RESEARCHES ON THE MECHANISM OF INDUCED CHROMOSOME REARRANGEMENTS IN DROSOPHILA MELANOGASTER.

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CONTENTS.

THE PROBLEM

The problem of the way in which structural changes of chromosomes, that is, changes in the linear arrangement of their genes, are brought about has recently had much light thrown upon it through a series of studies on the effects of high energy radiation.

The latest works - genetical ones by Muller and co-workers (1938, 39, 40) including Belgovsky (1939) on Drosophila and cytological ones by Bauer and co-workers (1938, 39) also on Drosophila (salivary glands) and by Sax (1938, 39, 40) and Faberge (1940) on Tradescantia micropores - to quote only the most important - leave no doubt that gross structural rearrangements induced by radiation come about by a process occurring in two distinct stages: a) primary changes, produced by individual ionisations, and b) combination of the primary affects. It is inferred that these two stages consist respectively of: a) breakage of the chromonemata in at least two points, and, b) fusion of the broken ends two by two. Presumably this fusion may occur either in the original way (restitution), in which case the effect of breakage is only temporary, or in a new way, giving a viable or inviable rearrangement. There is evidence

that an individual successful ion or pair of ions produced by high energy radiation - no matter of what wave length within the range from radium rays to soft X-rays - is responsible for the production of a break. One of the aims of the present paper is to present further data in support of this proposition.

The breakage frequency, however, varies for different parts of the chromonema, being higher for heterochromatic regions (Muller & Prokovyeva, 1935; Muller et al. 1937; Belgovsky & Muller, 1937; Belgovsky, 1938; Kaufmann, 1939; Prokovyeva & Khostova, 1939). It seems not to be influenceable by temperature, a fact which argues for a localized effect of the successful ion (Muller, 1939).

Evidence from different angles suggests that - provided the above view on the mechanism of structural changes be correct - the rejoining of broken ends is limited in space, in the sense that the position of two breaks relative to one another influences the chance of occurrence of a rearrangement (Sax, 1940; Bauer, 1939; Muller, 1938, 39).

In Tradescantia micropores protraction of the period in which a certain total dose is given, either by the reduction of its intensity with a compensatory increase in its duration, or by its fractionation into two or more portions with intermediate resting periods, has the effect of reducing the number of recombinations,

i.e. of favouring "restitution" a fact which shows that in this material the time between the primary and the secondary stages of the process - presumably breakage and fusion - is limited. In particular Sax (1940) succeeded in determining the maximum time-spacing between two fractions of the dose after which a further increase in the spacing does not reduce the frequency of rearrangements any more. This time was about 1 hour in his material: this means that within this relatively brief time, all the broken ends capable of fusion have rejoined - a small remainder being no longer capable of it - or at least that all the "primary changes" have interacted or become incapable of interaction. The same author, in collaboration with Enzeman (Sax & Enzeman, 1939) and Faberge (1940) have shown that a rise in temperature affects the frequency of recombinations in this material in the same way as a protraction of the time of treatment, no doubt because of the increased activity of combination induced in a given time.

In respect of both effects of protraction of the treatment in time and of temperature on the rejoining of broken ends, Drosophila sperm behaves in an exceptional manner. As for the time factor, the results of Muller and co-workers (Muller, 1939, 40) are quite striking: no difference whatsoever was obtained either by protracting the treatment up to

one month by fractionation, or by varying the intensity from 0.05r up to 250r per minute or by ageing the treated sperm for one month before fertilization. Results of Catcheside (1939) on fractionation agree with these. All these rather astonishing results could be explained only by assuming that in the sperm the primary changes (presumably the broken ends) retain for an indefinite time their full capability of interacting (joining together), and that the interaction (rejoining) does not occur until the fertilization of the egg or some time after (Muller 1938, 39, 40). Proof of this from another direction was given when a translocation was found between a chromosome of an irradiated sperm and one, presumably broken spontaneously, of the egg fertilized by it (Sidky, 1939). The different behaviour of the affected chromosomes (presumably of their broken ends) in sperm as compared with cells of other types, must be attributed to some temporary biochemical condition.

In harmony with this inability of broken ends to unite during the spermatozoon stage, it has been found by Muller and co-workers (Muller, 1939, 40) that differences in temperature applied over a long time at this stage fail to affect the frequency of recombination.

One of the ways, whereby the mechanism of structural changes has been investigated by different

authors, is through a study of the curve relating the frequency of induced rearrangements to the dosage of irradiation. Most of the contrasting ideas championed - up to less than a year ago - by the different authors, derived from the fact that this dosage-frequency curve expresses the results of a complex relationship between 1) the primary effects of irradiation, presumably the individual breaks, 2) the many possible ways in which the affected chromosomes can join with each other, and 3) effects of differential viability, some of which are dependent, and some not, on the type of rejoining.

In Drosophila for instance, results by genetical methods showed that between 1000 r and 4 or 5000 r the dosage-frequency relationship was expressed by an exponential curve, passing through the origin, and having an exponent higher than 1 and lower than 2. Now, at least, if two primary changes, independently produced, are necessary for a gross rearrangement and if each results from a single ionisation it was expected that the frequency of rearrangements should increase approximately with the 2nd power of the dosage of irradiation. On the other hand, on the so-called "contact" hypothesis, advocated by Serebrovsky, according to which rearrangements are produced when two chromosomes, or two different regions of the same chromosome, not yet broken but already in contact,

are affected by the radiation, an increase of the frequency of rearrangements roughly proportional to the 1st power of the dosage would have been expected, supposing the exchange to be induced by a single ionisation.

Valuable contributions to the solution of this problem were given by Stadler (1936), Haldane (1935-written communication to Muller) and Catcheside (1938). The latter theoretically worked out tables showing the expected results of the interaction of the different effects already described, if certain limiting assumptions are made to simplify the problem. These tables, when considered in a more extended and generalised form as worked out by Muller in collaboration with the writer, show that, on the hypothesis of each break being independently produced by one ionisation, the curve relating the dosage with the frequency of obtained rearrangements (of the type of reciprocal translocations) among total surviving individuals should be an S-shaped one. At the lowest part, the curve should have an exponent approaching 2, and this exponent should gradually diminish to 1 and even less as the dosage increases. Thus the power of approximately 1. 5 experimentally found, between 1000 and 4 or 5000 r, becomes explainable. In addition, experimental evidence that at very low dosages the power approaches 2 has lately been produced by Muller and

co-workers (Muller 1939, 40).

Catcheside's calculations were based on three assumptions which admittedly do not hold: 1) that only one break per chromosome is produced; 2) that fusion between broken ends is random; 3) that no broken end fails to rejoin with another broken end. For each break, three ways of fusion were recognised as possible: a) with the other broken end at the same breakage point, i.e. reconstituting the original arrangement (restitution); b) with a broken end of another chromosome, complementary in respect of having a centromere or a telomere (eucentric translocation); c) with a broken end of another chromosome, not complementary (acentric-dicentric, i.e. aneucentric translocation). The latter, and only these, were considered inviable combinations.

Assumption 1) is evidently only a working postulate, made for the purpose of simplifying the calculations and, for low dosages at least, it could not influence the results greatly. That Assumption 2) cannot hold is suggested by evidence obtained by Muller and co-workers, Catcheside, Bauer and Sax independently, by different methods. In fact the "proximity" of two breaks increases the chance of a rearrangement, decreasing that of "restitution". As for Assumption 3) few pertinent data have hitherto

been available. One of the aims of the present investigation concerns itself with this point, being to ascertain whether or not, and, if the answer be the affirmative, then with what frequency broken ends induced in the sperm of Drosophila melanogaster fail to rejoin, either in the original or in a newway, with other broken ends.

