the non-homologous chromosomes and an exchange of the detached fragments.

(2)/

(2) Deletion-Insertion (also called Insertional):

Three breaks, two in the donor chromosome and one in the recipient. The detached interstitial fragment gets inserted between the two breakage ends of the latter chromosome, and the two end pieces of the first chromosome join together.

(3) Mutual Deletion-Insertion (also called Mutual Insertional): Four breaks, two in each of the non-homologous chromosomes and an exchange of the detached interstitial fragments by insertion.

More multiple breakage would of course give more complex possibilities.

These types are illustrated in Diagrams NOS. 4 and 5. We took as examples translocations involving only two chromosomes for the sake of simplicity. The same principles apply to translocations involving more than two chromosomes.

Translocations have proved and promise still to be of great importance in genetical studies. They supplied new and convincing evidence for the theory/

theory of linear arrangement of the genes. They also proved to give a more adequate means of determining the relative spacings of the genes than the crossing-over frequency method. The latter has been successful in giving us the correct sequence of the genes but not the relative distances. Translocations might also prove important in the study of the mechanism of evolution. Many species or other natural groups differ from each other in the shapes and sizes of their chromosomes and it is probable that many of these differences have arisen through translocation in the evolutionary history of these groups. Strong evidence for this was found in studies of the chromosome structure of organisms of the same species collected from different geographical areas.

A clear knowledge of the mechanism of the production of gene mutations and of gene rearrangements by radiation might be of considerable importance in genetics. It would naturally give an approach to the understanding of the action of X-rays and other rays in the production of these chromosomal changes and their possible production by radiation in nature, but/

but it might also aid in an understanding of the properties of the hereditary material itself. Moreover, it would be easier to determine the relation between the different types of genic modifications and the role that each plays in evolution. In brief, it is to throw light on the mechanism of genic and chromosomal modifications that the study of the quantitative relation between the dosage of irradiation given and the frequency of induced chromosome and gene changes is carried out.

Hypotheses of the Mechanism of Gene Rearrangements:

The question of the relation between the dosage used and the rate of gene mutations produced can be taken as settled. It was found, as mentioned above, that a direct proportionality exists between the two. Attention has since been turned vigourously to attacking the problem of the relation between the dosage and the frequency of gene rearrangements. This problem, because of its having a more complicated nature/

nature than that concerning gene mutations, has aroused a good deal of controversy. It is in the hope of helping towards settling this question that the present work on the frequency of translocations produced in relation to dosages of irradiation, which will be detailed later, has been taken up.

From the finding of a direct proportionality between the radiation dosage and the gene mutation rate, it was concluded that each individual mutation is caused by an individual favourable ionization, that is to say a single cause or a single "hit" for each mutation. But, as explained above, a translocation - or any inversion or a deletion - requires a minimum of two breaks before it can take place. How does this situation compare with that of gene mutations?

At an early stage of attacking this problem two alternatives presented themselves according to Muller (Painter and Muller 1929). One of these presents an analogy to the crossing-over mechanism, namely the involved chromosomes - non-homologous in the case of translocations - come in contact with or in/

in close proximity to each other, in some way such as lying across each other, and both break at their point of contact or nearest point as a result (direct or indirect) of one common disturbance, e.g. one single ionization. This is essentially the same as the theory advanced by Serebrovsky (1929) and later elaborated by Dubinin (1930). The other alternative is that the breaks occur independently, as results of independent causes, i.e. independent favourable ionization. In both cases the breakage ends may reunite again where they parted, thus resulting in the reconstruction of the original chromosomes, or they may unite in new ways, giving rise to gene rearrangements.

The first of the two alternatives mentioned above is called the "Contact hypothesis" and the second is called the "Breakage first hypothesis". A combination of both of the above alternatives is also conceivable. Thus, gene rearrangements might be produced by one common ionization while others were produced by independent ones; this might in fact/

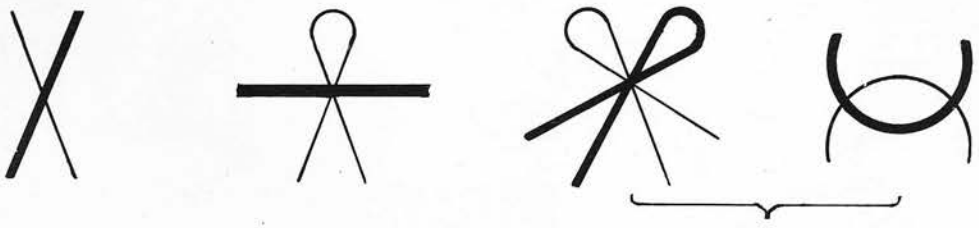
fact even apply to different breaks involved in the same case of multiple-break rearrangement.

If all the breaks involved in a given exchange were due to one common cause and this were a common ionization, we should expect the relation between the dosage and the frequency of gene rearrangements to be that of direct proportionality, as in the case of gene mutations. But if the breaks were due to independent causes, the coincidence of two breaks necessary to allow an exchange of the broken ends would vary in frequency nearly as the square of the dosage (until the breaks became so frequent that multiple breaks had to be taken into account), since the two causes have equal and independent chances. On the other hand, if both common cause and independent causes play a part, a relationship different from both the first and the square power - in fact an intermediate one - would be expected.

According to the contact hypothesis, for a mutual translocation the two chromosomes must be lying across each other or otherwise in contact, or at/

at least very near each other at the two points to be broken. For an insertional translocation, three points must nearly coincide, two on one chromosome and the third on the other. For a mutual insertional translocation, four points - two on each chromosome - must come nearly in contact. Simplified configurations are illustrated in the following diagram NO 1

Diagram NO. 1



A SIMPLIFIED DIAGRAMMATIC ILLUSTRATION OF THE CHROMOSOME CONFIGURATIONS REQUIRED AT THE TIME OF IRRADIATION ACCORDING TO THE "CONTACT HYPOTHESIS" IN THE CASE OF TRANSLOCATIONS INVOLVING ONLY TWO CHROMOSOMES.

It/

It would seem difficult to explain the occurrence of many cases of multiple exchange by the three- or four-way crosses in the manner mentioned above. The matter becomes more complicated still if we consider translocations involving more than four breakages, as have been found by Dubinin and Khvostova, Stone, etcetera.

Offermann has however suggested (Muller 1935) that the point of crossing of two strands might mechanically serve to catch a third strand within the notch so formed, thus making a three-strand contact; and by an extension of this idea - involving the concept of some external force twisting a whole group of strands into a kind of mutual crossing or "knot" at one point - Dubinin and Khvostova (1935) would explain their more complex multiple-break rearrangements.

But even if the strands to be broken come actually into contact or very near each other, the distance between the centres of the strands at these places would still be enough to make it more likely that/

that the points to break will be struck by independent electrons, unless they can be affected secondarily by intermediate events originated by the same electron and spreading out from that as a centre (on the "bomb" hypothesis, as Muller (1937) called it).

Previous Works./

Critical Review of Previous Works.

The works in which data were obtained with a view to throwing light on which of the proposed mechanisms actually obtains may now be briefly referred to.

It has already been mentioned that Oliver, in 1930-32, in his work on the frequency of gene mutations also recorded the cases of gene rearrangements - which he called chromosome abnormalities (C A) - detected by disturbances in the rate of crossing-over. The frequency of these chromosome abnormalities did not vary as the square of the dosage. They suggested a higher rate of variation of frequency than that of the dosage itself. In some cases, however, the apparent disturbance in crossing-over was thought to be due to the presence of two different lethals in one X-chromosome, for here cross-overs between the two lethals would be lethals too. Oliver concluded that there was probably a dirdct proportionality between the dosages of X-rays/

X-rays and the frequency of gene rearrangements. But it was evident that more investigations into the subject were called for.

As the results of the above-mentioned works did not clearly indicate an increase in the frequency of rearrangement with increase of dosage greater than that expected on a proportionality basis, Muller at first (1932) favoured the contact hypothesis as against the breakage hypothesis. Stadler (1932), on the other hand, though he did not positively exclude the contact hypothesis, supported the breakage hypothesis. He advocated the idea of the persisting of detached fragments, even through several cell generations, and later permitting delayed attachments, resulting in the occurrence of translocations, some after the treatment as the fragments come in contact with each other in the course of intracellular movement.

Then in 1933, Muller, Koerner and Maggie Vogt working in Berlin carried out experiments on the relation of the frequency of deletions to dosages of/

of X-rays. They found, as reported by Muller (1936, 1937, 1938 a, b), that deletions do not vary in frequency as the square of the dosage. Nevertheless the frequency was not in direct proportion to the dosage, i.e. to the first power of the dosage. They found that it varied about as the 1.5 power of the dosage - that is to say, more than the first power and less than the square power of the dosage. This led them to conclude that some at least of the rearrangements were probably caused by independent breaks.

Shapiro and Neuhaus (1933) observed that the frequency of translocations increases more rapidly than would be expected with a direct proportionality and less than expected with a proportionality to the square of the dose, but their data were statistically insufficient.

Then Belgovsky, studying the relationship between the frequency of translocations and the dosage in a large-scale experiment done under the direction of Muller (publicly reported at a meeting of/

of geneticists in Moscow, 1935, published in 1937), arrived at the same conclusions as Muller, Koerner, and Vogt. He found that the frequencies of translocations at two different dosages, one four times as great as the other, has shown a higher increase than the expected on the basis of a direct proportionality between frequency of induced rearrangements and dosage, as should be the case according to the contact hypothesis. The increase in the frequency was also lower than the square of the dosage, which was the relation which had been expected as a first approximation on the breakage hypothesis. As, according to the contact hypothesis, rearrangements might, alternatively, be expected to keep similar relation to dosage as the frequency of interchanges in ordinary crossing-over, he carried out experiments to investigate this point. He used four doses of X-rays, each one double the one directly lower. Comparing the curve for/

for frequency of ordinary crossing-overs with that for the frequency of rearrangements, he found that they differ significantly.

Using inversions, Berg, Panshin, and Borisoff in Leningrad (work of 1935, unpublished, quoted by Muller, 1938 a. b.) found a lack of proportionality between the frequency of these gene rearrangements and the dosage. They confirmed the 1.5 relationship referred to above.

Khvostova and Gavrilova (1935), working on the frequency of translocations involving the fourth chromosome in relation to the dosage, concluded that there is a linear relationship between the dosage and the chromosome interchanges produced. They used the recessive character cubitus interruptus which had been found by Dubinin and Sidorov (1934) to be manifested as a position effect by translocations involving the fourth chromosome. It must be borne in mind, however, that the detectability of the character cubitus interruptus depends greatly on temperature/

temperature conditions. Moreover, their results represent the summation of separate experiments in which the measurement of the dosage and the relative numbers of translocations at different, supposedly corresponding doses may have been subject to variation. Again, it must be noted that unless the flies to be given the lighter dose are divided into portions which are treated consecutively at the same time as those undergoing the heavier treatment, as will be explained in "Material and Method" of the present work, one cannot be reasonably sure of getting the desired relation of one dose to the other, owing to the variations which may occur in the dose during the treatment.

The same two authors - Khvostova and Gavrilova - in 1938 claimed to have obtained again direct proportionality between the dosage and the frequency of translocations, this time by means of the position effect of the gene for $Plum^{D}$ (the change of the brown eye colour into red and mottled) which was/

was discovered by Dubinin in 1936. Whereas, they reported, when they used the linkage method - the method used in the present work and which will be described later - they found a disproportionately high increase of aberration using doses of 4000 r and 6000 r units.

It might be mentioned here that recent work on the same lines (Muller and A. I. Makki, 1939, in progress) contradicts the above results, and, in fact, seems to suggest the 1.5 relationship found in the case of deletions in the work of Muller, Koerner, and Vogt referred to above.

Catcheside in 1938 reported on his experiments on the dosage-structural changes relation. He used the cytological method based on the study of the salivary gland chromosomes of F_1 Drosophila melanogaster female larvae derived from X-rayed fathers. Doses of 1000 r, 2000 r, and 3000 r were used. The scale of the experiment was not a large one. He interprets his results as showing that the frequency of/

of induced structural changes of chromosomes has a relation of direct linear proportionality with the X-ray dosage.

Karl Sax (1938), in cytological studies of the chromosome aberrations induced by X-rays in Tradescantia, has gone into the question of the relationship between these aberrations and the dosage. He found that this relationship was not a simple one. His data confirm the 1.5 power relationship. He found at the same time that the proportion of single breaks increases directly with the increase of the dosage. When he compared the curve for the chromosome aberrations induced at different doses with the theoretical curves based on the equations for one hit [$B = 1 - e^{-an}$] and for two hits [$B = 1 - e^{-an}(1 + an)$] (Wyckoff and Rivers/

B = chromosome aberrations.
a = the probability that an electron will hit the object.
n = the number of electrons shot at the object.
If two hits are necessary, the survival ratio is $e^{-an}(1 + an)$.

Rivers, 1930), he found that the observed values approach, but do not coincide with, the theoretical curve based on the assumption that two hits are necessary to break two chromosomes or two arms of one chromosome. But he offers as a possible explanation the suggestion that some of the dicentric and ring chromosomes may be produced by a single hit.

Hans Bauer, M. Demerec, and B. P. Kaufmann (1938) studied chromosomal rearrangements in Drosophila by salivary gland chromosome analysis of F_1 larvae obtained from untreated females mated to X-rayed males. Dosages between 1000 r and 5000 r units were used. They found that with the increase of the dosage the percentage of altered sperm increases more rapidly than would be expected with direct proportionality. The curve produced resembles an S-shaped curve, being steepest in the interval between 2000 r and 3000 r units.

Lastly, Buzzati-Traversa (Drosophila Information Service, January 1939), in a short note, reports/

reports finding direct proportionality between X-ray dosage and translocations between the second and the third chromosomes of Drosophila melanogaster. The scale on which the experiment was carried out is not mentioned, nor are details of the technique given: judging by the standard error given, however, his figures do not strongly support the direct proportionality relationship.

The works reviewed above are those bearing on the subject of the quantitative relationship between the dosage of irradiation and the frequency of gene rearrangements in general. Now there must be looked into some of the works dealing with the relative numbers of rearrangements involving different numbers and distributions of breaks at a given dose.

Dubinin and Khvostova in 1935 published a very interesting paper reporting on their cytological studies of the configurations of some complex rearrangements in the salivary gland chromosomes. Here/

Here they confirmed the conception of Muller (which they had not previously recognised) that reunion can only occur between breakage ends. They criticised, however, the conclusions of Muller and Belgovsky, based on the latters' work on the relationship between the frequency of translocations and of deletions and the dosage of X-rays (reported on by them at a public meeting at the Institute of Genetics, Academy of Sciences, Moscow, 1935, and published in 1937, reviewed above), maintaining on the contrary that these authors' findings of a proportionality of the frequency of rearrangements to a power of the dosage intermediate between one and two do not really support the breakage hypothesis, for Dubinin and Khvostova thought that, according to the breakage hypothesis, the exponent expressing the relation should have been the square. (This, however, we shall see later, is not necessarily the case, as the relation varies with the dose used.) They undertook to make a cytological study of translocations involving three/

three chromosomes, in the hope of obtaining distinctive structural findings which might explain the mechanism of their origin. Some of the translocations they examined proved to be of a very complex nature, which, according to the contact hypothesis, would require in certain cases as many as eight threads intersecting at the same point. This can be explained on the contact theory only on the special assumption, originally suggested by Offermann (Muller, 1935 a.), that there is a tendency for more threads to come together at a point where two are already together. Though they did not decide for one hypothesis against the other, they nevertheless favoured the contact hypothesis, adopting this explanation (which they described as the formation of a "knot") for the complex cases in question.

Kirssanov (1937) under the direction of Dubinin was the first to attempt a comparison of the frequency of translocations involving two chromosomes to those involving more than two chromosomes as calculated/

calculated both according to the contact and the breakage hypotheses. He then compared his calculated frequencies with those actually obtained by himself, by Muller and Altenburg, and by Stone, Patterson, Bedicheck, and Suche. (Only the data of the latter four authors are really of a large enough magnitude to give data of value for this purpose, however.)

Although he concluded that the results fitted the expectation on the contact hypothesis (of which he adopts a special form, based on the analogy of crossing-over) and not on the breakage hypothesis, a re-examination of the evidence (Muller, unpublished), based only on those classes large enough to be of value shows that they deviate more than he thought from the "contact" expectation, in the direction of the "breakage" expectation, and that they are in fact consistent with the latter.

Catcheside (1938), working on maize in an attempt to throw light on the mechanism of induction of rearrangements, X-rayed the pollen with 3000 r and applied/

applied it to untreated ears. Then he observed the number of F_1 showing rings of four or more chromosomes (indicating heterozygous translocations). He also compared his results with similar ones obtained by Stadler (1937) with a dose of 1333 r units. Pointing out that the dose used by himself was probably under 1000 r units, Catcheside concluded that there was a relation of direct proportionality between the dosage and the rate of induced structural changes. From his data, he also concluded that, while the absolute frequency of rings of six and of two rings of four chromosomes rises markedly with increased dosage, yet the ratio of their frequencies remains constantly near equality as it would on the contact theory. Moreover, individuals with two rings of four chromosomes were in fact slightly more frequent than those with one ring of six chromosomes, as they might well be on the contact theory, whereas on the breakage theory the latter should be more frequent, provided his assumption be correct that the reunions of broken ends/

ends occur at random. For these reasons he regards his data as favouring the contact hypothesis. The assumption last mentioned and certain others (such as neglecting the possibility of more than one break per chromosome occurring) are very questionable, however, especially in the light of recent work, and for this reason recalculations of the expectations on the breakage hypothesis, in which the effects of changes in these assumptions are taken into account, are at present being carried out in our laboratory.

Baur, Demerec and Kaufmann (1938) in their above-mentioned work on gene rearrangements studied among other things the question of the distribution of breaks among the chromosomes in cases of multiple breakage. They found different chromosome arms of comparable length to have much the same breakage frequency. Considering the spacings of two breaks within any given chromosome arm, they found that the values noted correspond, in general, with a random distribution of breaks within the euchromatic sections of/

of the chromosomes, with the possible exception of distal regions where the frequency of breaks may be slightly higher. In heterochromatic regions, on the other hand, breaks are more frequent than in euchromatic regions of similar salivary lengths (as much previous work had shown.)

On the basis of their studies of the manner of distribution of multiple breaks among the chromosomes, in different cases of gene rearrangements, they claimed that rearrangements, in which the breaks ~~on a random distribution~~ are concentrated in a smaller number of chromosome arms, are more frequent, in comparison with the cases in which the same number of breaks are scattered, than would be expected on a random distribution of breaks. Their calculations, however, fail to make allowances for the fact that the number of viable (monocentric) rearrangements obtainable is greater when the breaks are concentrated than otherwise. Nevertheless we shall see later that it is probably true that there is a tendency to "concentration of breaks"/

breaks" though this could not legitimately have been inferred from their evidence by their method of reasoning. As the cause of this supposed effect, they surmised that there is a tendency for the breaks themselves to occur oftener within the same chromosome. To us it appears more reasonable to infer that when the breaks happen to be closer together the proportion of viable gene rearrangements in comparison withthat of restitution is greater, i.e. that a proximity effect exists in the process of reunion, not in that of breakage.

Objectives/

Objectives of Present Experiments.

Answers to two main questions were aimed at in carrying out the experiments to be detailed presently:

Firstly, what is the quantitative relationship between the frequency of gene rearrangements induced and the dosage given? Does the frequency of gene rearrangements vary according to the first power of the dosage as might be expected on the basis of the contact hypothesis? Does it vary according to the square of the dosage as might have been expected as a first approximation on the basis of the breakage theory? - or is the relation ship different from both the first and the square power? If the latter, what is this new relationship and what does it imply? For this purpose two doses, one four times the other in order to give a marked difference, were used. As large a scale as the circumstances permitted was aimed at.

Secondly/

Secondly, what is the relation between the frequency of the double-break translocations and the frequency of the multiple-break translocations? Is this relation that to be expected on the basis of a random distribution of breaks and random union? For this purpose the ring-shaped chromosome supplies a good tool. As mentioned before, a minimum of two breaks, one in each of the non-homologous chromosomes is required before a translocation involving these two chromosomes can take place. This applies only to the non-ring chromosomes. In the case of a translocation involving a ring-shaped chromosome and a non-ring chromosome, there is a minimum of three breaks. The distribution of the breaks may be either one break in the ring and two breaks in the non-ring chromosome - in which case the detached piece of the latter gets inserted into the former - or two breaks in the ring and one in the non-ring, with the insertion occurring into the non-ring chromosome.

For/

For the ring, a stock was used in which the males had their X-chromosome ring-shaped, the ring chromosome being that denoted "Xc2", found by Beadle. All the translocations involving this ring-shaped-X-chromosome and an autosome will represent multiple-break translocations. If along with this we get the frequency of translocations involving an X non-ring-chromosome and an autosome, this figure will cover both multiple- and double-break translocation. By subtracting the former frequency from the latter, we have the frequency of double-break translocation, which can then be compared with that of multiple-break translocations.

Material and Method./

Material and Method.

For males having the ring X-chromosome, Beadle's stock was used in which the males have the so-called Xc^2 ring and the females have attached X's homozygous for yellow. For controls, males from the wild-type Oregon K stock were used. The irradiation was carried out with the Institute's X-ray apparatus, which has a water-cooled Coolidge tube 6 K.V. with rectified H.T. The flies were irradiated at a distance of 12 cm. from the X-ray target.

In the earlier series, where no dosimeter was used, the X-ray output had been approximately determined by previous tests in which the sex-linked lethal mutation rate was ascertained by the ClB method. The time of raying in our experiments was then adjusted so as to give the more heavily treated series as nearly 4000 r units as possible. There were, however, fluctuations of the dose, from experiment/

experiment to experiment, as may be seen from the table. Later, beginning with series 5, when a dosimeter became available, the heavy dose was held at 4000 r.

To achieve the purposes of the two previously mentioned problems, three classes of irradiated males were used. They are as follows:

1. Males with ring-chromosomes (Xc^2) were given the "heavy" dose (of as nearly as possible 4000 r units as just explained). These were called H-H.

2. Similar males were given the "light" dose, which was just one-quarter of the heavy dose; these were called L-H.

3. Oregon R. males were given the heavy dose simultaneously; these were called H-4.

Comparison of the translocation frequency involving the X-chromosomes in classes 1 and 3 allowed an attack on the first problem, as it disclos-

ed/

disclosed the frequency of multiple-break translocations as compared with that of double-break ones at the high dose. Comparison of the frequency of translocations involving only the autosomes in classes 1 ~~and 2~~ versus 2 allowed an attack on the second problem, which concerned the frequency of translocations resultant upon the two different doses. From this second result it was then possible to estimate the approximate number of X-chromosome translocations of the non-ring X at the lower dose as well - and thus to gain an idea of the frequency relation of multiple- to double-break translocations involving the X at the lower dose for the first problem.

In order to be reasonably sure that one dose was really four times the other, the flies receiving the lighter dose were divided into four lots and these lots were X-rayed consecutively at the same time as the flies receiving the heavier dose were being treated. The need for this method derives/

derives from the fact that, as the dosage per unit of time is liable to vary through the time of treatment, treating the flies which are to get the lighter dose apart from those which are to get the heavier dose, or treating them all in one particular relative portion of the time required for the heavy dose, would not permit their treatment to share the variation which occurs in the irradiation during the whole time for the heavy dose. This technique of subdividing the material for the lighter dose had been originated by Offerman.

In order to detect translocations, the treated males in each class were mated to $\frac{y}{y}$ bw e ey virgin females of the stock constructed for the purpose of such tests by Muller in 1931, i.e. the females having attached X-chromosomes homozygous for yellow, and their three pairs of autosomes homozygous for brown, ebony, and eyeless, respectively. These matings were done in mass cultures in bottles. For a reasonable output 50 males and 50 females per bottle were used for the heavy dose and 10 of each sex for the /

the light dose. The cultures were kept at 22.5° Centigrade. After four days the flies were transferred into fresh bottles, where they were left for another three days. Then the males were discarded and the females were again transferred into fresh bottles. Three broods were thus obtained, and the males were not left with the females for more than seven days. This technique was decided upon after considering the work on the duration of the effects of X-rays on male germ cells in Drosophila melanogaster done by B. B. Harris and by F. B. Hanson and F. Heys independently, the first showing only a small drop in the percentage of lethal mutations when the sperm cells were obtained from males eight days after irradiation, and the second showing no drop until after fourteen days.

The F_1 males, heterozygous for bw, e, and ey, and so phaenotypically wild-type (Diagrams 3 & 4) were backcrossed individually in small vials to virgin $\bar{y}\bar{y}$ bw e ey females. Their progeny, i.e. F_2 flies, were examined for translocations in the manner/

manner explained later in this paper. The food in these vials was made up according to the formula of Offermann and Schmidt (Drosophila Information Service, 1935), as this allowed a large enough output to make the recognition of a translocation certain in the great majority of cases.

For the results to be significant, the experiment had to be carried out on a large scale. For practical purposes, therefore, it was divided into consecutive "series", each series containing as nearly as possible the same proportions of H-R, L-R, and H-+. The total number of F_1 cultures aimed at for each batch was 2000. Of course, not all these cultures would give F_2, as some would be sterile. So this number was not always attained. Taking into consideration that the increase in dosage results in increase in number of translocations produced and also considering the results obtained by other workers on the relation between the dosage and the frequency of translocations, it was decided to/

to use the following formula for the desired number of F_1 cultures of each class in one series.

$$\underset{1760}{8 \times (L\text{-}R)} + \underset{220}{\times (H\text{-}R)} + \underset{110}{\tfrac{1}{2} \times (H\text{-}+)}$$

$$2090$$

F_1 cultures.

For the production of males for these F_1 cultures, it was decided, after some experimentation, to X-ray the parent males in the following numbers:

120 males with ring-chromosome given the light dose;
250 males with ring-chromosome given the heavy dose;
60 Oregon K. males given the heavy dose.

In order (1) that, within each series, the different classes will have approximately the same numerical relations to each other as in the other series throughout the experiment, and (2) to keep the numbers of X-chromosome translocations found in the different lots of each series approximately equal (to minimise/

minimise the error of the comparisons), it was decided to examine first the cultures of the class H-R, their number being the smaller, then to examine cultures of the class L-R till approximately the same number of translocations were obtained as were obtained from H-R and then to examine the H-+ series (with the object of obtaining here a somewhat greater absolute number of X-chromosome translocations than in H-R).

Analysis./

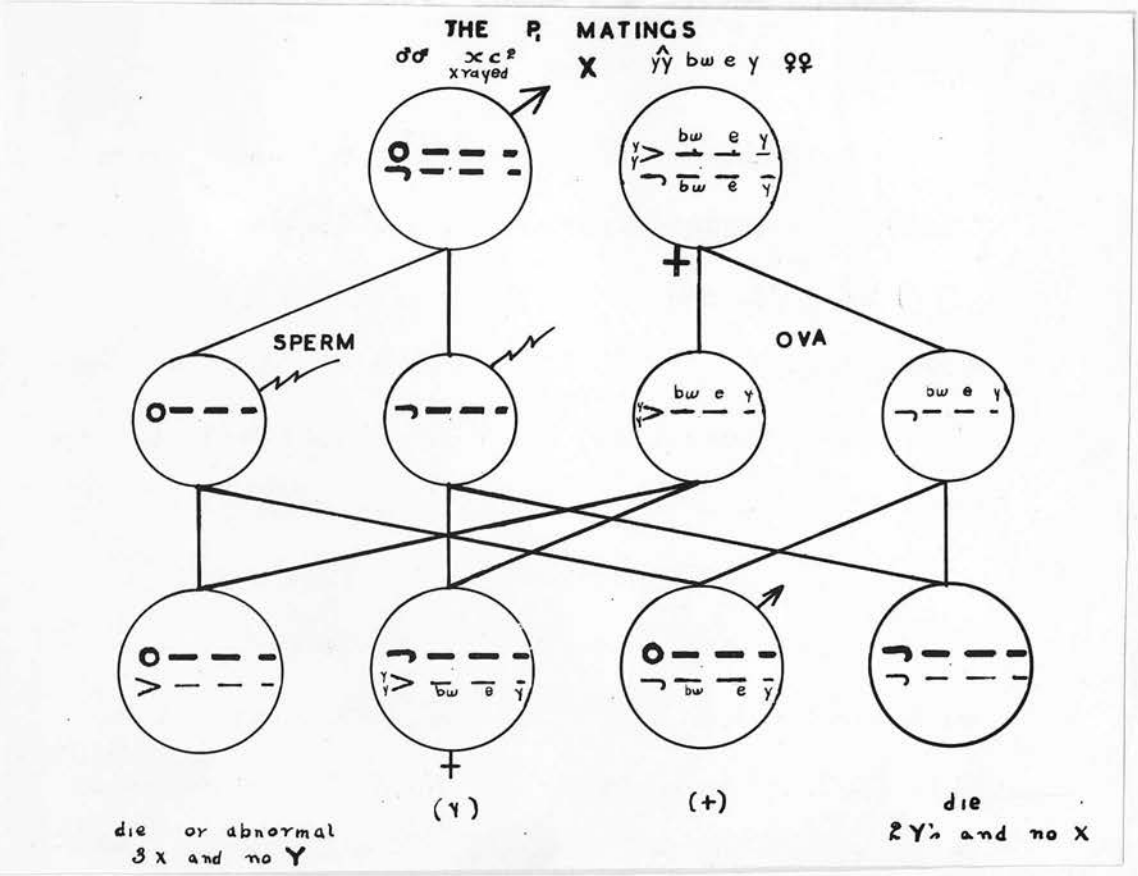
(Over)

Diagram No. 2 .

The P_1 Matings.

Illustrating the mating of the P_1 males whose sperms were X-rayed (in the diagram the case of those with the ring-shaped-X-chromosome is shown, but it will be the same in the case of the non-ring-X-chromosome) with non-irradiated females with attached X-chromosome and carrying gene markers on all the chromosomes. The X-rayed chromosomes are marked heavily in the diagram to distinguish them from the untreated chromosomes. Note that only one class of the F_1 males survives. These males are heterozygous for the marker genes supplied by the mothers' ova while the other set of chromosomes, supplied by the fathers' sperms, is treated and carries the normal alleles of the markers.

Diagram NO 2


THE P1 MATINGS
♂♂ xc²
X Y a y e d
X
ŷŷ bw e y ♀♀
SPERM
OVA
bw e y
bw e y
(Y)
(+)
die or abnormal
3 X and no Y
die
2 Y's and no X

Analysis of the Method for detecting Translocations.

Diagram No. 2 shows the parent matings, i.e. the matings of X-rayed males with the $\overline{yy}$ bw e cy virgin females. As is seen, heterozygous wild-type males are produced in F_1. Half their chromosomes are from the father - these, which had been X-rayed, are heavily marked - and the other half are from the untreated mother. These males may carry translocations produced in their father's germ cells by the X-rays. There is no danger of their being confused with males arising as a result of any non-virginity of the mother, as the latter males would be bw e cy.

In Diagram No. 3 is shown the result of the individual backcrossing of one of these heterozygous wild-type F_1 males, not containing a translocation, to $\overline{yy}$ bw e cy virgin females. The different possible modes of distribution of the chromosomes

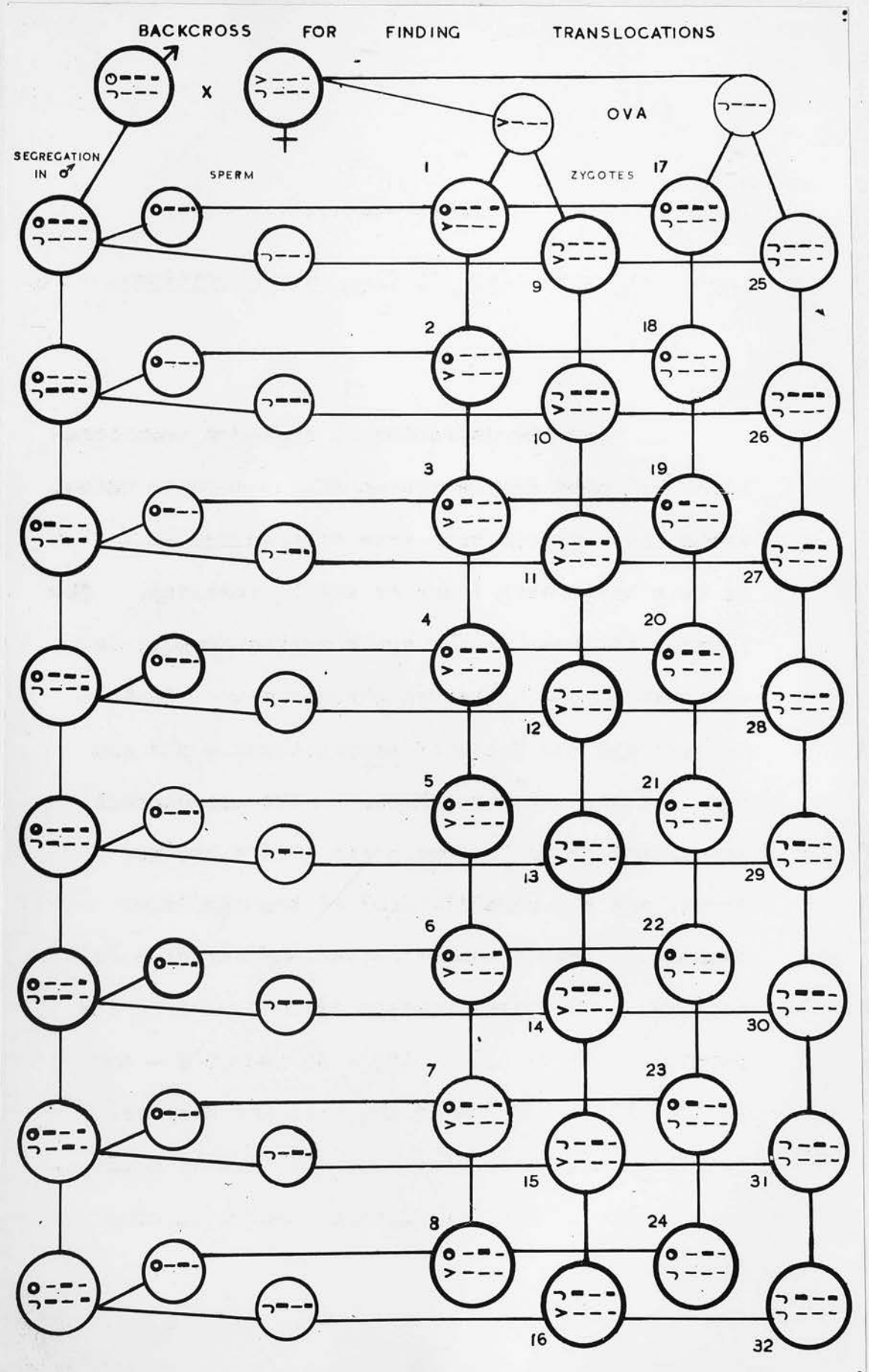
(Over)

Diagram No. 3 .