Experimental evidence obtainedmby Muller et al. (1937), Belgovsky & Muller (1937) and Belgovsky (1938) through an appropriate genetic technique, has confirmed the idea, that in Drosophila chromosomes without telomeres are either not formed by the action of radiation, or, what is far more likely, that they are generally not capable of functioning indefinitely through a period in which a number of cell divisions have occurred. Prokovyeva (1937) in addition, showed cytologically that various cases of apparent terminal deletions in the X, giving lethal y ac deficiencies, located very near the left end of this chromosome, were actually intercalary deletions, the very terminal bands being still present. A later genetical analysis by Raffel (1938) of the same stocks, showed that lJ^{1}, a lethal located still more terminally than the "deficient" y ac, is in many cases still present, thus confirming the inference that these cases are really other minute deletions or minute rearrangements. Some additional evidence of the same kind is

given in the present paper. In plant material (maiz) on the other hand, single-break deletions may be produced and the resulting truncated chromosome be capable of normal functioning as if the broken end assumed the unipolar properties of a regular "telomere" (Stadler, 1939; McClintock, 1939). As some results of Sax (1939) on Tradescantia point in the same direction, it is possible that this constitutes one of the general differences between the properties of the chromosomes in higher plants and animals, though the case of Ascaris shows that animals too can in special cases have facultative telomeres.

Taking it as sufficiently proved that chromosomes without a permanent telomere are never found in Drosophila, it must be asked why this is so. One possible answer would be that a broken end never fails to rejoin with another broken end either its previous partner or one derived from a different break. But if this is not so, i.e. if in some, more or less frequent cases, a broken end fails to join in either of the above ways, the two parts (one acentric and the other monocentric) of the broken chromosome must get lost by some mechanism or be lethal to the zygote.

The cytological and genetical works of McClintock (1938 a,b) on maiz, and the cytological one of Carlson (1938) on Cortophaga are suggestive of what the mechanism of loss may be: namely when the acentric

and the monocentric parts of the broken chromosome undergo mitotic division, the resulting sister chromatid fuse at the point of breakage and respectively give a V-shaped acentric fragment of double size and a dicentric chromosome. The acentric fragment may be transmitted for a few cell generations, but sooner or later becomes lost in the cytoplasm. The dicentric chromosome results in the formation of an anaphase bridge. McClintock (1938) had already proved that in maiz, at least when the dicentric chromosome is ring-shaped, repeated breakages occur at the anaphases of successive mitoses, so that the dicentric ring changes in size, is not necessarily lethal, for the tissues carrying it, and eventually is lost. Other evidence by the same author showed that small- sized ring chromosomes are very easily lost through lagging at anaphase. On McClintock's interpretation, if the movement towards opposite poles is initiated by forces acting at the centromere region but continued by other forces exerted on the chromosome when they are some distance away from the equatorial plate, these small dicentric rings may be too small to reach this distance. Thus the final process of loss of an <u>initially</u> large dicentric ring may be of the same kind. In fact every time that anaphase breakage results in the formation of one small and one large daughter ring, the former has a

high chance of being lost. A process of this kind has not yet been followed through successive cell generations in other material and for dicentric non-ring chromosomes; it is therefore a working hypothesis to assume that, in its main lines, some such process may take place in Drosophila and both with ring and non-ring chromosomes. Further, the possibility must be considered that in Drosophila breakage of the dicentric does not occur. In this case an alternative method of loss would be the failure of the dicentric chromosome to be included in either daughter nucleus because of delay or failure to break as in the case of McClintock's small rings.

Now, if the ends derived from breaks produced by irradiation in the chromosome of Drosophila sperm sometimes fail to rejoin in time, a fusion of sister chromatids at the point of breakage may occur at the first splitting following irradiation, and either cause immediate loss or start the process of repeated breakage at anaphase. As these cases, if they exist, should be produced by single breaks, in contrast with all gross rearrangements which, on the hypothesis we favour, require two or more breaks, the dosage-frequency relationship should be different from that of the latter. If the "primary changes" already mentioned are really the breakages then these losses, being due to single breakage, should vary in frequency no more than the first power of the dose. The finding of such

a result - contrasting so much with that found for gross rearrangements - would thus help to fill the gaps in the structure of evidence in support of the "breakage first" theory of chromosome rearrangements.

The main group of problems which it is proposed to investigate here is accordingly as follows:
a) whether or not irradiation of the Drosophila sperm gives an appreciable proportion of chromosome losses referable to broken ends which fail to rejoin, either in the original way or with ends from other breaks;
b) if the answer be the affirmative, what is the frequency-dosage relationship for these losses and what light may it throw on the validity of our interpretation that losses are due to simple breaks; and
c) what light may it throw on the interpretation of the shape of the frequency-dosage curve for gross rearrangements. On the technical side the problem is therefore that of detecting losses of whole chromosomes, produced when a dicentric chromosome originates by fusion of sister chromatids broken at the same point.

In connection with the technical problem just mentioned, another consideration arises. Following irradiation dicentric chromosomes and acentric fragments are undoubtedly produced in great numbers by fusion not between chromatids of the same chromo-

some, but between different chromosomes, as a result of two break rearrangements of the type known in Drosophila literature as translocations. A technique which allowed the detection of these two-chromosomes losses also, might show whether or not the behaviour of the two types is the same in respect of resulting in chromosome losses or in effects upon the viability of the zygote.

EXPERIMENTAL PART

A) POSSIBLE WAYS OF ATTACK.

Among the possible methods of detecting genetically the loss of a chromosome, the first one would think of is the utilisation of the mechanisms of sex determination(x) In fact, the loss of the X chromosome could be detected by its effects on the sex-ratio and that of the Y by its effects on the fertility of the males.(Bridges 1916; Safir 1921; Stern 1929; Stern & Ogura 1931; Neuhaus 1939).

Both criteria however present serious practical difficulties, one of which is the great number of cultures required to obtain statistically sound results if the frequency of induced losses is low relatively to the normal variation of the sex-ratio or to the frequency of spontaneous cases of sterility in the males. Another is that effects simulating losses can be produced in several ways. Methods based on the sex-ratio or upon effects on fertility in the males were therefore discarded and other ways of attack for detecting losses of the X or Y chromosome and of the autosomes, respectively, were considered.

(x) A method of this type has in fact been used by Bauer (1939) in a paper recently issued. See. "Discussion".

The first criterion, for detecting losses of X or Y chromosomes, is based on the possibility of distinguishing attached -X females carrying a Y from those which do not carry it, by two genetical means:
1) by the action of the extra Y, usually present in attached -X females, in suppressing or reducing the variegated phenotypical manifestation of the so called (Muller 1930, 1935) "eversporting displacements", that is, of genes connected with large chromosome rearrangements with one break in heterochromatic regions;
2) by the use of markers that have been attached to the Y chromosome.