Backcross for Finding Translocations.

For the detection of whatever translocations occurred in the sperms of the X-rayed males, their heterozygous sons were backcrossed to $\overline{yy}$ bw e ey females (same as the P_1 females). The X-rayed chromosomes are again marked heavily in contrast to the untreated chromosomes. In the diagram all the types of segregations - old and new - of the male are shown. The chromosomal constitutions of the sperm and of the ova are shown, and the constitutions of the resulting zygotes. The types are given numbers from (1) to (32). The same numbers will be used in the papers. Types (1) to (8) - 3X and no Y - and (25) to (32) - 2Y and no X - will not survive. What the remaining types would be like if a translocation took place is shown in the succeeding diagrams.

Diagram NO 3


BACKCROSS FOR FINDING TRANSLOCATIONS
X
OVA
SEGREGATION IN ♂
SPERM
ZYGOTES
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32

chromosomes at spermatogenesis is shown, with the resultant production of sperm having different old or new combinations. Following this, the different types produced in F_2 are shown. For reference, they have been given numbers from (1) to (32). These numbers will also be used throughout the text and in the diagrams showing translocations, and will always be bracketed as a means of distinction.

Now in Diagram No. 3 the types numbered from (1) to (8), having three X-chromosomes and no Y-chromosome, would either not develop at all or would develop abnormally. Also, types (25) to (32), having two Y-chromosomes and no X-chromosome, would not develop. The surviving types are those from (9) to (24), those from (9) to (16) being females, and those from (17) to (24), males. Their constitution will be as follows:

(9) /

(9) $\overline{yy}$ bw e ey (female)

(10) $\overline{yy}$ bw$^+$ e$^+$ ey$^+$ "

(11) $\overline{yy}$ bw e$^+$ ey$^+$ "

(12) $\overline{yy}$ bw e ey$^+$ "

(13) $\overline{yy}$ bw$^+$ e ey "

(14) $\overline{yy}$ bw$^+$ e$^+$ ey "

(15) $\overline{yy}$ bw e$^+$ ey "

(16) $\overline{yy}$ bw$^+$ e ey$^+$ "

(17) bw$^+$ e$^+$ ey$^+$ (male)

(18) bw e ey "

(19) bw$^+$ e$^+$ ey "

(20) bw$^+$ e ey "

(21) bw e$^+$ ey$^+$ "

(22) bw e ey$^+$ "

(23) bw$^+$ e ey$^+$ "

(24) bw e$^+$ ey "

It is apparent that in cases where no translocation has occurred there is random assortment of the characters in question in F_2.

Taking/

(over)

Table No. 1 .

The Case of No Translocation:

Analysis of the surviving types in F_2 taking the characters in pairs.

Note the random assortment of characters. Also note the 1 : 1 : 1 : 1 ratio of the classes (a) with the two characters present, (b) with the two characters absent, (c) and (d) with one of the two characters present and the other absent. Then the 1 : 1 ratio of the two classes (A) with either both characters present or both absent ((a) + (b)), and (B) with one of the two characters present and the other absent ((c) + (d)).

y and bw

y bw (females)	y⁺ bw⁺ (males)	y bw⁺ (females)	y⁺ bw (males)
(9)	(17)	(10)	(18)
(11)	(19)	(13)	(21)
(12)	(20)	(14)	(22)
(15)	(23)	(16)	(24)
4	4	4	4

1 : 1

y and e

y e (females)	y⁺ e⁺ (males)	y e⁺ (females)	y⁺ e (males)
(9)	(17)	(10)	(18)
(12)	(20)	(11)	(19)
(13)	(21)	(14)	(22)
(16)	(24)	(15)	(23)
4	4	4	4

1 : 1

y and ey

y ey (females)	y⁺ ey⁺ (males)	y ey⁺ (females)	y⁺ ey (males)
(9)	(17)	(10)	(18)
(13)	(20)	(11)	(19)
(14)	(21)	(12)	(20)
(15)	(24)	(16)	(24)
4	4	4	4

1 : 1

bw and e

bw e (females)	(males)	bw⁺e⁺ (females)	(males)	bw e⁺ (females)	(males)	bw⁺ e (females)	(males)
(9)	(18)	(10)	(17)	(11)	(21)	(13)	(19)
(12)	(22)	(14)	(20)	(15)	(24)	(16)	(23)
2	2	2	2	2	2	2	2
4		4		4		4	

1 : 1

bw and ey

bw ey (females)	(males)	bw⁺ ey⁺ (females)	(males)	bw ey⁺ (females)	(males)	bw⁺ ey (females)	(males)
(9)	(18)	(10)	(17)	(11)	(21)	(13)	(19)
(12)	(24)	(16)	(23)	(12)	(22)	(14)	(20)
2	2	2	2	2	2	2	2
4		4		4		4	

1 : 1

e and ey

e ey females	males	e⁺ ey⁺ females	males	e ey⁺ females	males	e⁺ ey females	males
(9)	(18)	(10)	(17)	(12)	(22)	(14)	(20)
(13)	(19)	(11)	(21)	(16)	(23)	(15)	(24)
2	2	2	2	2	2	2	2
4		4		4		4	

1 : 1

Taking the characters in pairs, there are to be expected equal numbers of flies showing both mutant characters or neither and showing either one and not the other, as seen in Table No. 1.

We may now turn to cases in which translocation has taken place. Diagram No.4 shows translocations involving the second chromosome - carrying the gene for e^4. The diagram shows the three different ways in which the translocation could take place, namely mutual, deletion-insertion (more briefly termed "insertional") with either the second or the third chromosome as donor, and mutual deletion-insertion. Only translocations involving large enough pieces of the chromosomes to cause the death of the individual in cases of deficiency and duplication are considered. In these F_2 flies, in addition to the absence of types (1) to (8) and (25) to (32) as in the non-translocation case, types (11), (13), (15), (16), (19), (21), (23), and (24) do not survive owing to their contained deficiency and/or duplication. As to the eight/

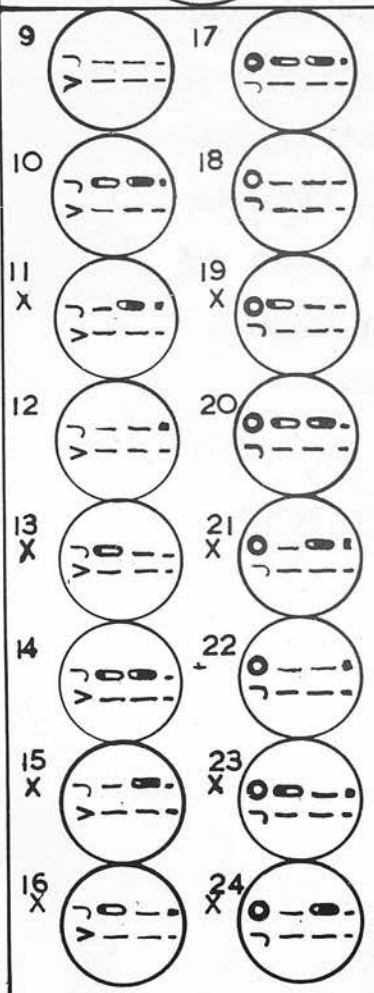
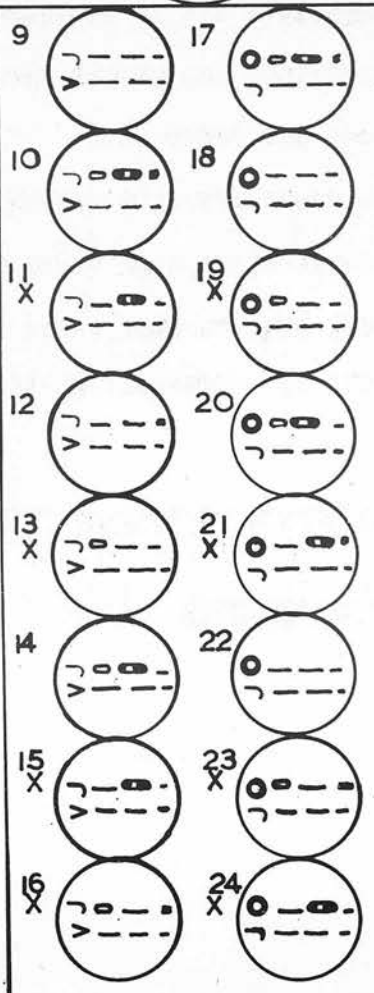
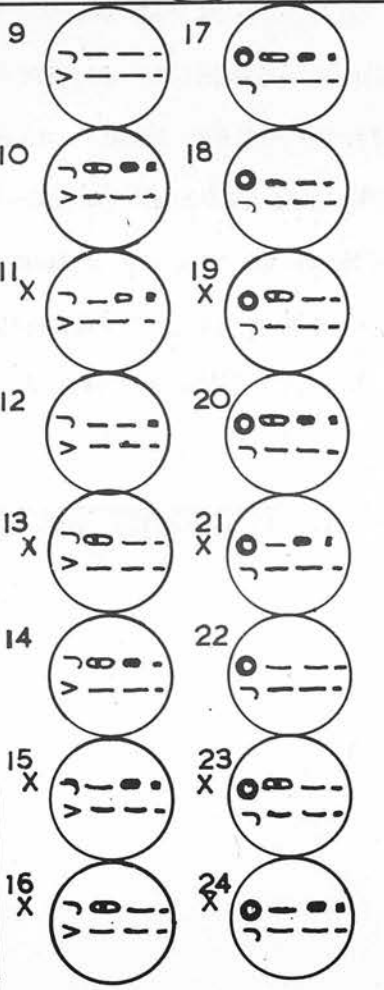
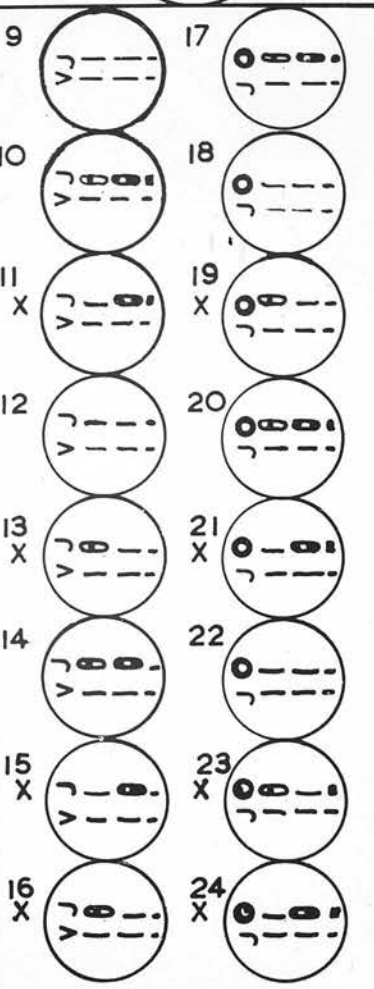
(Over)

Diagram No. 4.

Translocations involving the Second and Third Chromosomes.

The second chromosome is shown as a white band and the third chromosome as a black band so that the exchanges may be easily traced. The three major types of translocations, namely Mutual, Deletion-insertion, and Mutual deletion-insertion, are illustrated and dealt with separately. Only cases are considered where the fragments involved are large enough for duplication and/or deficiency to bring about the non-survival of the individual; these are marked with crosses in the diagram. Classes (1) to (8) and (25) to (32) are not included as they do not survive (see previous diagram).

TRANSLOCATIONS INVOLVING THE 2ND & 3RD CHROMOSOMES (LARGE PIECE IN EACH CASE)

TYPE OF TRANS-LOCATIONS	MUTUAL	DELETION – INSERTION		MUTUAL DELETION – INSERTION
		2ND DONOR	3RD DONOR	
F_1 ♂ WITH TRANS-LOCATIONS				
F_2				

eight surviving types, their constitution is as follows:

(9)	$\overline{yy}$ bw e ey	(female)
(10)	$\overline{yy}$ bw$^+$ e$^+$ ey$^+$	"
(12)	$\overline{yy}$ bw e ey$^+$	"
(14)	$\overline{yy}$ bw$^+$ e$^+$ ey	"
(17)	y$^+$ bw$^+$ e$^+$ ey$^+$	(male)
(18)	y$^+$ bw e ey	"
(20)	y$^+$ bw$^+$ e$^+$ ey	"
(22)	y$^+$ bw e ey$^+$	"

Table No.2./

(Over)/

Table No. 2.

The case of a translocation involving the second and the third chromosomes. Analysis of surviving types taking the characters in pairs.

Note the random assortment of characters and the 1 : 1 ratio between classes referred to in the previous table, except in the case of brown and ebony. Note that brown and ebony are either present together or absent together. In other words the ratio between the classes bw e + bw^{+}e^{+} and bw e^{+} + bw^{+}e = 1 : 0.

y and bw

y bw (females)	y* bw* (males)	y bw* (females)	y* bw (males)
(9) (12)	(17) (20)	(10) (14)	(16) (22)
2	2	2	2
4		4	
1	:	1	

y and e

y e (females)	y* e* (males)	y e* (females)	y* e (males)
(9) (12)	(17) (20)	(10) (14)	(16) (22)
2	2	2	2
4		4	
1	:	1	

y and ey

y ey (females)	y* ey* (males)	y ey* (females)	y* ey (males)
(9) (14)	(17) (22)	(10) (12)	(18) (20)
2	2	2	2
4		4	
1	:	1	

bw and e

bw e (females) (males)	bw* e* (females) (males)	bw e* (females) (males)	bw* e (females) (males)
(9) (18) (12) (22)	(10) (17) (14) (20)		
2 2	2 2		
4	4		
1	:	0	

bw and ey

bw ey (females) (males)	bw* ey* (females) (males)	bw ey* (females) (males)	bw* ey (females) (males)
(9) (18)	(10) (17)	(12) (22)	(14) (20)
1 1	1 1	1 1	1 1
2	2	2	2
4		4	
1	:	1	

e and ey

e ey (females) (males)	e* ey* (females) (males)	e ey* (females) (males)	e* ey (females) (males)
(9) (18)	(10) (17)	(12) (22)	(14) (20)
1 1	1 1	1 1	1 1
2	2	2	2
4		4	
1	:	1	

Table No. 2

Taking each pair of characters separately and constructing Table No. 2 from the list, we notice that we now fail to get the ratio 1 : 1 where the pair of characters bw and e are considered, and get 1 : 0 instead; that is, the two classes bw e^+ and bw^+ and e are missing, brown and ebony being either present together or absent together in every surviving individual. The reason for this is that where the chromosomes involved do not segregate together, hyperploids or hypoploids are formed, which do not live.

This principle ordinarily applies just as well to translocations involving any other two chromosomes. In the case of a translocation involving the second and fourth chromosomes, we find that brown and eyeless are either both present or both absent. In the case of a translocation involving the third and fourth chromosomes, ebony and eyeless are either present together or absent together.

Also/

Also in cases of translocations involving the ordinary - not ring-shaped - X-chromosome and any of the autosomes, this same principle applies. In translocations involving the X-chromosome and the second chromosome, all the males will be bw^+ and all the females bw; in those involving the X-chromosome and the third chromosome, all males will be e^+ and all females e; and in those involving the X-chromosome and the fourth chromosome, all males will be ey^+ and all females ey.