Another way, for detecting losses in the autosomes, utilizes the well known fact that segregation in triploid females gives origin to a considerable portion of eggs, disomic for one but not for both large autosomes. (Bridges 1921; Mather 1935; Darlington 1937; Sturtevant & Beadle 1939). These eggs, when fertilised by a sperm containing the haploid set, produce inviable zygotes (trisomic for one autosome, but not for both). If one of these eggs, instead of being fertilised by a normal sperm, were fertilised by a treated sperm in which the chromosome that was in excess in the egg had been potentially eliminated, it would seem conceivable for a viable (diploid) zygote to be

obtained. It is easy to imagine how, with different markers in the triploid mothers and in the diploid treated fathers, the flies arising through such a process could be detected.

B) GENETICAL TECHNIQUE ADOPTED FOR DETECTING LOSSES OF THE X AND Y CHROMOSOMES.

Attached -X females ($\underline{XX}/Y$)[X] produce two kinds of eggs: one carrying the $\underline{XX}$, the other carrying the Y chromosome. The first type of eggs give origin, when fertilized by a Y bearing sperm, to a $\underline{XX}/Y$ female matroclinous for sex-linked genes, and, when fertilized by an X bearing sperm, to a $\underline{XX}/X$ (triplo-X) female. The triplo-X females have very low viability and seldom reach the image stage. The second type of egg produced an inviable zygote (YY) when fertilized by a Y bearing sperm, and a X/Y male, patroclinous for sex-linked genes, when fertilized by an X bearing sperm. In conclusion therefore only half the zygotes from $\underline{XX}/Y$ females mated to X/Y males develop regularly, namely the $\underline{XX}/Y$ daughters

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(X) As recommended in D.I.S., underlining indicates, in this notation, attachment of the two parts underlined. When the two parts are homozygous, only one may be indicated: for instance $\underline{y\ v\ f}$ means attached -X homozygous for y v f.

(matroclinous) and the X/Y sons (patroclinous).

If loss of the X chromosome can be induced in an X-bearing sperm and this happens to fertilize an XX egg, the resulting zygote will be a perfectly viable XX/O female, in general phenotypically indistinguishable from an XX/Y one. The same result will be obtained if the loss of the Y chromosome is induced in a Y-bearing sperm. Then the possibility of detecting losses of X or Y chromosomes induced in the sperm, depends upon suitable methods of distinguishing, by inspection, XX/O from the ordinary XX/Y females.

The use of the sc Y^L chromosome.

An excellent opportunity in this direction was provided by the special scY^L chromosome found by Miss Lamy (Crew and Lamy, 1940), which probably originated by crossing over between the short arm of a Y and the distal end of the inverted sc^{Sl} X-chromosome. (see also: Sidorov 1939. M.S.) It was described as a rod-shaped chromosome with nearly terminal spindle fiber attachment carrying the whole set of fertility genes of the long arm of the Y and having a very small (indetectable in mitotic metaphase plates) portion of the Xchromosome attached to the other side of the centromere. This transferred portion

of the X is composed of all the loci which are to the left of the left break in the sc^{Sl} inversion (Muller, D.I.S. 3), i.e. it extends from the distal end of the X to the gene scute inclusive. The presence of this X chromosome component carrying markers, the most important of which is the wild type allelomorph of yellow, makes the scY^{L} very valuable in studies in which it is necessary to detect the distribution of a Y-chromosome. In fact the detection of XX/O females in F_1 can be achieved by the use of P_1 males carrying the scY^{L} and of P_1 females with an XX marked by recessive allelomorphs of wild type genes carried by the scY^{L}. If the recessive marker in the XX is y (0. 0, yellow body colour) a cross between males with the scY^{L} and y females will give daughters all with grey body colour, y being "covered" by its wild type allelomorph carried in the sc- component of the paternal scY^{L}. Only females XX/O originating when the scY^{L}, or the X, is "lost", will show the yellow body colour. The P_1 males must, of course, have the Y^{S} (short arm of the Y) attached to their X chromosome, so as to have the whole set of genes carried by the Y and necessary for the fertility of the males. As preliminary tests had shown that the frequency of induced losses of X and Y was not high

(of the order of 0.5% with 1000r) it was necessary to choose a stock with a very low spontaneous frequency of those events which lead to a loss or which give the same phenotypical effects as a loss, namely: a) non -disjunction in the males; b) mutation to yellow in the sc-component of the scY^L c) release into circulation in the stock of an extra Y, or of parts of Y, not having the marking sc-component.

The first stock tested, kindly furnished by Miss Lamy, to whom the writer is deeply indebted, was of the following composition: FEMALES y w f/scY^L that is with attached -X, marked by the recessives y (yellow body colour 0.0) w (white eyes 1.5), f(forked bristles, 56.7); MALES yXY^S/scY^L, that is with the X attached to the short arm of the Y (Y^S) and marked by the recessive y. Both males and females were of grey body colour because of the y being covered by its wild type allelomorph carried in the sc-component of the scY^L. The females were therefore phenotypically white and forked, and the males wild type.

In mass cultures of the stock some phenotypically y males, y w f females and wild type females cropped out. The phenotypically y males and y w f females, respectively tested for fertility and for fertility of their sons, proved in different cases

to be non-disjunctional (carrying no Y) or to carry a whole Y, a Y^S or a Y^L without the sc-component. The presence of a whole Y in circulation in the stock could easily be explained by a crossing-over in the male between the scY^L and the XY^S giving an X with sc-duplication and a whole Y. Although this event must be rare, nothing would stop the whole Y from spreading in the stock once it had originated. Segregation from both the scY^L and the XY^S in the males, or from both the XX and the scY^L in the females, would give it a chance of being phenotypically detected through the presence of individuals in which y was "uncovered".

The presence in the stock of a Y^L deprived of the y^+ marker could also be explained easily as a spontaneous "mutation" to y (or better a minute rearrangement) in the sc-component of the scY^L. The rate of this spontaneous "mutation" has been measured by Levit (1931, unpublished), Belgovsky (1938, 39) and Makki (1939 unpublished) as some 1 in 10,000 in an inverted X chromosome (sc^8) in which, as in the scY^L, the y^+ locus is very close to a portion of originally proximal heterochromatic region.

The origination of the Y^S, instead of the Y^L, was not so obvious until the following fact was observed by Miss Lamy and confirmed by the writer:

the phenotypically wild type females were not all, as expected, cases of "detachment" of the XX of the mothers; some, tested, proved to be of the composition $\underline{y}/scY^L$, namely newly originated XX females with their attached -X of the same type as those carried by their yXY^S/scY^L fathers. A fact of the same kind has already been briefly reported by Neuhaus (1936). The origin of these attached XX females can be explained by a crossing over between sister chromatids in the paternal XY^S chromosome, its complementary product must be a free Y^S, probably double (Y^SY^S). This accounts for the presence of the Y^S in the stock. (x) (see foot not next page.)

Six generations of brother-sister pair matings with culling of the families which showed groups of some of the already described exceptions, proved that even the primary frequency of these exceptions (some 1.5%) was far too high for making this stock suitable.

Frequency of primary exceptional flies in the stock

y w f/scY^L & yXY^S/scY^L

Regular females phenotype: w f	Exceptions (phenotypes) ++♀♀	ywf♀♀	y ♂♂
1255	1	18	2

As the exceptional yellow females were proved, by the means of the test of fertility of their sons,

to be mostly XX/O, that is, products of non-disjunction in the males, the problem arose: is this high frequency of non-disjunction in the males caused by the scY^L, its sc-component disturbing the meiotic process through competitive pairing (Dobzhansky 1933), or is it an effect of the particular XY^S chromosome used? The fact that non-disjunction in the females, as shown by the frequency of exceptional yellow males, is considerably lower than in the males, was already suggestive of the greater likelihood of the second alternative. This was confirmed when a new stock was built up with the same y w f/scY^L females, but using males with an XY^S chromosome of different origin marked by the recessive forked. The causes of the different behaviour of the two stocks, probably connected with some difference in the chromocentral region, are being investigated.

After six generations of multiplication by means of brother-sister pair matings, and selection for a low frequency of exceptions, the new stock gave the following satisfactory results:

(x) Incidentally, this mode of new origination of attached -X which has been successively detected in several different XY^S and XY^L stocks can be of great practical use for the building of any kind of XX stock. (see Lamy & Pontecorvo, 1940, D.I.S.14 in the press). If this may be the origin of attached -X in nature, then the XX chromosomes are not necessarily iso-chromosomes (Darlington, 1939). Perhaps investigations in this direction could afford a test for deciding between such an origin for these cases and that proposed by Darlington (misdivision of the centromere.)

	No. of flies
Regular females	6419
Exceptions classified phenotypically:	
Females y w f	3
" f	2
Males w f	1 (detachment of the XX)

The phenotypically forked females could have been either y w f/f XY^S females produced by the detachment of the XX, or patroclinous XX females of new origination. Of the two found, one did not breed, and the other proved to have attached -X, possibly derived, as already suggested, by sister-chromatid crossing-over in the fXY^S chromosome of the father. A more exact picture of the frequency with which exceptional (y w f) females arise in this stock can be obtained if we add to these preliminary figures those obtained in the controls of all the sets of experiments reported later on in which crosses of this nature were made:

Regular (grey) females	21467
Exceptional (yellow) "	16
% of exceptions	0.065 ± 0.017

Tests of fertility of the sons of these exceptional flies (reported later) show that about half of them are without any part of the Y and therefore probably

produced by non-disjunction in the males. The remainder carry, in different cases, a scY^L mutated to y, or a whole Y originated by crossing over in the male, or a Y^S, probably a double one, derived from the same type of crossing over in the male which gives rise, as a complementary cross-over, to the attached X's of the patroclinous female exceptions.

In conclusion, this stock, females y w f/scY^L; males fXYS/scY^L, proved to be very suitable because of the low frequency with which exceptional (non-disjunctional and other) females arise in it, and was the most extensively used in the subsequent experiments. To avoid the dissemination through this stock of whole Y-chromosomes, or of either of the arms of the Y, originating in the manner above explained, the stock was maintained by the inbreeding of individual pairs of brothers and sisters, with continual elimination of families in which an undue proportion of exceptions appeared.

An attempt was successively made to produce a stock with a translocation between the scY^L and the second or third chromosomes. A stock of this kind might have offered the opportunity of distinguishing between the losses of the XY^S and those of the scY^L chromosomes, because in the case of a translocation between the scY^L and an autosome, for instance,

any potential losses, induced in the sperm, of a chromosome composed of a part of the scY^L and a part of an autosome, would always have given an inviable aneuploid zygote leaving only losses of the XYS chromosome to be recorded. For this purpose, males of composition fXY^S/scY^L; Cy L^4; D CXF were made up be means of a series of crosses, and then were mated, after irradiation, to y w f/scY^L females. The F_1 females, possessing one or both of the paternal second or third chromosomes marked by the dominant genes (in which crossing over is practically suppressed by a series of inversions) were individually backcrossed, and the translocations between scY^L and an autosome were detected by the resulting apparent sex linkage (all the sons and none of the daughters showing the dominant autosomal marker). Five translocations including the scY^L and an autosome (two with the second and three with the third) were obtained, but only one gave fertile males, thus confirming the results obtained by Neuhaus (1939) with a different technique, namely that most of the breaks in the Y produce sterility mutations or position effects. The only fertile translocation found proved to be totally unsuitable on account of its high frequency of non-disjunction. It was not thought worth while

to repeat this attempt because of the small chance of obtaining a fertile translocation giving a sufficiently low amount of non-disjunction.

A well known translocation, T (1;4) B^S provided the opportunity of investigating, from a somewhat different point of approach, the same problem, namely the distribution of the frequency of induced losses among different parts of the X and Y. T (1;4) B^S, the translocation associated with Stone's Bar, is a translocation between the X and the fourth chromosome, viable and fertile in homozygous condition, and inseparable from a very extreme Bar allel. The break in the X (Painter & Muller, 1929, Mackensen, 1935) is between forked and Bar. The break in the fourth is distal to all the known loci. Thus the X chromosome is divided into two portions; the proximal extends from the centromere to B, with just the tip of the right arm of the fourth attached beyond Bar. Females hyperploid for this portion have almost normal viability and fertility, while males have rather poor viability and are sterile. The distal portion of the X (from f to the left end) is attached close to the right of f to the major portion of the fourth chromosome, in which the centromere is included. Females hyperploid for this portion have

the very low viability usually shown by triplo -X's. The salivary gland length of the two portions is about 5 of Bridge's divisions for the proximal and 16 for the distal one (the latter including 1 division from the 4th and 15 from the X). The mitotic metaphase length, on the other hand, is about the same for both portions (Muller & Painter, 1932; Painter, 1934), on account of the disproportionate metaphase size of the heterochromatic proximal region of the X. In the male the proximal portion generally segregates from the Y and the other goes at random (Muller & Painter, op cit; see also our data, page 29). If therefore B^S males are crossed to XX females, two main types of XX daughters are obtained: XX/Y, as in any other XX stock, and XX/$4RB^S$.

To use this translocation for our purpose, it was necessary to have it present in males carrying the scY^L instead of the ordinary Y chromosome. But males with the scY^L, to be fertile, must carry the complementary part of the Y, that is, the Y^S. It was therefore necessary to build up a stock in which the $4RB^S$ chromosome would have the Y^S attached. This was effected by means of the following series of crosses, framed so as to allow the occurrence of an easily detectable crossing over to the right of B, between a B^S and a fXY^S chromosome:

females $\frac{XL\ 4L}{XL\ 4L}$; $\frac{4R\ B^S}{4R\ B^S}$ x males fXY^S/scY^L

females $\frac{XL\ 4L}{fXY^S}$; $\frac{4R\ B^S}{}$ x males fXY^S/scY^L

(Among the Bar males, only crossovers are fertile.) $\frac{XL\ 4L\ ;\ 4R\ B^S\ Y^S}{sc\ Y^L}$

A single fertile male crossed with y w f/scY^L females formed the progenitor of the desired stock.

For the same reasons as already mentioned for the fXY^S/scY^L stock, this stock could only have been utilizable if it gave a low frequency of non-disjunction. After some generations of brother-sister pair matings, the following measurements of its segregation were made:

Segregation in the stock B^SY^S/scY^L ♂♂ & y w f/scY^L ♀♀

females				males
y w f/scY^L	y w f/$B^S\ Y^S$	y w f/B^SY^S/scY^L	y w f/(?)	
1917	1596	4	3	1419

The difference between the numbers of males of Bar and of non-Bar females is certainly due in large measure to the lower viability connected with the presence of B, in different relative doses. The y w f/scY^L/B^SY^S females are produced by the scY^L and the B^SY^S undergoing non-disjunction and at the same time separating

from the XL 4L. The phenotypically y w f females can either be non-disjunctional y w f/O, produced by the scY^L, the B^SY^S and the XL 4L all going to the same pole, or contain a normal Y or part of it that originated in the ways already mentioned for the other stock. However, the frequency of origination of these females - which constitute the undesirable feature of the stock for the present purpose - is sufficiently low ($\frac{3}{1917} = 0.16 \pm 0.09$) to allow the stock to be utilized. It is interesting to note that this frequency is but slightly different in this and in the fXY^S stock. The translocation between X and 4th seems to have little appreciable effect in increasing non-disjunction between the Y and the proximal part of the X. The smallness of the fragment of the 4th chromosome attached to XR, probably causing very little, if any, competitive pairing, perhaps accounts for this fact.