Among the F_2 flies, from the translocational cases, involving the X- and second chromosomes, for example, there will be both an absence of types (1) to (8) and (25) to (32) (as in non-translocational cases) and of types (10), (13), (14), (16), (18), (21), (22), and (24), owing to their contained deficiency and/or duplication. The genetical constitution of the surviving types is shown in the following list:

(9)/

(9) $\overline{yy}$ bw e ey - (female)

(11) $\overline{yy}$ bw e^+ ey^+ "

(12) $\overline{yy}$ bw e ey^+ "

(15) $\overline{yy}$ bw e^+ ey "

(17) y^+ bw^+ e^+ ey^+ (male)

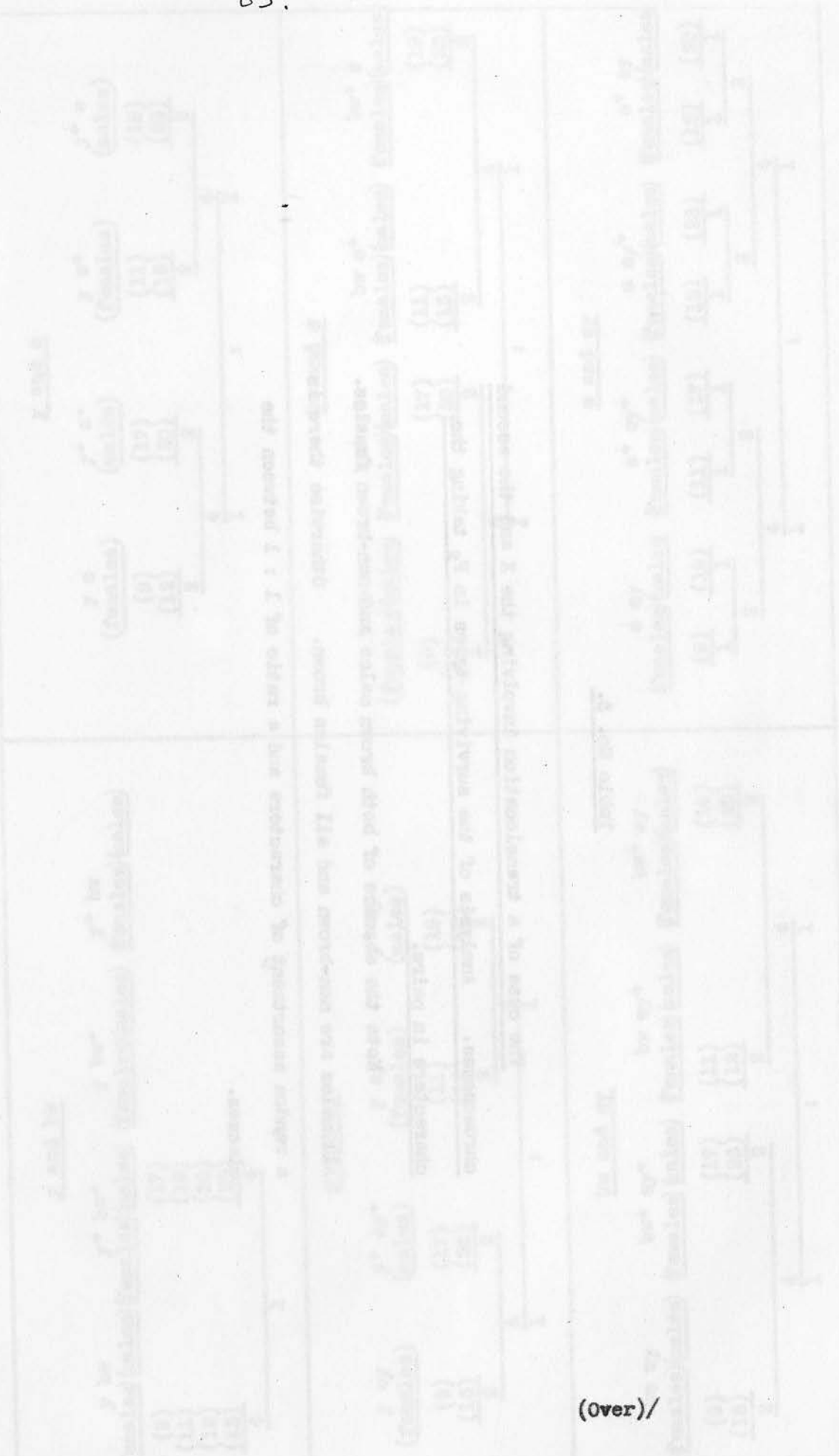
(19) y^+ bw^+ e ey "

(20) y^+ bw^+ e^+ ey "

(23) y^+ bw^+ e ey^+ "

Constructing Table No. 3 from the above list, we notice the absence of both brown males and non-brown females.

Translocations involving the ring-shaped-X-chromosome need special consideration, however. This is due to the fact that a minimum of three breaks is required before a viable translocation involving a ring-chromosome and another chromosome can be formed. An example of a translocation involving the ring-X and the second chromosome is given in Diagram No. 5 . We see that a simple mutual translocation,/



(Over)/

Table No. 3.

The case of a translocation involving the X and the second chromosomes. Analysis of the surviving types in F_2 taking the characters in pairs.

Note the absence of both brown males and non-brown females. All males are non-brown and all females brown. Otherwise there is a random assortment of characters and a ratio of 1 : 1 between the classes.

y and bw

y bw		y⁺ bw⁺		y bw⁺		y⁺ bw	
(females)	(males)	(females)	(males)	(females)	(males)	(females)	(males)
(9)		(17)					
(11)		(19)					
(12)		(20)					
(15)		(23)					
4		4					

1 : 0

y and e

y e		y⁺ e⁺		y e⁺		y⁺ e	
(females)		(males)		(females)		(males)	
(9)		(17)		(11)		(19)	
(12)		(20)		(15)		(23)	
2		2		2		2	
4				4			

1 : 1

y and ey

y ey		y⁺ ey⁺		y ey⁺		y⁺ ey	
(females)		(males)		(females)		(males)	
(9)		(17)		(11)		(19)	
(15)		(25)		(12)		(20)	
2		2		2		2	
4				4			

1 : 1

bw and e

bw e		bw⁺ e⁺		bw e⁺		bw⁺ e	
(females)	(males)	(females)	(males)	(females)	(males)	(females)	(males)
(9)		(17)		(11)		(19)	
(12)		(20)		(15)		(23)	
2		2		2		2	
4				4			

1 : 1

bw and ey

bw wy		bw⁺ ey⁺		bw wy⁺		bw⁺ ey	
(females)	(males)	(females)	(males)	(females)	(males)	(females)	(males)
(9)		(17)		(11)		(19)	
(15)		(25)		(12)		(20)	
2		2		2		2	
4				4			

1 : 1

e and ey

e ey		e⁺ ey⁺		e ey⁺		e⁺ ey	
(females)	(males)	(females)	(males)	(females)	(males)	(females)	(males)
(9)	(19)	(11)	(17)	(12)	(25)	(15)	(20)
1	1	1	1	1	1	1	1
2		2		2		2	
4				4			

1 : 1

64

Slide No 3

(Over)

Diagram No. 5.

Translocations involving the Ring-shaped-X-chromosome and the Second Chromosome.

The ring chromosome is shown in white and the second in black. The diagram is constructed on the same lines as the previous one. Note the absence of the mutual translocations owing to the formation of a dicentric chromosome which gets lost. Types which do not survive owing to duplication and/or deficiency are marked with a cross.

TRANSLOCATIONS INVOLVING THE RING AND 2ND CHROMOSOMES

TYPE OF TRANS-LOCATIONS	MUTUAL	DELETION—INSERTION		MUTUAL DELETION- INSERTION
$F_1 ♂$ WITH TRANS-LOCATIONS	DICENTRIC; LOST			
F_2		9, 10 X, 11, 12, 13 X, 14 X, 15, 16 X; 17, 18 X, 19, 20, 21 X, 22 X, 23, 24 X	9, 10 X, 11, 12, 13 X, 14 X, 15, 16 X; 17, 18 X, 19, 20, 21 X, 22 X, 23, 24 X	9, 10 X, 11, 12, 13 X, 14 X, 15, 16 X; 17, 18 X, 19, 20, 21 X, 22 X, 23, 24 X

translocation, entailing only two breaks, cannot survive as it results in a dicentric chromosome, that is, a chromosome with two centromeres; such a chromosome must eventually get lost, by being pulled in both directions at once at some mitosis. The remaining translocation types, and the resulting F_2 flies, are, however, the same as in translocations involving an ordinary - not ring-shaped - X-chromosome and an autosome. The insertional types do not result in the production of dicentric chromosomes (provided the inserted piece does not include a centromere).

Table No. 3 applies to the F_2 results from a viable translocation involving the ring-X as well as to those from an ordinary X.

A key for detecting translocations according to segregation of characters is given in Table No. 4.

Table No. 4 ./

Table No. 4.

Key for detecting translocations according to segregation of characters.

Type of Translocation.	Segregation of Characters.
Involving the X-chromosome (ring or non-ring) and the second chromosome.	All males bw^+, all females bw.
Involving the X-chromosome (ring or non-ring) and the third chromosome.	All males e^+, all females bw.
Involving the X-chromosome (ring or non-ring) and the fourth chromosome.	All males ey^+, all females ey.
Involving the second and the third chromosomes.	The flies either bw e or bw^+e^+ but not bw e^+ or bw^+ e.
Involving the third and the fourth chromosomes.	The flies either e ey or e^+ ey^+ but not bw ey^+ or bw^+ ey.
Involving the second and the fourth chromosomes.	The flies either bw ey or bw^+ ey^+ but not bw ey^+ or bw^+ ey.
No translocation involved.	All possible combinations present.

In more complicated types of translocations involving three chromosomes at once, both criteria for the cases in point apply at once.

Results./

Results.

A tabulated summary of the results obtained with a total of 12,167 F_1 cultures.

TRANSLOCATION FREQUENCIES IN RELATION TO RADIATION DOSE.

(Heavy Dose = 4 x Light Dose.)

Experiment No.	Series	Fertile F_1 Cultures	Translocations involving X-2	X-3	X-2-3	X-4	2-3	2-4	3-4
1	L-R	1132					12		
	H-R	160		1	1		14		
	H-+	26					3		
2	L-R	1176			1		28		1
	H-R	156	1				19		
	H-+	59	1				5		
3	L-R	863		1			4	2	
	H-R	141		1			6	1	
	H-+	54		2			2		
4	L-R	1238					8		2
	H-R	136					8	1	
	H-+	83			1	1	7		2
5	L-R	1492				1	20		
	H-R	195			1		20	1	1
	H-+	89	2	1			10		
6	L-R	1480					20	1	1
	H-R	207					20	1	
	H-+	96					8		2
7	L-R	1362		1			8		
	H-R	192					11		
	H-+	82					7		
8	L-R	1453	2	*			18	1	
	H-R	186		1			16		
	H-+	109		2			7		
Total.	L-R	10196	2	2	1	1	118	4	4
	H-R	1373	1	3	2	0	114	4	1
	H-+	598	3	5	1	1	49	0	4

L-R = results from light dose applied to males with ring X-chromosome.
H-R = " " heavy " " " " " " "
H-+ = " " heavy " " " " " normal "

* An atypical unique case of translocation occurring between paternal and maternal chromosomes. (See page)

Analysis of the Results.

Quantitative relationship between the dosage of irradiation and the frequency of gene rearrangements.

From the above table summarising the results obtained from the experiments, we can determine the quantitative relationship between the frequency of translocations and the dosage of X-rays between the doses 1000 and 4000 r units.

The frequencies of translocations involving exclusively the autosomes in the experiments where the ring X-chromosome was used were as follows, for the two doses:

Dose	Fertile Cultures Examined	No. of observed Translocations between the Second and Third Chromosomes.	Percentage of observed Translocations
Light (1000 r)	10,196	118	1.16 ± 0.116
Heavy (4000 r)	1,373	114	8.30 ± 0.76
Total.	11,569	232	

The/

The relation between the dose and the percentage of these translocations obtained can be calculated with sufficient accuracy by fitting the formula

$$F_d = F_0 d^x$$

where F = percentage of translocations, and

d = dose in r units,

to our experimental data. The value for x here was found to be 1.42. This exponent is significantly different from both the first and the second powers but not significantly different from the (approximately) 1.5 power found by some workers for doses similar to those used in the present work. This will be seen from the following calculations, where the number of translocations with the heavy dose was taken as a basis and the number expected for the low dose on the assumption of different values of the exponent was calculated from it, assuming that the total number of cultures at the low dose remained that which had actually been dealt with at the high dose.

Assumed/

Assumed Value of Power of the Dose to which Translocation Frequency is Proportional.	Number of Translocations for the Low Dose.		Difference.	Difference / Error approx.
	Expected.	Found.		
1	29 ± 3.0	16 ± 1.6	-13 ± 3.3	4
1.5	14 ± 1.5	16 ± 1.6	+2 ± 2.3	1
2	7 ± 0.8	16 ± 1.6	+9 ± 1.7	5

Multiple/

Multiple Breaks.

(a) Test for randomness of distribution among chromosomes:

The data presented show the following observations (where "R" represents the ring-chromosome):- R-II-III and X-II-III are considered as translocations involving R or X. chromosome and an autosome.

Translocations R-II, R-III, R-II-III (requiring at least two breaks in one chromosome, within the same arm, and one in another chromosome)

= 6 in 1,373 = 0.4,37 ±0.0,58 ‰

Translocations X-II-III (requiring at least one break in each of three separate chromosomes)

= 1 in 598 = 0.1,67 ±0.0,53 ‰

If we assume that the breaks are distributed at random, and that the breakage frequencies of the five/

five ~~main~~ arms of the major chromosomes, consisting of the X (or ring), second, and third chromosomes, are as a first approximation the same, the probability of the distribution of three breaks one in the ring and two in one autosomal arm (or vice versa), as compared with the distribution of the three breaks - one, and one and one - among the X, second, and third chromosomes, can be calculated by the expansion of the polynomials $(1_X + 1_a + 1_a + 1_a + 1_a)^3$, and $(1X + 2A + 2A)^3$, in which a, a, a, a represents the four arms of the autosomes, while A and A represent the whole autosome.

This probability works out as $\frac{12}{125}$ for the R^2a^1, $\frac{12}{125}$ for the R^1a^2, and $\frac{24}{125}$ for the $X^1 A^1 A^1$. This makes $\frac{12 + 12}{24}$, or 1 for the ratio of $R^2a^1 + R^1a^2$ to $X^1 A^1 A^1$ cases. In addition each break in the X (or ring) chromosome has as a conservative assumption (according to an estimate made by Muller) a chance of 50% (probably more nearly 75%) of causing ~~the~~ lethality on account of position effect. Therefore the combinations/

combinations $R^1\alpha^2$ and $X^1\text{-}A^1\text{-}A^1$ (where the index numbers represent the number of breaks) must be reduced to half, while the combinations $R^2\alpha^1$ (on account of their chance of lethality due to either of the two breaks) to $(.5)^2$. Making these reductions, the proportion of the combinations of breaks $R^2\alpha^1$ or $R^1\alpha^2$ to those of type $X^1\text{-}A^1\text{-}A^1$ surviving the lethal position-effect becomes 3 : 4, or 0.75.

It must next be noted that, on the assumption that broken ends rejoin at random, out of the fifteen ways in which the fragments formed by the three breakages can combine when distributed as $X^1\text{-}A^1\text{-}A^1$ or as $R^2\alpha^1$ (or $R^1\alpha^2$), only two ways give triple translocations in the first case and the same number give insertional translocations in the second case (such translocations only are included here, since those of other types involving a ring-chromosome form inviable (dicentric) chromosomes).

Since the same factor, $\frac{2}{15}$, applies to both types of cases, the above-mentioned ratio of 3 : 4 in the distribution of the breaks coincides also with the expected/

expected ratio of observable $R^2\alpha^1$ (plus $R^1\alpha^2$) translocations to $X^1-A^1-A^1$ translocations. The proportion actually found is, however, $\frac{4.32\ (R\ A)}{1.67\ (X-A-A)}$ = 2.61 ± 0.91 instead of 0.75. This would indicate a frequency of translocations caused by more nearly adjacent breaks (two in one chromosome arm) 3.5 times as great as expected.

But although the difference between found and expected is approximately twice its standard error, and therefore might be taken as statistically significant, it must be considered that in fact some other data indicate that usually the X-A-A translocations occur with a higher frequency than here found. Therefore the conclusion drawn needs confirmation before/finally accepted being.