The use of a dominant variegated allel of brown:

The other method adopted for discriminating XX/Y from XX/O females, and detecting cases of loss of the X or Y chromosomes, is based on the effect that an extra Y, and in general an increased proportion of heterochromatin in the genome, has in

suppressing the "eversporting" or variegated phenotypical expression of genes whose loci have, through structural change, become juxtaposed to an heterochromatic region.

Mosaicism of expression is apparently a general effect of this particular kind of rearrangement on all genes which happen to be not far from the break. Genetic and cytological evidence by Noujdin (1938) Prokovyeva (1939) and Schultz (1939) has shown strikingly that the effect spreads from the heterochromatic region, the closer a gene - transferred from another region - being to it, the more extreme the variegation and the greater the area of the mutant effect in the mosaic. The mechanism of this process, which in some cases at least seems to be a process of inactivation, is not known. Recent cytological and biophysical work by Caspersson (1939) in collaboration with Schultz (1939), as well as cytological work by Prokovyeva (1939) shows that variegation is associated with an increase of the chromatic substance - nucleic acid according to Caspersson - in the region of the salivary gland chromosomes near the breakage point.

Whatever the cause of this mosaicism, the effect of two factors on the degree of its phenotypical manifestation are fairly well known (Gowen & Gay 1933 a & b, 1934; Muller 1935; Glass 1932; Schultz & Dobzhansky 1934; Schultz 1936, 1939; Surrarrer 1935, 1938; Dubinin & Heptner 1935; Noujdin 1936,1938).

These factors are: temperature and the proportion of heterochromatin in the chromosomal set. Both a rise in temperature and an increase in heterochromatin produce a reduction of the variegation.

The autosomal variegated genes most suitable for our purpose, were to be sought among the numerous dominant variegated allels of brown eye colour which are all produced when a large rearrangement occurs with one break near the brown locus (bw, 2nd chromosome locus 104.5) and the other in a heterochromatic region. Two of these dominant browns were available to us, namely bw^{V7} and the mutant called "A" by Dubinin & Heptner (op cit.), which Muller calls bw^{VA} (brown variegated A), for uniformity with the rest of the series to which he has found it to belong.

The first is inseparable from translocation T (2; 3) bw^{V7}. The breaks in 2R are at the bw locus and at the right of the spindle attachment, all the large portion comprised being inverted. The rearrangement connected with the second has not been thoroughly investigated, but some genetic data by Dubinin & Heptner (op cit), and results obtained by Muller and by the writer indicate a large inversion in 2R. Crossing over in 2L is not affected.

The eye colour in flies carrying no extra heterochromatin and reared at 24-25° C. is red-brown in bw^{V7} and dark-brown in bw^{VA}, the variegation in

both being so extreme that only a careful examination shows some spots of the wild type (red) colour. In combination with v (vermilion eye colour, X chromosome 33.0) both give a very pale yellow background flecked with small spots of nearly vermilion colour. In old flies the background becomes darker, a very light chocolate colour. The ratio of spotted to yellow area is variable from one fly to another and between the two eyes in the same fly. This variability is rather large in the bw^{V7}, which gives in addition some flies with a finely mixed pattern of vermilion and yellow, resulting in an apparently uniform dilution of vermilion (somewhat like the combination of v with carnation). In bw^{VA}, on the other hand, most of the eyes show very few or no tiny spots of vermilion, the whole eye appearing as pale yellow.

In both stocks a sufficient amount of extra heterochromatin, for instance an extra Y - as in XXY females or in XYY males - leads to suppression of the variegation, so that the eye shows almost the same colour as if the dominant brown were not present. For instance, in attached -X flies, homozigous for v and carrying in the second chromosome the dominant brown, the effect of the extra Y is to give a vermilion eye colour almost indistinguishable from that of flies without dominant brown. If however there is no extra

Y, the yellowish colour appears. It is in fact this easily detectable phenotypic effect of the Y chromosome on which we base the use in our experiment of XX females, homozigous for vermilion, carrying in their second chromosome a dominant variegated brown allel.

The following desiderata had however to be met before a choice could be made between the two available dominant browns and before a suitable and balanced stock of the chosen allel could be made up :

a) Both the suppression of variegation by the extra Y and the variegation itself, when there is no extra Y, must be as extreme as possible, excluding any over-lapping expression of the two conditions.

b) The effects of the presence of extra parts of the Y or of the X (particularly of the proximal heterochromatic region) must be known.

c) As all the dominant browns are lethal, or nearly so, in homozigous condition, the "balancing" of the chromosome carrying the dominant brown is necessary. And in achieving this it must be kept in mind that the inversion associated with the bw might be of over-lapping configuration with respect to an inversion used for balancing it and thus might allow some crossing over.

On point a) a preliminary test led to the elimination of bw^{V7}, because although in the great

majority of cases the extra Y suppresses variegation completely, there still remains far too high a frequency of cases of incomplete suppression which may lead to doubts in the classification of the flies. The frequency of these doubtful cases in bw^{VA}, on the other hand, is very low: in a preliminary test, none in some 1500 $\frac{y\ v\ f/Y;\ bw^{VA}}{+}$ females reared at 24 C. The absence of the extra Y gives a very clear cut effect in this stock.

The results of tests with parts of the Y are reported below:

Effect of extra portions of X or Y chromosome in flies carrying bw^{VA} (reared at 24-25 C).

Composition of the X & Y chromosomes	Phenotypical effect on the eye
A) Females	
y v f/Y	vermilion, no yellow variegation
y v f/Y^{bb^-}	vermilion and yellow, mottled
y v f/Y^S	extreme yellow
y v f/Y^L	yellow, with some occasional vermilion fine mottling
y v f/scY^L	vermilion, no yellow variegation
y v f/4R B^SYS	variable from orange to yellow
y v f/Del.14	variable orange-vermilion
y v f/0	extreme yellow
B) Males	
v/Y	yellow, with few vermilion spots
Xc^2/Y	dark-brown, few red (wild type) spots
XY^L/Y	dark-brown, slight red mottling
yvfY^L/Y	yellow, slight vermilion mottling
fXY^S/Y	dark-brown, slight red (wild type) mottling
yvfY^S/Y	vermilion, almost no yellow variegation
sc^{J1}/Y/Del 14	mottled brown and red

These results, along with others obtained with deleted X & Y chromosomes, are the object of a separate investigation. Suffice it here to point out that a purely quantitative effect of extra heterochromatin does not, as generally assumed, seem to account entirely for these and other results (obtained with deleted Y-and X-chromosomes). For the present purpose however, the finding that at 24° C both an extra whole Y and an extra scY^{L} suppress almost completely the variegation in attached X females homozigous for vermilion and carrying bw^{VA} in their second chromosome, indicated the feasibility of using this marker, provided it were kept in mind that extra parts of the X or Y might give non predictable effects.