(b)/

(b) The relative frequencies of double and multiple breaks:

From our data with H-R and H-+, we have:-

R-II + R-III + R-II-III = 6 out of 1,373

= 0.437 ± 0.058 %

(at least two breaks in R and one in an autosome, or two breaks in the same arm of an autosome and one break in R)

X-II + X-III + X-II-III = 9 out of 598

= 1.505 ± 0.157 %

(at least one break in the X and one in an autosome).

If we subtracted from the frequency of X-II, X-III, X-II-III translocations (two or more breaks) the frequency of R-II, R-III, R-II-III (three or more breaks), it might be supposed at first glance that we would obtain the frequency of translocations involving only two breaks each. Before a subtraction is made for this purpose, however, we must make allowance for the/

the fact already mentioned that in the case of the ring only insertional translocations survive and can be detected. On the hypothesis of random reunion of parts, an equal number of nuclei having their three breaks distributed ~~in the same way~~ as $R^1a^2 \sim R^2a^1$ result in non-insertional translocations, which would survive and be detectable as translocations in ordinary cases but which would form dicentric chromosomes and be lost in the case of the ring.

We thus obtain 1.505% - (.437 x 2)% = .631% which may be considered with some approximation as the frequency of those X-II, X-III, X-II-III translocations which involve only two breaks in the arms concerned in the translocation. (In our further calculations, however, we ignore the fact that a certain proportion of these breaks were present in other arms as well; it is hoped later to estimate the amount of error involved in doing this.)

Now by the expansion of the polynomial $(1_X + 2_{A_1} + 2_{A_2})^2$ we find that there are $\frac{8}{25}$ cases in which any two breaks taken at random happen to lie one/

one in X and one in one of the autosomes (A_1 or A_2). Of these cases only a fraction survive on account of the lethal position effect of breakage in the X-chromosome. A conservative assumption in this case would be to take the lowest survival ration found for this effect, which (according to estimation by Muller) is approximately $\frac{1}{8}$. In addition we must take into account that one of the possible ways in which the fragments resulting from two breaks (one in each chromosome) can combine, only 33% give viable translocations, (the rest giving inviable chromosomes and restitutions). We can, therefore, calculate all the possible combinations between these fragments, of which the corrected observed frequency of 0.631% is only a fraction, as follows:-

$$\frac{0.631 \times 4 \times \frac{25}{8}}{0.33 \times 1.33} = 18\%$$

This total frequency may then be taken as the ordinate corresponding to X = 2 in a Poisson distribution of the number of nuclei containing different numbers/

numbers (X) of breaks. - We can easily find, by consulting the Poisson tables and interpolating, the frequency corresponding to X = 3 in the same distribution. This frequency, which is found to be 5.8%, represents that of all the possible combinations which contains three breaks in the haploid nucleus containing an X-chromosome (ignoring the fourth chromosome).

But only those cases of three breaks which are of types R^2a^1 or R^1a^2 (where 'a' indicates a chromosomal arm and the index figure refers to the number of breaks) can give the translocations in question. These types of distributions of the breaks are found from the expansion of the polynomial $(1_R + 1_a + 1_a + 1_a)^3$ to have a probability of $\frac{12}{125}$ for the type R^2a^1 and $\frac{12}{125}$ for the type R^1a^2. Of the latter, one-quarter only survives on account of the already mentioned injurious position effect, while of the former only $(\frac{1}{4})^2$ survives as explained before. In addition, only $\frac{2}{15}$ of the three-break rearrangements involving the ring and one of the autosomes/

autosomes give viable detectable translocations, as already explained. Therefore, we have

$$5.8\% \times \frac{12}{125} (0.25 + 0.06) \times \frac{2}{15} = .023\%$$

This is the expected frequency of the three-break ring translocations on the assumptions that :

(1) The breaks are distributed at random;

(2) The breakage ends join at random;

(3) The lethal position effect of the breakage of the X-chromosome causes the death of 75% of individuals (a higher viability would give a lower calculated frequency);

(4) The breakage length of the X and the autosomes is as 1 : 2 : 2; and

(5) The method of calculation is reasonably correct.

If we now compare the "expected" frequency so calculated, 0.023%, with the observed value, 0.437%, we see that the latter is about nineteen times as great as the former. It must be taken into account, however, that our calculated frequency is that of exactly three-break cases, whereas our found frequency is/

is that of <u>at least</u> three breaks. Surely, however, we could not consider anything like $\frac{18}{19}$ of our observed cases to be of more than three breaks (in view of the large number of two-break and no-break progeny). Hence, it seems probable that there are really far more translocations of types R^2a^1 and R^1a^2 than the assumption of random reunion will allow.

It is realised, however, that the above method of attack represents only a rough approximation, and the attempt is being made in our laboratory to work out the expected frequencies of different types of double-and multiple-break translocations, on the assumption of random reunion, in a more exact way, for comparison with observed figures. The more exact treatment of this matter, however, must be reserved for a later publication.

The same method of treatment may be extended to the light dose. But as no L-+ was included in the experiment, the total frequency of translocations involving X with II and X with III with the light dose is here calculated from the observed frequency of translocations/

translocations II-III at that dose, by assuming (as an approximation) that the ratio of these classes to each other would be the same as that observed between them at the heavy dose. This gives a calculated frequency for X-II + X-III + X-II-III of 0.213%. This frequency of X-chromosome translocations may then be compared with the data on R translocations obtained in the L-R series, which for R-II + R-III + R-II-III is $\frac{5}{10196} = 0.048\%$.

$0.213\% - (2 \times 0.048\% = 0.117\%$, frequency of translocations between X and II, III, or II-III, involving only two breaks.

$\frac{0.117\% \times 4 \times \frac{25}{6}}{0.33 \times 1.33} = 3.34\%$, frequency for two breaks.

The corresponding X = 3 frequency in the Poisson series is 0.33%.

$0.33\% \times \frac{12}{125} (0.25+0.06) \times \frac{2}{15} = 0.0013\%$ (calculated viable ring translocations involving no superfluous breaks).

THe/

The found frequency of-all ring chromosome translocations for this dose, being 0.048%, is 37 times as great as expected. This discrepancy (which is far beyond the limits of statistical error for this case) then helps greatly to confirm our inference, based on the high ~~low~~-dose data, that the multiple-break translocations (involving two breaks in one arm) are disproportionately frequent incomparison with the cases of lower rank.

(c) The relation between the frequencies of Ring Translocations at the low and at the high dose:

We have made calculations showing that on the breakage theory the relation of ring chromosome translocations at the low and high doses to be expected assuming random breakage and reunion would be sufficiently well represented by a finding of four and eight respectively as the number of ring chromosome translocations in the light and heavy series instead/

instead of six and six as actually observed in an experiment. However, the statistical error of the expectation, four and eight, is sufficiently high, thus easily to have resulted in observed values of six and six. Thus a conclusion adverse to the breakage theory cannot be drawn from this result.

Discussion./

Discussion.

The quantitative relationship between the frequency of gene rearrangements induced by radiation and the dosage.

Using translocations and X-ray dosages of 1000 and 4000 r units for this study, the data presented above indicate the following conclusions:

The observed frequency of translocations is not proportional to the dosage.

It does not vary according to the square of the dosage.

It increases more rapidly with increasing dosage than it would in the case of direct proportionality to the dosage, but less rapidly than would be expected if it was proportional to the square of the dose.

Between the dosages mentioned above, the increase in the frequency seems to be proportional to approximately the 1.5 power of the dose.

These results are in harmony with those obtained/

obtained by several previous workers and contradictory to those reported by some others, as mentioned under the heading "Previous Works".

The fact that the frequency rises more rapidly than the dosage suggests that the contact hypothesis is not valid - at least, not in all the cases of gene rearrangements. If all the breaks involved in any "primary" gene rearrangement were produced by the same ionization - by one "hit" - we should have observed a proportionality between the frequency of rearrangements and the dosage, at lower dosages, and an even less rapid rise in frequency than in the dosage, at higher dosages, as in the case of gene mutations. By "primary" gene rearrangement, is meant those involving one contact - double or multiple - and one electron. This will include cases of double or multiple transfer, but will not include cases in which there were two or more independent exchanges involving, in part, the same chromosomes.

If/

If we admit that individual ionizations produce the breakages, the results mentioned above lead us to the conclusion that many or all of these breakages occur independently in the different chromatin regions involved in gross rearrangements, and that union of pieces occurs subsequently. In other words, in the majority of cases at least, the two breaks - which are the minimum required before a gene rearrangement can take place - are produced by two independent ionizations, - by two "hits". In this case we should, as a first approximation, expect the frequency of gene rearrangements in the irradiated gametes to increase according to the square of the radiation dosage. In fact, if two independently originated breaks are necessary for a gene rearrangement, the probability of having the two breaks in the same nucleus with a dose which gives an average of p breaks per nucleus, where p is a very small frequency, is nearly $\frac{p^2}{2}$. If we double the dose, the probability becomes $\frac{(2p)^2}{2}$, which is four times/

times that with the original dose, and so on, until the dosage/(and p) becomes so high that multiple breaks can no longer be neglected. Why then do we find the above-mentioned increase in proportion to approximately the 1.5 power of the dosage? According to the hypothesis that the breaks occur independently, as results of individual ionizations, the increase in the number of breaks will be proportional to the increase of the dosage. Moreover, the increase of the dosage increases the percentage of nuclei with two breaks in relation to those with one break. Still more does it increase those with three breaks in relation to those with two, and again more those with four breaks in relation to those with three, and so on. On our assumption that the breaks are produced at random, this progressive increase in the multiple breaks can be calculated.

As was pointed out by Catcheside (1938) and by Haldane (1936, ex litt, unpublished), the frequencies of single, double, and multiple breaks of different/

different ranks, for any given dose producing a given mean number of breaks per nucleus, form a Poisson series. We give in the table below an example of these series for various possible mean numbers of breaks. Our table includes the series for some mean values smaller than those of Catcheside, since, as we shall see, these represent conditions of some importance, and the numbers are given to a further place beyond the decimal point than his, in order that the ratios of these numbers to each other may be ascertained more accurately.

Table No. 5 ./

Table No. 5 .

Mean number of breaks per nucleus (prop. to dosage).	Percentage of nuclei with 0...6+ breaks.						
	0	1	2	3	4	5	6+
0.05	95.12	4.76	0.12	-	-	-	-
0.1	90.5	9.05	0.45	-	-	-	-
0.2	81.83	16.37	1.64	0.13	-	-	-
0.4	67.0	26.8	5.4	0.7	0.1	-	-
0.8	44.9	35.9	14.4	3.8	0.8	0.1	-
1.6	20.2	32.3	25.8	13.7	5.5	1.8	0.6
3.2	4.0	13.0	20.9	22.3	17.8	11.4	10.5
6.4	0.2	1.0	3.4	7.3	11.6	14.8	61.3

(The percentages of nuclei for 0.05, 0.1, and 0.2 mean breaks per nucleus were calculated according to the formula of frequency in the Poisson distribution $f_x = e^{-m} \times \frac{m^x}{x!}$

where f = frequency of nuclei having given number of breaks,

x = breaks per nucleus,

e = base of natural logarithms,

m = mean breaks per nucleus

as the percentages for 0.05 mean were absent, and to obtain more accurate figures for those for 0.1 mean and for 0.2 mean.

From/

From the table we note that ~~while~~ when the mean number of breaks per nucleus - which is proportional to the dose - increases for instance from 0.05 to 0.1, i.e. the dose was doubled, while the percentage of nuclei with one break was ~~also~~ only approximately doubled, but that of nuclei with two breaks increased approximately four times. The same principle will be found to hold true when we compare the percentage of nuclei with two breaks with that for three breaks, the latter in turn with that for four breaks, etcetera, and this applies all the way down the table, that is, with increasing dose.

But there is no reason to suppose that detached chromosome fragments would always unite in such a way as to form new viable chromosomes involving gene rearrangements. It seems probable that the breakage ends may unite where they parted, forming the original chromosomes with no gene rearrangements ("restitution"). In some case, the fragments might conceivably miss uniting and get lost, as a result of union/

union of chromatids (McClintock, 1938) by their adjoining broken ends. The fragments with no spindle attachment might unite forming acentric chromosomes, while those with spindle attachments united forming dicentric chromosomes. Both acentric and dicentric chromosomes are inviable. Finally, the chromosome fragments might unite in a new way giving viable chromosomes involving gene rearrangements.

The simplest assumptions concerning the rules governing the reattachments are those originally made by Haldane (1935, ex litt, unpublished) and by Catcheside (1938), (1) that all breakage ends rejoin, and (2) that they rejoin at random. On the basis of these two assumptions, Haldane (op. cit.) deduced general formulae for the frequencies of various kinds of rearrangements among the viable offspring. Similar results have been published by Catcheside (1938) in the form of a table showing the frequencies that would obtain in the case of given mean numbers of/

of breaks per nucleus.. This table (which has been copied and somewhat extended below) is founded on the further assumption (known to be not strictly accurate but giving greater simplicity in calculation) that (3) the breaks occur one in each chromosome, so that only interchanges are being dealt with . In the table the relative distribution is shown of the following three classes where the fragments unite to form (a) the original chromosomes, (b) inviable chromosomes, (c) viable translocated chromosomes. The formulae used for calculating these figures are -

$$\text{Proportion original} = \frac{1}{1.3.5.7. \ldots. (2n-1)}$$

$$\text{Proportion translocated} = \frac{n! - 1}{1.3.5.7. \ldots. (2n-1)}$$

$$\text{Proportion inviable} = \frac{1.3.5.7. \ldots. (2n-1) - n!}{1.3.5.7. \ldots. (2n-1)}$$

where n = number of breaks per nucleus.

Table No. 6./

Table No. 6.

Breaks per Nucleus.	Proportional contribution to		
	Original Chromosomes	Inviable Chromosomes	Translocated Chromosomes
1	1.00	0	0
2	0.33	0.33	0.33
3	0.07	0.60	0.33
4	0.01	0.77	0.22
5	0.001	0.88	0.12
6	Nearly 0	0.93	0.07
7	-	0.96	0.04
8	-	0.98	0.02
9	-	0.99	0.01
10	-	0.994	0.006

These figures show clearly that with increase of the number of breaks the class of the inviable chromosome increases greatly at the expense of the other two classes. For example, for the increase from two

breaks/

breaks per nucleus to three breaks, the inviable chromosomes class increases from 0.33 to 0.60 while there is no increase in the translocated chromosomes class. Again, with the increase of the number of breaks per nucleus to four, the inviable chromosomes class increases to 0.77. This principle continues to hold as the number of breaks per nucleus increases.

So from the two tables last given above, we can draw the two following conclusions:

(1) The higher the dose, the higher is the number of nuclei with multiple breaks relative to those with double breaks; and

(2) The higher the number of nuclei with multiple breaks, the higher is the proportion of inviable to viable translocated chromosomes.

These two conclusions combined would mean that with the increase of the dosage the proportion of the inviable translocations increases more than that of the viable ones. Thus, as we calculate the frequency/

frequency of translocations among the viable offspring we find that it rises less, with increase in dosage, than the frequency of translocations among the total offspring (viable plus inviable). It therefore departs from the square relationship even more than would be expected merely on the basis of the "saturation effect" (coincidence of more than enough breaks to give a translocation), and thus the existence of the 1.5 rule ~~at these~~ between our doses becomes understandable.

But the lower the dosages used, the fewer are the multiple breaks, relatively to the double breaks, and so the nearer the power of the dose - according to which the frequency of gene rearrangements varies - to the square. Mutatis mutandis, the higher the dosages above those which were used, the further below the 1.5 must this power become if our premises are correct. This will be noticed clearly in the following table, which was constructed according to the following analyses of the contributions/

contributions to the three classes of nuclei (viable non-translocated, viable translocated, and inviable) which would be made under conditions of irradiation, giving as examples 0.05, 0.1, and 0.2 mean number of breaks per nucleus.

For mean of 0.05 breaks per nucleus/

For mean of 0.05 breaks per nucleus.

No. of Breaks per Nucleus.	Percentage of Nuclei.	Contributions to nuclei with		
		Normal Chromosomes	Inviable Chromosomes	Translocated Chromosomes
0	95.12	95.12	-	-
1	4.76	4.76	-	-
2	0.12	0.04	0.04	0.04
		99.92	0.04	0.04
			100.00	

For mean of 0.1 breaks per nucleus.

No. of Breaks per Nucleus.	Percentage of Nuclei.	Contributions to nuclei with		
		Normal Chromosomes	Inviable Chromosomes	Translocated Chromosomes
0	90.50	90.50	-	-
1	9.05	9.05	-	-
2	0.45	0.15	0.15	0.15
		99.70	0.15	0.15
			100.00	

For mean of 0.2 breaks per nucleus.

No. of Breaks per Nucleus.	Percentage of Nuclei.	Contributions to nuclei with		
		Normal Chromosomes	Inviable Chromosomes	Translocated Chromosomes
0	81.83	81.83	-	-
1	16.37	16.37	-	-
2	1.64	0.54	0.54	0.54
3	0.13	0.009	0.078	0.043
		98.749	0.618	0.583
			100.000	

From/

From similar calculations for other means, the following table for the percentage of survivors with translocations is constructed:

Table No. 7.

Mean Breaks per Nucleus	Percentage individuals with			Percentage Individuals Surviving	Percentage Survivors Translocated
	Normal Chromosomes	Inviable Chromosomes	Translocated Chromosomes		
0.05	99.92	0.04	0.04	99.86	0.04
0.1	99.70	0.15	0.15	99.85	0.15
0.2	98.75	0.62	0.58	99.33	0.58
0.4	95.6	2.5	2.0	97.6	2.1
0.8	85.8	7.8	6.3	92.1	6.7
1.6	62.2	23.2	14.5	76.8	18.8
3.2	25.8	53.9	20.3	46.1	44.0
6.4	3.1	86.3	10.6	13.7	77.4

It/

It may be seen from the table that the relation between the dosage and the frequency of translocations to be expected - if the assumptions made hold good - follows an S-shaped curve. For the very lowest doses the relation is approximately quadratic, departing from this, gradually passing through all the powers between the second and the first (thus the approximately 1.5 power found in the intermediate portion), and then becoming convex (power <1) and tending to be asymptotic to the dosage.

The results in this table, then, agree with our observed data in giving an increase in the translocation frequency proportional to the 1.5 power of the dosage, for a range of frequencies like that studied (that produced between 1000 and 4000 r units). But according to the theoretical calculations above, we should expect that at the lower dosages increase of the frequency of translocations should be more nearly according/

according to the square of the dosage. Muller and S. P. Ray-Chaudhuri (1939, in progress), working on the frequency of translocations with lower dosages of radium and X-rays than those used for this experiment, are apparently getting this result, i.e. an approximation to the frequencies varying as the square of the dose of irradiation.

The assumptions may now be discussed that were made before the theoretical calculations mentioned above were carried. The first assumption was that all breakage ends rejoin. This was tested by a special method (Muller, as yet unpublished) and found not to be the case. But the frequency with which the chromosomes fail to rejoin is too small to produce any serious effect on the results of the calculations. The second assumption was that the breakage ends rejoin at random. This means that the original recombinations, the viable gene rearrangements and the inviable rearrangements - acentric and dicentric/

dicentric chromosomes - have the same probability of occurring for each case of two breaks. It seems reasonable to suppose that there should not - other conditions being equal - be a preference for a breakage end to join with another forming viable (monocentric) rather than inviable (acentric or di- or polycentric) chromosomes. What instead is quite possible, and seems not unlikely to occur, is a greater tendency for breakage ends to rejoin in the original way, owing to proximity. This, however, would not alter the general shape of the curve.

The third assumption was that there was but one break per chromosome, so that we are dealing only with translocations. This assumption is actually unjustifiable and certainly incorrect, but it too will not seriously alter the general shape of the curve being dealt with, and as the recalculation which would be necessary in the absence of this assumption is a rather involved one (now being worked out in our laboratory), we are for the time being using Catcheside's/

Catcheside's table to illustrate the general trend expected in the results on the breakage theory.

Now we may turn to the contact hypothesis. According to this hypothesis, as mentioned before, one "hit" produces all the breaks required for any one primary gene rearrangement. If we assume that for each X-ray hit the contributions to nuclei with normal, inviable, and translocated chromosomes are, on the average, x, y, and z respectively, we can calculate the percentage of individuals with normal, inviable, and translocated chromosomes, and from that the percentage of translocation individuals among survivors, for different mean numbers of hits per nucleus. These probabilities could be obtained by applying the Poisson distribution algebraically to the expression in x, y, and z before proceeding to use numerical values of the Poisson coefficients. The probabilities thus obtained are:

$$\text{Normal} = e^{-n(y+z)};$$

$$\text{Inviable} = 1 - e^{-ny};$$

$$\text{Translocated} = e^{-ny} - e^{-n(y+z)}$$

(Dr. D. E. Lea, see Catcheside, 1938).

The/

The values are given in the following table:

Table No. 8 .

Mean Breaks per Nucleus	Percentage individuals with			Percentage Individuals Surviving	Percentage Survivors Translocated
	Normal Chromosomes	Inviable Chromosomes	Translocated Chromosomes		
0.05	96.75	1.64	1.61	98.36	1.64
0.1	93.61	3.25	3.14	96.75	3.25
0.2	87.63	6.39	5.98	93.61	6.36
0.4	76.80	12.37	10.83	87.63	12.36
0.8	58.98	23.20	17.82	76.80	23.20
1.6	34.78	41.02	24.20	58.98	41.20
3.2	12.10	65.22	22.68	34.78	65.35
6.4	1.46	87.90	10.64	12.10	88.00

Thus/

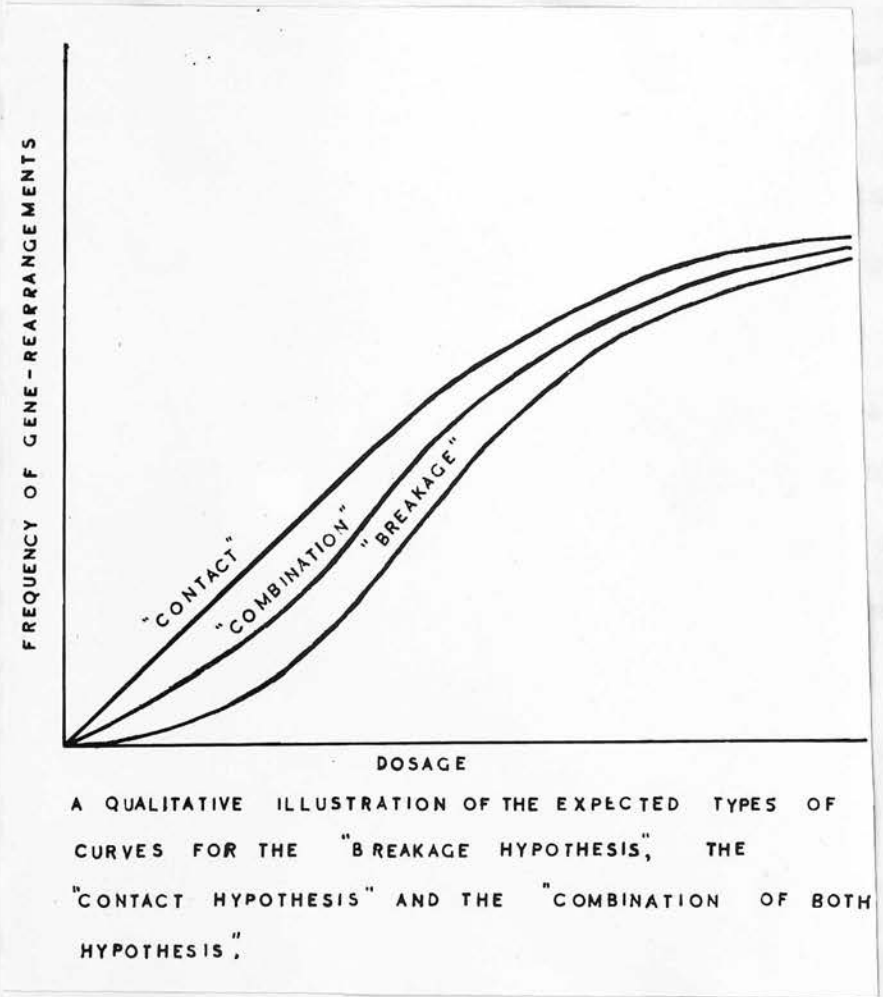
Thus, the curve resulting on the contact hypothesis is approximately linear in the very earliest part and thereafter becomes less than ~~the~~ linear, as the "saturation effect" (of two primary translocations forming one more complicated "secondary" one) is encountered. This then is exactly the same as the curve for gene mutations.

If the case is a combination between the breakage and the contact hypothesis, it will necessarily approximate more and more to the linear curve caused by the contact translocations as we follow it back towards the origin, and will partake more and more of the nature of a curve dependent on the breakage scheme alone as we follow it upward in the other direction from the region where it is more obviously intermediate in nature between the two.

A qualitative illustration of the expected types of curves for the "breakage hypothesis", the "contact hypothesis", and the "combination of both hypothesis" is given in Diagram No. 6.

Diagram No. 6./

Diagram NO 6



A QUALITATIVE ILLUSTRATION OF THE EXPECTED TYPES OF CURVES FOR THE "BREAKAGE HYPOTHESIS", THE "CONTACT HYPOTHESIS" AND THE "COMBINATION OF BOTH HYPOTHESIS".

Multiple breaks /

Multiple Breaks.

Under "Analysis of Results, and Conclusions" this question has been discussed at length.

As a result of comparing the frequencies of translocations involving the X-chromosome and two autosomes (i.e. requiring at least one break per chromosome) with that of translocations involving the ring-chromosome and an autosome (requiring at least two breaks within the same arm of one chromosome and one break in another chromosome), a provisional suggestion of a proximity effect on the union of broken ends was put forward. This is left open to contradiction or confirmation by further data and analyses.

Multiple-break rearrangements appear, in our data involving the ring-chromosome, to occur oftener in relation to double-break rearrangements than a random distribution would allow. This result too seems best explained as resulting from an effect of proximity on unionof ends. This could work in two ways to give a result like that observed. First, it might/

might make recombination, as opposed to restitution, more likely when two breaks were in the same chromosome arm than when they were in different chromosomes, and so favour translocations of the type which would be observable in ring-chromosomes as compared with ordinary "mutual" translocations. Secondly, it would happen that, in nuclei containing three or more breaks, if a recombination first took place between any end derived from one break and that derived from another break, there would then be a greater chance of any end derived from a third break undergoing recombination with one of the as yet ununited ends of the first or second break, than if the first step had been a restitution of one of the original chromosomes derived from the first or second break. For, if proximity counted in the unions, the greater proximity usually existing between ends derived from the same original break would ordinarily favour restitution as against recombination, hence when restitution of two ends had been prevented in the way above described, their/

their recombination with still other ends would be more apt to occur. As a result, multiple-breakage translocations would be more frequent in relation to double-breakage translocations than a random distribution would allow.

An Atypical Unique Case/

AN ATYPICAL UNIQUE CASE OF TRANSLOCATION OCCURRING BETWEEN PATERNAL AND MATERNAL CHROMOSOMES.

Out of the 10,196 L-R F_1 cultures, one - in the last series - gave a progeny showing an unexpected type of segregation of characters. This forms, in fact, the only case yet recorded of a very special type, which has for several years been an object expected, and for the obtaining of which special experiments had been devised[1] by those who favour the breakage hypothesis of rearrangements.

In this case all the males were ebony and the great majority of females were non-ebony. A translocation involving the paternal R and III chromosomes/

---

[1] Altenburg had in 1935, while on a visit to the Institute of Genetics, Moscow, proposed the institution of an experiment in which both males and females should be irradiated and bred immediately, with the aim of finding just such a case, as evidence for the breakage theory. Circumstances have till now, however, prevented the carrying through of such an experiment.

chromosomes would be expected to result in exactly the contrary, namely all the males would have been non-ebony and the females ebony.

The most reasonable explanation of this unique case appears to be the following:

(1) The ring-shaped X in the irradiated spermatozoon from which the translocation-containing F_1 male was derived was broken at two points by the treatment.

(2) The third chromosome, carrying the gene for ebony, in the egg which this spermatozoon fertilised, happened to have become broken spontaneously (not as a result of the X-ray treatment) at one point.

(3) After fertilisation, the interstitial fragment, detached from the ring, became inserted in the gap produced by the breakage of the maternal third chromosome, while the remainder of the X joined together again (deletion). This direction of the exchange is indicated by the presence of some exceptional - ebony - females among the progeny.

Less/

Less likely possibilities are: (1) Crossing-over between the third chromosomes in an embryonic cell of F_1 from which the entire germ tract was derived. Such crossing-over has been recorded on only two or three occasions in the whole of the Drosophila literature, and it would have to be supposed that in this case it happened to involve the very chromosome which had just beforehand undergone one of those rare recombinations that was of the type adapted to give translocations involving the ring. (2) Recombination between the paternal ring and maternal III occurring by means of a contact mechanism, presumably because of a considerably delayed after-effect of the treatment on the paternal chromatin.

It is obvious that, on account of the greater plausibility of the first explanation given, this case adds to the evidence in favour of the breakage hypothesis as opposed to that of contact. Because of this bearing, as well as because of its interest in relation to certain other points, such as that of a delayed union/

union of detached chromosomal fragments, it is being subjected to various tests, and a separate paper dealing with the results will be published in due time.

Summary./

Summary.

(1) The data obtained from a large-scale experiment on the frequency of translocations with two dosages of X-rays, one four times the other, are reported. A ring-shaped-X-chromosome as well as a non-ring-X-chromosome was used, to make possible a comparison between the frequencies of double- and multiple-break translocations.

(2) The data show clearly that the total frequency of translocations varies approximately as the 1.5 power of the dosage, for the range of dosages used (1000 r to 4000 r). The exponent of the dosage to which the frequency is proportional is definitely higher than one and lower than two.

(3) Both the breakage and the contact hypothesis are discussed in detail, and it is shown that the results under (2) are those to be expected on the breakage hypothesis only.

(4) A case is recorded in which translocation occurred between one chromosome (the ring X) of a treated spermatozoon and another (the third) of the egg/

egg which that spermatozoon fertilised. The treated chromosome underwent two breaks, the other probably but one. This case is held to give further support to the breakage hypothesis of translocations.

(5) The results indicate that multiple-break rearrangements occur oftener in relation to double-break rearrangements than a random distribution would allow.

(6) A method is given for estimating the relative frequencies to be expected for ring-chromosome translocations, and for translocations involving three ordinary chromosomes at once, if breakage and reunion of chromosomes took place at random. Applied to the present data they indicate that reunion occurs oftener between ends derived from the same than from different chromosome arms, but larger numbers will be needed to decide this question.

(7) A provisional suggestion of a proximity effect in the reunion of broken ends is put forward to explain the results under (4) and (5).

Acknowledgments./

Acknowledgments.

The author wishes to express his gratitude to Professor F.A.E. Crew for his kind hospitality and continuous encouragement, to Dr. H.J. Muller for his supervision, constructive criticisms and suggestions, and keen interest, and to Dr. G. Pontecorvo for his valuable and appreciated help in the mathematical part of the paper.

References Cited./

References Cited.

BAUR, H., DEMEREC, M., and KAUFMANN, B.P. 1938. X-ray induced chromosomal alterations in Drosophila melanogaster. Genetics, 23 : 610-630.

BELGOVSKY, M.L. 1937. Dependence of translocation frequency in Drosophila melanogaster upon the X-ray dosage. Trudy Inst. Genet., No. 11 : 93-124.

BUZZATI-TRAVERSA, A. 1939. Direct proportionality between X-ray dosage and translocations between the 2 and 3 chromosomes in D. melanogaster. Drosophila Information Service, No. 11 : 43.

CATCHESIDE, D.G. 1938. The effect of X-ray dosage upon the frequency of induced structural changes in the chromosomes of Drosophila melanogaster. J. Genet., 36 : 307-320.

CATCHESIDE, D.G. 1938. The bearing of the frequencies of X-ray induced interchanges in maize upon the mechanism of their induction. J. Genet., 36 : 321-328.

DUBININ, N.P. 1936. A new type of position effect. Biol. Ž. (Mosk.), 5 : 851-874.

DUBININ, N.P., and KHVOSTOVA, V.V. 1935. The mechanism of occurrence of complex chromosome rearrangements. Biol. Ž. (Mosk.), 4 : 935-975. (English text : 967-975.)

DUBININ, N.P., and SIDOROV, B.V. 1934. Relation between the effect of the gene and its position in the system. Biol. Ž. (Mosk.), 2 : 307-331.

HANSON, F.B., and HEYS, F. 1929. Duration of the effects of X-rays on male germ cells in D. melanogaster. Amer. Nat., 63 : 511-516.

HARRIS/

HARRIS, B.B. 1929. The effects of aging of X-rayed males upon mutation frequency in Drosophila. J. Hered., 20 : 299-302.

KAUFMANN, B.P., and HEITZ, E. 1937. Frequency of induced breaks in chromosomes of D. melanogaster. Proc. Nat. Acad. Sci. Wash., 23 : 484-488.

KHVOSTOVA, V.V., and GAVRILOVA, A.A. 1935. A new method of detecting translocations. Biol. Z. (Mosk.), 4 : 905-916.

KHVOSTOVA, V.V., and GAVRILOVA, A.A. 1938. Relation between the number of translocations in D. melanogaster and the X-ray dosages. Biol. Z. (Mosk.), 7 : 381-390.

KIRSSANOW, B.A. 1937. Der Entstehungsmechanismus der Translokationen bei Drosophila melanogaster. Biol. Z. (Mosk.), 6 : 837-906.