The"balancing" of the bw^{VA} was first attempted with the Curly-containing second chromosome generally designated as "In (2L) Cy, Cy - In (2R)Cy", which has both arms marked - the left by the dominant Cy (Curled wings; 8.5 homozygous lethal) and the right by L^{4} (Lobed eye; 72) - and carries one inversion in each arm almost completely suppressing the appearance of viable crossovers when the homologous chromosome has the normal gene sequence. But when used to balance the bw^{VA} however this Curly-containing chromosome proved to be unsuitable as it gave a high (non exactly measured) frequency of crossovers between

bw^{VA} and L^4. Although all the classes of crossover flies here arising were easily distinguishable (those carrying Cy, L^4 and bw^{VA} all on the same chromosome are also distinguishable by the abnormal appearance caused by their being aneuploid) the stock might have required continuous selection.

It was therefore attempted to balance bw^{VA} with a chromosome of normal arrangement carrying a dominant marker in a region in which, supposedly, the appearance of crossovers was hindered by the inversion associated with bw^{VA}. This inversion would have one break near bw (104.5) and another near the centromere (55): a marker half-way between the two would presumably be satisfactory. Lobe 2 (L^2, 72.- eye very much reduced in size and slightly rough) was tested and proved suitable, as practically no crossovers with bw^{VA} occurred. A stock was accordingly constructed in which the females had attached -X, homozigous for yellow (y), vermilion (v) forked (f) and in which the two second chromosomes carried, respectively, bw^{VA} and L^2 (symbolic representation $\underline{y\ v\ f}/Y$; bw^{VA}/L^2). The males had normal X and Y chromosomes but second chromosomes like the females. Later, vermilion was introduced into the X chromosome of the males, so as to detect more easily, by means of the resulting yellow mottled colour, the presence of the bw^{VA}, since the reduction

in size and the roughening of the eye caused by L^2 interferes too much with the expression of bw^{VA} when v is not present. This stock is fairly well balanced as a consequence of the non-occurrence of crossovers between L^2 and bw^{VA}, and of the fact, that bw^{VA} proved to be almost completely lethal and completely sterile in both sexes in homozygous condition. As for the homozygous L^2 flies they have a low viability. Thus only the heterozygous bw^{VA}/L^2 flies develop in great numbers and reproduce themselves regularly, so that the stock goes on without need of selection.(x) It was observed that the homozigous bw^{VA} attached -X females, although carrying an extra Y, show extreme variegation. Although only 1/4 of the fertilized eggs are capable of development (1/2 being inviable YY or XXX zygotes, and 1/2 almost inviable homozigous bw^{VA} or L^2), the stock proved to be sufficiently fertile and of very good viability.

(x) Towards the end of the experiment here reported, the stock was further improved by inserting the dominant Bl (locus 54.8, lethal when homozygous) into the chromosome with L^2, so as to eliminate even that small proportion of homozygous L^2 flies the appearance of which had been previously unavoidable.

C) GENETICAL METHODS ADOPTED FOR DETECTING LOSSES OF THE 2nd AND 3rd CHROMOSOME.

Triploid females produce gametes in which the chromosomes range from the haploid to the diploid set. As for the two large autosomes, only eggs with both of them in diploid or haploid number can give, when fertilized by a haploid sperm, viable zygotes. The eggs containing one autosome in diploid number, and the other in haploid number give origin to inviable trisomic zygotes (Bridges, 1921). Now if an egg of this aneuploid type could be fertilized by an aneuploid sperm in which a potential loss of the autosome homologous to that hyperploid in the egg had been induced, the zygote might survive. In fact, its genome would be a normal diploid one, in which both chromosomes of one pair were derived from the triploid mother.

Starting from this idea, an experiment was planned and suitable stocks constructed. As the exceptional flies would have at least one pair of major autosomes (sometimes both pairs) of purely maternal origin, it was decided to mark the triploid mothers with recessives in the autosomes, and to mate them to treated males with the dominant allelomorphs, for this would result in all the F1 exceptional flies

showing the corresponding maternal recessive character, here homozygous, instead of the paternal dominant character, present in heterozigous condition in all the regular flies. The recessives brown eye (bw) and ebony body (e, 3rd chromosome 70.7) were chosen, because of easy identifiability and relatively good viability.

The stock of triploids in which these recessives had to be introduced had the following composition: females $\underline{y}/sc^{Sl}$ and males sc^{Sl}, that is, the triploid females had attached X's homozygous for y and a third free X chromosome carrying the double inversion associated with sc^{Sl} (outer inversion with left break just to the right of sc-o.o- and the right break to the right of bb- 66.0- and block A); the diploid males had the sc^{Sl} chromosome and a normal Y. The sc^{Sl} inversion almost entirely suppresses crossovers throughout the whole X chromosome, so that the composition of the attached Xs as well as of the sc^{Sl} chromosome, is preserved. The progeny of such a stock is constituted of: a) $\underline{y}/sc^{Sl}$ phenotypically wild type triploid females; b) sc^{Sl} diploid males (extreme scute); c) $\underline{y}$ diploid females (yellow); d) sc^{Sl} homozigous diploid females (extreme scute); e) $\underline{y}$ ^{Sl} intersexes; f) sc^{Sl} homozigous intersexes; g) $\underline{y}/sc$ triple X diploids (superfemales); h) sc^{Sl} supermales.

Types d) to h) are sterile, g) and h) have in addition an extremely low viability. The stock needs selection of triploid females at every generation, otherwise the y diploids, being of much higher fertility than the triploids, would immediately supplant them. The yellow body colour of these diploids makes selection very easy, and so affords opportunity of preserving the stock.

To construct a homozigous bw;e stock of triploids, it was necessary first to construct a stock having males with an X-chromosome that does not cross over, and with second and third chromosomes homozigous for bw and e respectively. The stock in question consisted of y; bw; e females and sc^{Sl}; bw; e males. At first these males had an extremely low fertility and some generations were necessary before natural selection of a system of modifiers gave the stock a suitable fertility. By repeated backcrossing of the triploid females to these males, homozygosity for bw and e was finally obtained in the triploids.

The resulting stock of triploids (y/sc^{Sl};bw;e triploid females and sc^{Sl};bw;e diploid males) has a satisfactory fertility and viability. Through non-disjunction, however, many males and females have extra Y chromosomes (vide infra). The chance of crossing over in the triploids between y and Y is

therefore not remote, and the greatest care is necessary to eliminate these cases. In fact, from this detachment of the XX a free X, carrying yellow, is released into circulation. A female with one y bearing and one sc^{Sl} X-chromosomes is phenotypically wild type and can easily be mistaken for a triploid; such a mistake results in very rapid contamination of the stock by the production of y sons and more wild type diploid daughters.

The males to be treated and used in the experiment for crossing to these bw;e triploids need not contain mutant genes. It was thought, however, that the presence of the dominants Cy & L^4 in 2L and 2R, respectively, and of D, in 3L, all associated with crossing over suppressors - In (2L) Cy, In (2R) Cy in the 2nd chromosome, and the series of small inversions denoted as CXF in the 3rd - would have some advantage. In particular, opportunity should thereby be afforded of detecting some triploids, with deleted autosomes, having deletions so drastic as to be lethal to diploids. A stock of the composition v;$\frac{Cy\ L4}{+}$; $\frac{D\ CXF}{+}$ was accordingly synthesized, and the males of this stock were used for crossing to the triploids.

D) EXPERIMENT ON THE FREQUENCY DOSAGE RELATIONSHIP OF LOSSES OF THE X AND Y CHROMOSOMES.