McCLINTOCK, B. 1938. The fusion of broken ends of sister half-chromatids following chromatid breakage at meiotic anaphase. Res. Bull. Mo. Agric. Exp. Sta., No. 290 : 1-48.

MORGAN, L.V. 1932. Genetic behaviour of a closed X-chromosome of Drosophila. Proc. VI. Int. Congr. Genet., 2 : 135-137.

MULLER, H.J. 1927. Artificial transmutation of the gene. Science, 66 : 84-87.

MULLER, H.J. 1928. The problem of genic modification. Verh. d. V. Int. Kongr. Vererb. Biol., 1.

MULLER, H.J. 1929. The first cytological demonstration of translocation in Drosophila. Amer. Nat., 63 , 689 : 481-486.

MULLER, H.J. 1930. Types of visible variations induced by X-rays in Drosophila. J. Genet., 23 : 299-334.

MULLER/

MULLER, H.J. 1932. Further studies on the nature and causes of gene mutations. Proc. VI Int. Congr. Genet. : 213-255.

MULLER, H.J. 1937. The biological effects of radiation, with special reference to mutation. Congrès du Palais de la Découverte, Paris, October, 1937.

MULLER, H.J. 1938. The remaking of chromosomes. Collecting Net, 13 : 181-195.

MULLER, H.J., and ALTENBURG, E. 1928. Chromosome translocations produced by X-ray in Drosophila. Anat. Rec., 41 : 100.

MULLER, H.J., and ALTENBURG, E. 1930. The frequency of translocations produced by X-rays in Drosophila. Genetics, 15 : 283-311.

MULLER, H.J., NAKKI, A.I., and SIDKY, A.R. 1939. Gene rearrangement in relation to radiation dosage. Address to Genetical Society, London, Dec. 1938. Abstract in Proc. Genet. Soc. 1939 and J. Genet., 37, No. 3.

MULLER, H.J., and PAINTER, T. S. 1929. The cytological expression of changes in gene alignment produced by X-rays in Drosophila. Amer. Nat., 63 : 193-200.

MULLER, H.J., PROKOFYEVA, A., and RAFFEL, D. 1935. Minute intergenic rearrangement as a cause of apparent "gene mutation". Nature, 135 : 253-255.

MULLER, H.J., and STONE, W. 1930. Analysis of several induced gene rearrangements involving the X-chromosome of Drosophila. Anat. Rec., 47 : 393-394.

OFFERMANN, C.A., and SCHMIDT, I.K. 1935. Culture media for Drosophila. Drosophila Information Service, No. 3 : 52-54.

OLIVER/

OLIVER, C.P. 1930. Effect of varying the duration of X-ray upon the frequency of mutations. Science, 72 : 44-46.

OLIVER, C.P. 1932. An analysis of the effect of varying the duration of X-ray treatment upon the frequency of mutations. Z. Indukt. Abstamm.- u. Vererblehre, 61 : 447-448.

PAINTER, T.S., and MULLER, H.J. 1929. Parallel cytology and genetics of induced translocations and deletions in Drosophila. J. Hered., 20 : 287-298.

PATTERSON, J.T., STONE, W., BENDICHEN, S., and SUCHS, M. 1934. The production of translocations in Drosophila. Amer. Nat., 68 : 359-369.

SAPIRO, M., and NEUHAUS, M. 1933. Comparative analysis of mutation in female and male Drosophila. Biol. Z. (Mosk.), 2 : 425-446.

SAX, K. 1938. Chromosome aberrations induced by X-rays. Genetics, 23 : 494-516.

SCHULTZ, J., and CATCHESIDE, D.G. 1937. The nature of closed X-chromosomes in Drosophila melanogaster. J. Genet., 35 : 315-320.

SERGEJEVSKY, A.S. 1929. A general scheme for the origin of mutations. Amer. Nat., 63 : 374-378.

STADLER, L.J. 1933. On the genetic nature of induced mutations in plants. Proc. IV Int. Congr. Genet., 1 : 274-294.

STADLER, L.J. 1933. Genetic effects of X-rays in maize. Proc. Nat. Acad. Sci. Wash., 14 : 69-75.

A CASE OF GONOSOMIC MOSAICISM INVOLVING A LETHAL.

A. R. Sidky.

TABLE OF CONTENTS.

A CASE OF GONOSOMIC MOSAICISM INVOLVING A LETHAL.

A. R. Sidky,
Institute of Animal Genetics, University of Edinburgh.

In a section of an experiment on the frequency of translocations, males of Beadle's Xc^2 and $\overline{\overline{yy}}$ stock - where the males have a ring-shaped X-chromosome which they always get from their father as the females have attached X-chromosomes - were X-rayed and then mated to virgin females $\overline{\overline{XXY}}$[1], i.e. they have attached X-chromosomes

[1] These females were yy bw e ey, i.e. they have attached X-chromosomes homozygous for the gene yellow and the three pairs of autosomes are homozygous for brown, ebony, and eyeless respectively. T stock was constructed by Muller in 1931.

X-chromosomes and a Y-chromosome. The F_1 heterozygous males, which as was to be expected were phaenotypically wild type, were backcrossed individually to the same type of female $\overline{XX}Y$. The F_2 cultures were examined for detection of translocations.

One of these F_2 cultures contained only females - about seventy were counted - and not one single male. An attempt to explain this unusual result had to be made.

In the cross mentioned above, as the mother has attached X-chromosomes the sons will receive their ring-shaped-X-chromosome from their father and the daughters their attached X-chromosome from their mother.. The absence of males in the culture can only be explained by the presence of a lethal on the ring-shaped-X-chromosome of the father.

This lethal might have been one of the following:

(1) A lethal mutation either dominant or recessive on the original material of the ring-shaped-X-chromosome;

(2)/

(2) An autosomal-dominant lethal mutation, a piece of that autosome which carried the lethal being included in the ring as a result of a translocation involving the ring and that autosome, or a piece of the ring being included in the autosome;

(3) A bobbed lethal mutation on the ring-shaped-X-chromosome.

The questions of how the mutation arose, when it arose, and how it was carried by the father without affecting him will be discussed later.

Out of the three possible types of lethal mutations mentioned above the second possibility was discarded from the beginning, as, judging by the random assortment of the characters in the F_2 culture, no translocation was involved.

As regards the third possibility we have to assume that the mother had no Y-chromosome (or at least no Y containing the normal allele of bobbed). So the putative sons will carry the bobbed lethal on their ring-shaped-X-chromosome which they receive from their father, but will get no Y-chromosome (or no/

no effective chromosome) from their mother to suppress the bobbed lethal. They will consequently fail to develop. In this case the daughters will be different from their mother in having Y-chromosomes of ordinary types which they receive from their father. If this was the case, when these daughters are mated to their father they would be able to supply their sons with Y-chromosomes. So we would expect such matings to give rise to sons carrying the bobbed lethal on their ring-shaped-X-chromosome but having a Y-chromosome which suppresses the lethal. But when these daughters were actually mated to their father, only females were produced. So the possibility of a bobbed lethal had to be ruled out.

The first possibility, therefore, seems to be the right one. A lethal mutation - dominant or recessive - on the ring-shaped-X- chromosome will be received only by the sons - the mother having attached X-chromosomes - which will fail to develop. The daughters will be like the mother, i.e. having attached X-chromosomes and a Y-chromosome. When they/

they were mated to their father the same thing was simply repeated. Unfortunately, owing to the nature of the cross - the females having attached X-chromosomes - it was impossible to ascertain whether the lethal was a dominant or a recessive one.

Now we come to more interesting and unusual facts. This F_1 lethal-containing male looked normal in every respect. It was quite fertile, as in spite of its being mated to one female in a small vial it produced seventy females. But it produced not a single male. That must have been the result of the lethal being present throughout the gonads. Every cell of the gonads must have carried the lethal gene on the ring chromosome. This case was designated "gonosomic mosaic", since the soma must, in part at least, have been without the lethal, else the male would not have survived. This name can be applied to similar cases.

This case is similar to that found by Agol (1931) where a male carried in his somatic (eye) cells the genes apricot and the normal allele of ruby and possessed/

possessed genotypically by a mutation both apricot and ruby. When this male was mated to $\overline{XXY}$ females, he produced none but white-appearing sons. This white-appearing character was shown to be the result of the interaction of the gene for apricot and the new mutant gene ruby. This new mutant must then have been present in all the cells of the gonads.

The first proved case of Drosophila mosaic involving the gonads was reported by Muller (1920). A male $\frac{R'}{tt}$ from a stock with red eyes had one red and one white eye. When he was mated to $\frac{tt}{tt}$ females with red eyes, all the offspring had red eyes. These were mated together in mass cultures and produced females all of which had both eyes red and males half of which had both eyes red and the other half both eyes white. The new factor for white, therefore, must have been recessive and sex-linked.

Another interesting case of mosaicism involving the gonads is that studied by Crew and Lamy (1937). This was a pseudo-obscura "triplo-X" female which was phaenotypically normal. She was a "triplo-X"/

"triplo-X" in the sense that the three chromosomes were present in the same cell. The result of crossing-over experiments showed that the most plausible explanation is that the two ovaries of this female differed in respect of their X-chromosome content, both possessing the grand-paternal X-chromosome but each having a different grand-maternal X-chromosome. It was later suggested by the authors that this female must have been the result of a double-nuclear fertilisation, i.e. of two X-bearing sperm with two female pro-nuclei of different constitutions. As this female was phaenotypically normal it was concluded that mosaicism was restricted to the germ cells and possibly other internal tissues.

Another case of mosaicism worth mentioning here is that of Panshin. It is a bilateral mosaic male of Drosophila melanogaster whose left eye showed the character lz^{sp} (a strong allele of the gene for lozenge, lz) and whose right eye showed the character lz^{wa} ("lozenge weak", a weak allele of lz). It resulted from a cross between an X-rayed a y f male

and/

and a $\overline{YY}$ female. It is attributed to simultaneous origination of the two different alleles of the lozenge gene in two halves of a split X-chromosome of a spermatozoon.

Now we turn to the question of how this condition came about. In Agol's case, he rightly excluded the possibility of the new mutation, ruby, having been produced by the X-rays, the reason being that he got his unusual result - none but white sons - in the F_1 of the cross of the treated apricot male with $\overline{XX}Y$ females. This male, then, must have already had the new mutation, ruby, in all the cells of his gonads before he was treated.

In this present case there is every reason to suppose that X-rays were the responsible factor. The unusual result - absence of males - was in the F_2 of a treated male. So it is probable that the lethal mutation was produced by X-rays in the ring chromosome of a spermatozoon of the treated P_1 male. This spermatozoon, after fertilisation, gave rise to the F_1 "gonosomic" mosaic of this case.

The/

The X-ray action in producing mosaics, as Muller (1927) suggested in interpreting the origin of mosaics, may be either a "fractional effect" or an "after effect". For the "fractional effect" we must assume that the ring-shaped-X-chromosome was in a split state at the time the spermatozoon of the F_1 male was X-rayed and that the lethal mutation occurred on one of the daughter chromatids. Later on, from the daughter cell carrying the lethal mutation the gonads developed. On the other hand, the lethal mutation might have been the result of an after effect of X-rays during the ontogenesis of the ovum fertilised by the treated sperm, the gonads developing from the daughter cell carrying the lethal.

No definite proof of "after effect" has been obtained as yet. Patterson produced by X-ray sex-mosaics, in which the male parts carried, in addition to the marked maternal X, a broken or deleted X-chromosome. He assumed that the gametic chromosome must be in the two-strand stage at the time of treatment if a part of the fly is thus to receive a broken/

broken daughter X-chromosome and another part a whole X-chromosome, but the "after effect" interpretation would be possible here too. An intermediate alternative, in all these cases, would be, as Muller has pointed out to me, to suppose that breakage occured at once in the unsplit chromosome of the spermatozoon, but reunion later, after splitting, and that the two pieces might reunite differently.

On the other hand, we cannot absolutely exclude the possibility that this lethal mutation occurred spontaneously in the F_1 heterozygous male at the early stage of his embryonic development, probably the first or the second zygotic division in one of the daughter chromatids. Since, however, X-rays are known to cause mosaics, they were probably responsible in this case also for the mosaicism.

This present case is also of interest in connection with the question of whether the origin of the gonads is uni- or multicellular. It may be recalled that when our F_1 gonosomic mosaic male was mated to $\overline{XX}Y$ females not one son was produced along with the seventy/

seventy daughters. Also, when the daughters were backcrossed to their father (the gonosomic mosaic), again no sons were produced. It was then concluded that all the cells of the gonads of this male must have carried the lethal on the ring-shaped-X-chromosome. This will be difficult to explain with a multicellular origin of the gonads in this case. It suggests a unicellular origin. Agol's case, too, supports this view. When he mated his apricot male, which was also a gonosomic mosaic, to $\overline{XXY}$ females, he got none but white sons.

As mentioned previously, the gonosomic mosaic of this case was phaenotypically normal. He looked quite healthy and was fertile. Yet the breeding experiments which were carried can only be explained on the assumption that all the cells of the gonads carried the lethal. But does this mean that all the somatic cells were free from the lethal? There seems to be no reason to suppose so. In the light of the evidence of random movement of nuclei in the embryonic development brought forward by Parker (1936) and by others/

others, it will be difficult to assume that the daughter cells carrying the lethal produced nothing but the gonads. It also does not seem improbable that the gonosomic mosaic of this case should survive carrying the lethal not only in his gonads but also in some of his somatic cells. The normal somatic cells - those free from the lethal - may be supposed to counterbalance the effect of the abnormal cells carrying the lethal and thus cause the survival of this mosaic male and his phaenotypic normality.

Patterson in his work on sex differentiation found that the male parts of the gynandromorphs tolerate a much longer duplication than was found to be the case in non-mosaics. In this case the gynandromorphs had a normal diploid half and a hyperploid male half, while the non-mosaics were X-hyperploids. He also found that hypoploid tissue will survive with longer deficiences in mosaic than in non-mosaic flies. These findings support the view mentioned above that the lethal gonosomic mosaic of this case might have had the lethal contained in some of his somatic cells and was/

was able to tolerate them because of his normal somatic cells.

Also in Agol's case it is likely that beside the gonads some of the somatic cells contained the new mutant ruby. The reason why the eyes of the mosaic male were apricot and not white-appearing can be explained by assuming that none of the somatic cells containing the ruby gene took part in the production of the facets of the eyes.

Summary /

Summary.

A mosaic male is reported on which had a lethal in the X-chromosome throughout the germinal tissue, as he produced many daughters but not one son when mated to females with attached X's. On the other hand, his soma must, in part at least, have been free from the lethal, otherwise this male himself would not have survived.

Accordingly, this case has been designated a "gonosomic mosaic". It is suggested that this name may be used for cases where mosaicism is due to a difference in genetical constitution between all or a part of the soma and all or a part of the gonads.

One possible explanation for this case is that the X-chromosome in the spermatozoon from which the mosaic was derived was in a split condition and that the lethal occurred in one of the daughter chromatids (most probably as a result of X-rays, as the father was irradiated).

Two/

Two other possibilities presented themselves - (1) an autosomal dominant lethal which got attached to the X-chromosome by a translocation, and (2) a bobbed lethal in the X-chromosome. Both of these were experimentally proved non-valid in this case.

The question of whether the origin of the gonads was uni- or multicellular in this case was discussed, and it was decided that they must have had a unicellular origin.

It was argued that though the lethal was on the X-chromosome throughout the gonads, it could not be the case that all the somatic cells were free from the lethal. Some of them must have contained the lethal, and the normal healthy condition of the gonosomic mosaic was probably due to his being able to tolerate the lethal because of the part of the soma that was free from the lethal. It is suggested that the same could be applied to other similar cases.

acknowledgment

Acknowledgments.

An acknowledgment of indebtedness to Professor F.A.E. Crew for his kind hospitality and to Dr. H.J. Muller for his keen interest and constructive criticism is sincerely expressed.

References Cited./

REFERENCES CITED.

AGOL, I. N. 1931. A case demonstrating the absence of somatic induction in Drosophila. Amer. Nat., 65 : 88-92.

CREW, F.A.E., and LAMY, R. 1937. A fertile and phaenotypically normal triplo-X female of Drosophila pseudo-obscura (Race A). J.Genet., 34 : 91-96.

CREW, F.A.E., and LAMY, R. 1938. Mosaicism in Drosophila pseudo-obscura. J. Genet., 37 : 211-228.

MULLER, H. J. 1920. Further changes in the white-eye series of Drosophila and their bearing on the manner of occurrence of mutation. J. Exp. Zool., 31 : 443-473.

MULLER, H. J. 1927. Artificial transmutation of the gene. Science, 66 (1699) : 84-87.

MULLER, H. J. 1928. The problem of genic modification. Verh. d. 5. intern. Kongresses Vererbung, Bd. 1.

PANSHIN, I. 1935. The analysis of a bilateral mosaic mutation in Drosophila melanogaster. Trud. Lab. Genet., No. 10 : 227-232.

PARKS, Hal B. 1936. Cleavage patterns in Drosophila and mosaic formation. Ann. Ent. Soc. Amer., 29 : 350-392.

PATTERSON, J. T. 1933. The mechanism of mosaics formation in Drosophila. Genetics, 18 : 32-51.

PATTERSON/

PATTERSON, J. T. 1938. Sex differentiation. - Amer. Nat., 72 : 193-206.

TIMOFEEF-RESSOVSKY, N. W. 1931. Does X-ray treatment produce a genetic after effect. J. Hered., 22 : 221-223.

GENETICAL STUDIES

ON THE "SPHEROIDAL" MUTANT

OF DROSOPHILA FUNEBRIS.

A. R. Sidky.

TABLE OF CONTENTS.

GENETICAL STUDIES ON THE "SPHEROIDAL" MUTANT OF DROSOPHILA FUNEBRIS.

A. R. Sidky.

Introduction.

The mutant "spheroidal" which is the object of study in this paper was first noted by Donald and Lamy (1937) while studying the fecundity of Drosophila funebris. One female was found to give only a few eggs and these were abnormal in shape. Some of her daughters exhibited the same character.

The type of inheritance of the character "spheroidal" and the differences between the normal and the spheroidal eggs were studied (Crew and Auerbach, 1937). It was found that spheroidal (sph) was/

was a simple Mendelian recessive. The spheroidal egg is distinctly shorter and broader than the normal egg. The filaments are short, stiff, and undulated. The spheroidal eggs exhibit much less uniformity than do the normal eggs. Sometimes, particularly at the end of the spheroidal female's short laying period, the eggs are very pale, yellowish, and transparent, so that keen observation is required to detect them in the food.

A comparison of the fecundities of spheroidal and wild-type was made (Crew and Auerbach, 1937). The fecundity of sph was found to be very low, with a total output of about fifty eggs, in contrast to over one thousand for the wild-type. Also, a great difference in the fecundity curve was observed. The spheroidal female usually starts with her maximum performance, which is followed by a more or less rapid decrease. Egg-laying stops in less than a week./

week. - The wild-type female on the other hand reaches her maximum gradually, and this is followed by a progressive decrease.

The Problem.

Egg-size, egg-shape, and fecundity being quantitative characters, the degree of manifestation could be measured more accurately than is generally the case with purely qualitative characters. Such quantitative characters may prove very useful in studying the action of the gene. It was therefore decided to carry out a genetical analysis of it as far as possible.

In this paper the following points were studied:

(1)/

(1) The determination of the linkage group of the spheroidal gene;

(2) The establishment of the genetic independence or otherwise of low fecundity and abnormal egg-shape; and

(3) The determination of the linkage relations between these two characters, if they proved to be separable.

The Technique.

The genetical tests mentioned above were rendered difficult by the fact that "spheroidal" is a character which becomes visible only in the eggs laid by the female. No means were found to determine the genotype of females from their morphology. The/

The females had therefore to be cultured individually and the egg-shape observed. The only means of determining the genetical constitution of a male is to mate him to a sph female and observe the eggs laid by his daughters. The low fecundity of the sph females makes these tests still more difficult.

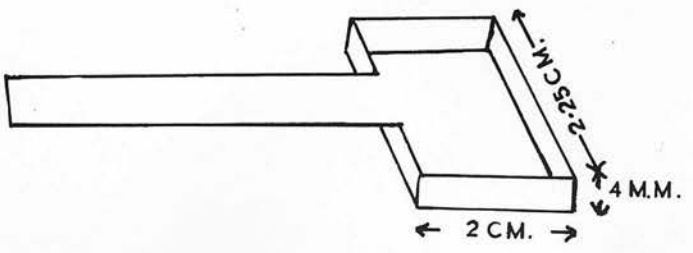
For observing the egg-shape, the females were made to lay their eggs on spoons containing food. The food used was prepared according to the formula suggested by Offermann and Schmidt (1935).

For carrying out genetical tests on the low fecundity character, egg counts were made at fixed intervals. Conditions such as temperature, handling, kind of food, amount of food (that is to say, the surface exposed), seeding with yeast, were kept as nearly identical as possible.

A special spoon was constructed by the author, as well as a method for seeding with yeast, as described below:

The/

- The spoon is made of aluminium of about 5 mm. thick. Its shape and dimensions are shown in the illustration.



NEW SPOON FOR EGG COUNTING.

The width of the spoon is so planned that it just fits the vials used. The one hundred and fifty ordered by the Institute were supplied at one penny each. The food is poured into the spoons in a liquid state through a funnel fitted with a rubber tube and a clamp. The food takes about one minute to harden. A large number of spoons can be prepared in a very short time, so that a few days' supply can be prepared and kept in a refrigerator. This spoon is superior to the paper ice cream spoon commonly used/

used in the following respects: (1) The main advantage is that, the surface being level, one can count all the eggs with one focussing and so save much time. (2) The food is of uniform thickness and the edges do not get dry and make counting or collecting the eggs difficult. (3) The food does not stick to the spoon, and when it is to be removed for incubation, etcetera, it can be lifted completely out of the spoon by merely inserting the point of a blade or a needle. No eggs are lost or crushed in the process. (4) Being made of aluminium it is very durable, stands any amount of boiling and sterilizing, and is very easily cleaned. It does away with the recoating with paraffin which is necessary with the paper spoons. The counting is done by marking the surface of the food with a needle and so dividing it into two or three rectangular areas according to the field of the binocular, and passing the spoon backwards and forwards while counting.

Instead of the usual method of allowing a drop/

drop of yeast to fall on the food, which results in the yeast growing into a large lump containing many eggs and so renders the counting difficult, painting the surface of the food with a thin suspension of yeast, using an ordinary small camel hair brush thus providing a uniform thin film of yeast, gave very good results. (Drosophila Information Service, No. 11, January 1939 : 55-56.)

The location of the chromosome carrying the gene for spheroidal.

The first part of the experiment was devoted to ascertaining which of the six pairs of chromosomes of Drosophila funebris carries the gene for spheroidal. A stock carrying the marker genes st cv ri vti^2 (obtained from Professor Timofeeff-Ressovsky, Berlin) was/

was used.

st = scarlet eye colour (second chromosome);

cv = curved wings (third chromosome);

ci = cubitus interruptus (third chromosome);

ri = radius incompletus (fourth chromosome);

vtf^2 = venae transversae incompletae (fifth chromosome).

The procedure of the matings was as follows, and is illustrated below by a diagram which shows also the actual result obtained.

Females homozygous for all the recessive marker genes were crossed to homozygous sph males. The F_2, being heterozygous for the marker genes and for sph, were wild-type in appearance, and the females laid normal eggs. Since a stock combining the marker genes and sph in homozygous condition was not available, the testing of recombination by the backcross method had to be carried out in two steps. The first consisted in crossing F_1 males - in which independence of linked genes cannot be simulated by crossing-over/

crossing-over - with females of the multiple recessive stock. Since their spermatozoa carry one chromosome only of each pair, there will be recombination of sph only with those genes with which it gives free assortment. The female progeny of this first backcross was, as expected, fifty per cent homozygous sph, the other half carrying sph in heterozygous condition. All those females were in addition heterozygous for one or more of the marker genes, but in the homozygous sph females the marker gene on the sph chromosome was necessarily missing, because it could not be furnished by the sperm. The second step of the backcross test consisted in mating $\frac{sph}{sph}$ as well as $\frac{sph}{+}$ females of this generation to males of the multiple recessive stock.

Excluding the rather unlikely possibility of sph being situated on the small sixth chromosome, one of the characters used (in the case of the third chromosome, two) would be expected to be lacking in the progeny of homozygous sph females. When the offspring/

offspring from females laying sph and normal eggs and mated to multiple recessive males was classified separately, this result was actually found in respect to vti^2, proving that sph is located on the vti chromosome, i.e. the fifth chromosome (Table No. 1).

From Table No. 1 we see that approximately half of the females gave spheroidal eggs and the other half gave normal eggs. Also we see that none of the progeny of any of the homozygous sph females showed the vti^2 character, while among the progeny of every female which laid normal eggs the vti^2 character was observed. On the other hand, free recombination with the other characters, i.e. st cv ci ri, was obtained. This showed conclusively that the spheroidal gene is on the same chromosome as the vti^2, namely the fifth chromosome.

Diagram illustrating matings./

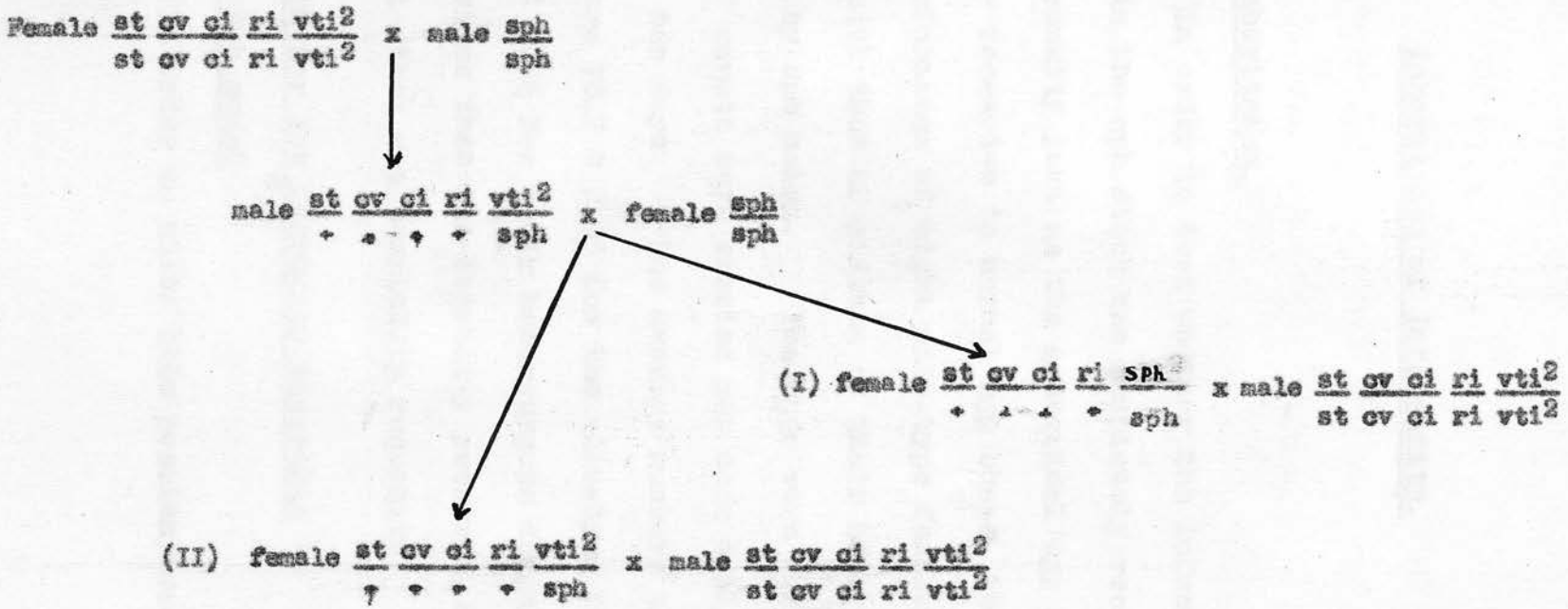
(Over)

Table No. 1.

The offspring from females laying spheroidal and normal eggs and mated to multiple recessive males are classified separately. Note that none of the progeny of any of the females laying spheroidal eggs - homozygous for sph - showed the vti^2 character, while this character was observed among the progeny of the females which laid normal eggs - heterozygous for sph.

	st								st*								
	cv ci				cv* ci*				cv ci				cv* ci*				
	ri		ri*		ri		ri*		ri		ri*		ri		ri*		
	vti^2	vti^{2*}	vti^2	vti^{2*}	vti^2	vti^{2*}	vti^2	vti^{2*}	vti^2	vti^{2*}	vti^2	vti^{2*}	vti^2	vti^{2*}	vti^2	vti^{2*}	
Spheroidal		1		1		1		1		1		1		1		1	To-tal
		1		1		1		1		1		1		1		1	
		1				1		1		1		1		1			
		1												1			
		1															
Total		5		2		2		3		3		3		4		2	24
Normal	1		1		1		1		1		1		1		1		Total.
	1		1		1		1		1		1		1		1		
			1		1		1		1		1		1		1		
			1				1		1		1				1		
							1								1		
Total	2		4		3		5		4		4		3		5		30

Diagram illustrating the matings and the actual result obtained.



The character vti^2 could obviously appear only among the progeny of this backcross.

Inheritance of Infecundity.

Type of inheritance.

In order to test whether the infecundity observed in the sph stock was completely recessive to normal fecundity just as the spheroidal egg shape was completely recessive to normal egg shape, the egg laying performance of eight wild-type females was compared with that of sixteen of their heterozygous daughters by sph males. The eggs were collected on spoons and counts were carried out once daily over a period of ten days. The average numbers of eggs per day were 78.7 ± 2.05 for the wild-type females, and 79.7 ± 1.66 for their heterozygous daughters. It was obvious that the infecundity occurring in the spheroidal stock was completely recessive.

Is the gene for low fecundity separable from the sph gene?

In order to solve this problem, males and females/

females from a cross $\frac{sph}{sph}$ by wild-type were back-crossed to sph flies. From the male backcross there resulted a number of daughters, seventy three of which were tested for egg shape and fecundity. Since no crossing-over occurs in the male, the abnormalities of egg shape and fecundity could only be found to separate if they were due to genes on different chromosomes. The actual results were thirty four females laying sph eggs, thirty eight females laying normal eggs. All females in the first class were infecund, with an average life performance of 54 $\pm$ 3.2 eggs; all females in the latter class were fecund, with an average eight-days performance of 557 $\pm$ 11.8 eggs. It was obvious that no separation between the abnormal egg-shape and low fecundity had occurred. This meant that the two characters were due either to one gene or to two linked genes. The female backcross made it possible to decide between these two alternatives. Among a total of forty five daughters examined for egg shape and/

and fecundity over a period of fourteen days, it yielded twenty six laying normal eggs, and nineteen, spheroidal. The deviation from the expected 1 : 1 ratio - 3.5 - is not significant, the standard error being $.5 \times \sqrt{45} = 3.4$. Of the nineteen sph females, three were fecund, and ~~they must~~ were assumed to have arisen through crossing-over between the genes for spheroidal and fecundity, which must therefore be located on the same chromosome pair. The reciprocal cross-over (normal infecund) was not found, but the figures are too small to decide whether the non-appearance of this class is simply due to chance or to an underlying interaction of genes.

The results described above are obviously susceptible of more than one interpretation. The fecund spheroidal flies obtained may have really been the result of crossing-over as suggested above; in the absence of the reciprocal class, however, this interpretation is open to doubt, until the idea of a specific/

specific interaction between the two genes which may be involved is established. Again, there is nothing to show that the infecundity associated with the spheroidal gene had not been merely affected by the introduction of specific modifiers in the course of the experiment.

A third possible explanation is that the "spheroidal-infecund" gene may be to some extent mutable in the direction of fecundity.

Further experiments may make it possible to decide which of these hypotheses is correct.

Summary./

Summary.

The mutation spheroidal of *Drosophila funebris*, which affects egg-size and was associated when first studied with low fecundity, was subjected to further genetical studies:

(1) The gene for spheroidal (sph) was found to be on the fifth chromosome.

(2) Both the characters spheroidal egg shape and low fecundity were found to be of the same linkage group.

(3) In order to find out whether the two characters were due either to one gene or to two linked genes, a female backcross was carried out. Only one class of cross-over, namely spheroidal fecund, was observed. Alternative interpretations are put forward, and further study is suggested to decide which is correct.

Acknowledgments./

Acknowledgments.

The author is grateful to Professor F.A.E. Crew for his hospitality and continuous encouragement, to Miss Auerbach for her advice and help, and to Miss Lamy for her valuable criticism.

References Cited./

References Cited.

BRIDGES, C.B., and MORGAN, T.H. 1923 a. The third chromosome group of mutant characters of Drosophila melanogaster. Publ. Carnegie Inst. Wash., No. 327 : 1-251.

CREW, F.A.E., and AUERBACH, C. 1937. "Spheroidal" a mutant in Drosophila funebris affecting egg-size and shape and fecundity. Proc. Roy. Soc. Edinburgh, 57 : 255-268.

OFFERMANN, C.A., and SCHMIDT, I.K. 1935. Culture media for Drosophila. Drosophila Information Service, No. 3 : 4.

SIDKY, A.R. 1939. New spoon for egg counting and method for seeding. Drosophila Information Service, No. 11 : 55-56.