The aim of this experiment was to ascertain whether or not losses of the X and Y chromosomes, induced by irradiation of the mature sperms, give, under suitable conditions, viable zygotes, and in the event of an affirmative answer to find the relationship between the dosage of irradiation and the frequency of these induced losses, with special reference to the question of whether this relationship conforms with the approximately linear one expected on the "breakage first" theory of structural change.

Experimental set-up.

X-rayed males of the stock previously described - fXY^S/scY^L - were crossed to $\underline{y\ w\ f}/scY^L$ females, and the exceptional yellow females appearing in F_1 were recorded.

The following technique was adopted: the males were collected, from inbred pair matings, 1-3 days after hatching and kept in food bottles at the temperature of $24\pm1^{\circ}C$ for 5 days. They were then thoroughly mixed and divided into three groups,

to be given two different X-ray doses and to be used as untreated material for the control, respectively. The control males were mated individually in vials, each male being put with two virgin females. The other males were X-rayed in gelatine capsules placed at a distance of 12 cm from the tungsten target of a broad-focus watercooled Coolidge tube.

The tube was operated at 8 milliAmperes and 70 Kilovolts; a 05 mm Al. filter was used. The intensity of irradiation on the capsules, calculable from the readings of an integrating jonisation chamber, was about 400 r per minute. Two doses were given, namely, 1000 and 4000 r.(x)

For the high dose the males were exposed to the irradiation for about 10'. For the low dose, the males were divided into four equal groups and each group irradiated, together with the males of the high dose, but up to only one quarter of the high dose. After each quarter (1000 r) one group of low-dose males was removed and another one substituted. This method, first adopted by Offermann in 1934 (unpublished) has the advantage of equalising to a

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(x) The relative values of the two doses (1:4) in this experiment have considerable accuracy, owing to the technique adopted. The same cannot be said of the absolute values. Some alterations were introduced in the measuring apparatus which might make these doses not exactly comparable with those of later experiments.

considerable extent variations in the conditions of treatment that might otherwise affect the high and low dose groups differentially.

Immediately after treatment, the males were mated individually in vials with virgin females; 6 females were placed in each vial, for the males treated with the high dose and 3 for the males treated with the low dose. These numbers were decided on as a result of previous tests which had shown that under these conditions the density of the cultures was approximately the same for the two doses. After 4 1/2 days the males were discarded and the females of each vial transferred to fresh vials. The cultures were maintained at a constant temperature of $24 \pm 0.5^{\circ}C$ and were examined when almost all the pupae had hatched.

The reason the system of individual matings was adopted in this first experiment was because it was then not yet definitely known whether or not the frequency of the already described types of crossing over which release whole Y-chromosomes or either of their two arms into circulation in the stock was high enough to be seriously disturbing. By means of individual matings it is possible to detect males carrying, instead or in addition to the scY^{L}, one of

those unmarked Y's or parts of a Y. In fact, males of these kinds should give a high number of phenotypically yellow daughters, which, on being tested in turn, would have revealed the presence of such a chromosome. No cases of this kind occurred however in 1020 individual matings of the above experiment. When more data on the frequency of the already described special types of crossing over in the stock used were known, it was realized that males, carrying a whole Y or part of a Y, are to be expected with an incidence of some 3 in 10,000, provided the stock is always multiplied, as it actually was, by inbred pair matings, thus excluding secondary occurrences.

Results.

The results of this experiment are shown in the following table:

DOSE in r	FEMALES			
	Regular (phen. w f)	Exceptional (phen. y w f)	Except. / Regul. X 100 Except.	
4000	4, 987	84 + 2½ (x)	1.68 ± 0.18	
1000	10, 427	56	0.54 ± 0.07	
Control	7, 071	5	0.07 ± 0.03	
Total	22, 485	146		

(x) Two cases of "fractionals" (for explanation see text).

Among the 86 exceptional flies found in the F_1 from the males treated with the high dosage, 2 were of mosaics of the "fractional" type (Muller, 1927). The body colour of these two flies was part grey and part yellow, the two parts each extending over about half of the body in a more or less bilaterally symmetrical pattern and being sharply delineated from one another. In both cases, however, the head was all of one colour, namely grey. As the results obtained in genetic analyses of these and the many other "fractionals" found in the subsequent experiments throw an interesting light on the mechanism of induced chromosome-rearrangements, a detailed discussion of the problem will be given in a later section. It is sufficient here to point out that for reasons which will be explained, these fractionals are not counted as exceptional units, but as halves.

In the table above the frequency of exceptional flies has been given as it is actually observed. It should be considered, however, that when a potential loss is induced in XY^S bearing sperms a zygotes which would not otherwise survive, because of their being triplo-X, are turned into viable exceptions. Therefore a more exact calculation of the frequency with which losses are induced in the sperms should make allowance for this extra number of viable flies.

The correction is of such negligible size (one order of magnitude smaller than the experimental error) that it does not seem worth while to introduce it.

Tests of the exceptional flies.

The exceptional flies are detected by their manifestation of the gene for yellow body colour, which, in the regular flies, is "covered" by the wild type allelomorph carried in the sc-component of the scY^L. In addition to the complete loss of XY^S or of scY^L, several other ways are conceivable whereby the same phenotypical effect can be produced, namely :

a) presence of a normal Y, or of one of its two arms, carrying no marker;

b) minute rearrangement in the sc-component of the scY^L chromosome producing the "mutation" to yellow of its wild type allelomorph;

c) large deletion of the fXY^S chromosome with left break to the left of the y^+ locus and right break between w^+ and the centromere;

d) large deletion of the fXY^S chromosome, producing a ring shaped chromosome, with left break between w^+ and the centromere and right break anywhere in the Y^S, but proximal in respect of at least one of its fertility genes, or producing a sterility mutation in it;

e) deletion of the scY^L - producing a ring shaped chromosome-with one break in the sc-component, proximal in respet to the y^+ locus, or producing mutation of it to yellow, and another break anywhere in the Y^L arm but proximal in respect of at least one of its fertility genes or producing a sterility mutation in it;

f) "mutation" (minute rearrangement) to y in the sc-component of the scY^L and simultaneous sterility mutation in the Y^L;

g) translocation of the fXY^S or scY^L to the 4th chromosome with acentric-dicentric reunion of the broken ends and loss of both chromosomes;

h) complex rearrangements resulting from the combination of the already described simple ones.

Cases a) are a consequence of crossing over in the male and therefore independent of the X ray treatment. The control series were carried out for the very purpose of checking this occurrence.

The occurrence of the other types had to be ascertained by tests carried out on the exceptional flies themselves. The following technique was adopted:

In cases b) and c) the Y^L arm of the scY^L chromosome, or the Y^S arm of the fXY^S chromosome, respectively, remain unaffected. An XX exceptional female carrying either will transmit it to her sons. If these sons carry [illegible], attached to their X chromosome, the complementary arm of the Y, they will have the whole set of fertility genes, and will be fertile. Therefore by mating each exceptional female to XY^S and XY^L males successively and testing the sons of each for fertility, it can be ascertained whether or not she carried one of the arms of the Y, and, in case she did, to identify it.

In cases d) and e) the fertility of genes

- several of which have been shown (Neuhaus (1939) to be located in the distal portions of each arm of the Y - are affected and therefore none of the proceeding tests is suitable. However the presence in the proximal regions, both of the XY^S and of the scY^L, of the bb^+ locus suggested a test based on the detection of this locus. A stock in which the X chromosome of the males is deficient for the bb^+ locus was utilized. Males of this stock - y sc4R, In sc^{Sl}, $sc^{Sl}L$ - obtained by Muller by crossing over between the sc^4 and the sc^{Sl} inverted chromosomes - are viable only if the deficiency of the bb^+ locus in the X is "covered" by its presence in the Y or elsewhere. From the cross of such a male with an exceptional nullo-Y attached -X female, no sons are produced except for a few <u>fertile</u> males resulting from non-disjunction of the X and Y in the father. If however the exceptional female tested carries a Y^S, a deleted fXY^S or a deleted scY^L in which the bb^+ locus or loci have not been affected, a number of males will be produced which will be <u>sterile</u>. In an XY^S chromosome there are two bb^+ loci, both proximally placed. In a scY^L chromosome at least one bb^+ locus is surely present in the proximal part of the sc-arm, probably no bb, or a very weak bb allel (Neuhaus, 1936) is present in its Y^L arm. If a ring is formed in the XY^S or in the scY^L as a result of two breaks occurring

one on each side of the centromere, followed by joining of the broken ends, there is a good chance for one of the breaks to have been distal in respect of the bb^+ locus. This chance must be high for rings formed from the XY^S, because of the two bb^+ loci originally present, one in each arm, in fairly proximal portions. Therefore it should often happen that at least one of the breaks be proximal in respect of the bb^+ locus in the arm in which it occurred. When the ring is formed in the scY^L, with its presumably single bb^+ locus, the chance of the bb^+ locus not being included in the ring is higher.

Case f),-a mutation in one of the fertility genes carried in the long arm of the scY^L chromosome coinciding with a y "mutation" in its sc-component-is also detectable by the test of the presence of the bb^+ locus.

Case g),-an aneucentric (acentric-dicentric) translocation of the XY^S or the scY^L with the 4th chromosome,-may produce through elimination of both the chromosomes, an exceptional (yellow) fly which would also be haplo-IV. The haplo-IV condition - when the zygote survives - is easily detectable as it gives a very clear "minute bristle" appearance to the fly. No such exception occurred in the experiment reported here.

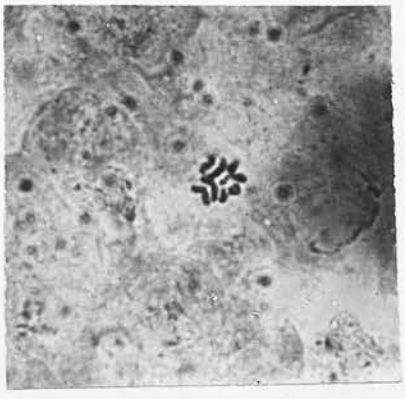
Fig. 2

A small V-shaped chromosome probably resulting from a large deletion in the X-arm of an X.Y^S chromosome and a simultaneous sterility mutation in its Y^S- arm.

Mitotic metaphase plate of a female carrying attached-X's and the deleted chromosome (Camera lucida drawing -X 3500).

facing page 52

a



b

Fig. I - A ring-shaped chromosome resulting from one break in each arm of the scY^{L}.
Mitotic metaphase plate of a female carrying attached -X's, a normal Y and the ring.
a) Camera lucida drawing - X3500 - of same plate as in microphotograph b) - X800.-

All the rearrangements described require one (case b) or two breaks (cases c, d, e, f, g). The occurrence of a three break rearrangement or other "three hit case is certainly less frequent. The simplest of the "three hit" types are large deletions including the locus of y^+ in the X arm of the XY^S accompanied by sterility mutation in the Y^S arm, or deletions affecting fertility genes in the Y^L arm of the scY^L accompanied by a yellow "mutation" in the sc- arm. Both types can be detected, with the same limitations as for the rings, by means of the test for the bb^+ locus.

The experimental evidence shows that all the types from a) to f), and in addition some of the more complex ones, of phenotypically exceptional flies carrying different parts of the scY^L or the XY^S actually occur as a consequence of X ray treatment. Fig 1. shows the metaphase plate of a ring resulting from one break in each arm of the $sc\text{-}Y^L$, and Fig 2 a small V-shaped chromosome probably resulting from a large deletion of the X and a simultaneous mutation of the Y^S in an XY^S chromosome.

In conclusion three tests had to be carried out with any given exceptional (yellow) female before it could be ascertained to which type she belonged:

1) Test for the presence of the whole set of fertility genes carried by the Y^L.

2) Test for the presence of the whole set of fertility genes carried by the Y^S.

3) Test for the presence of the bb^+ locus.

Exceptional females giving negative results with all the three tests can be considered as in the great majority of cases nullo-Y females. The same results are, to be sure, given when a ring chromosome not including the bb^+ locus is carried by the female as when a female is nullo-Y. But such cases are probably a small fraction of the total, judged by the small number of rings that do contain bb^+, when considered in connection with the tests of earlier workers on deleted X-chromosomes (Muller & Painter, 1929 and Muller & Gershenson - unpublished -) which show that in a large proportion of deleted X's having one break in the heterochromatic region the bb^+ locus has not been removed.

Practically, the three tests were carried out as follows :

The exceptional flies, when collected, were already inseminated by their fXY^S/scY^L brothers. A period of 4-5 days laying (each female being kept individually) was sufficient to give rise to enough offspring for the first test. The sons coming from any given culture of this "brood" were mated in mass (5-10 males with 5-10 females) to their sisters. In case they proved to be fertile, the test was verified by mating 5-10 males individually to 2-3 females (virgin)

so as to exclude cases of fertility due to the presence of the rather rare non-disjunctional males. A positive result from this test was considered as a proof that the exceptional fly had carried the whole set of fertility genes of the Y^L.

The same exceptional fly, which by its 4-5 days laying the eggs of the first brood had been discharged of a part of the sperm received in the first mating, was then mated in a second set of vials to males of the composition XY^L/Y^S. The two progenies were easily distinguishable because the daughters from the first type of mating, carrying the paternal scY^L, were phenotypically y^+ w f and the sons f, while the daughters of the second brood were y w f and the sons wild type. The sons derived from this second type of mating were tested for fertility in the manner already described for the ones from the first mating. A positive result of this test was considered as proof that the exceptional fly had carried the whole set of fertility genes of the Y^S.

After the second type of mating, the fly was transferred to fresh food for 4-5 days and then transferred again and mated for a third time, this time with y sc^4 sc^{Sl} (bb-deficient) males, marked in one of their second chromosomes by the dominants Cy and L^4. If no y sc^4sc^{Sl} sons appeared in the progeny, but many

females, half of which were Cy L^4, the result of the test for the presence of the bb^+ locus was considered as negative. Usually, however, even when the bb^+ was absent in the exceptional fly, a few sons appeared in consequence of the very high non-disjunction (at least 5%) occurring in the males of the y sc^4 sc^{S1} stock. In this case these sons were tested individually for fertility (presence of a whole Y) to confirm their non-disjunctional origin. When on the other hand many males appeared in the progeny and on being tested individually the great majority proved to be sterile, the result of the test was considered positive, and the conclusion drawn that the exceptional fly carried a deleted, probably ring shaped, scY^L or XY^S.

The impossibility of carrying out the above triple test on all the exceptional flies is obvious. Each fly, already some days old when collected, must give progeny with three different types of males.

DATA ON PERCENTAGE OF EXCEPTIONAL F_1 ♀♀ WHICH CONSTITUTE CASES OF COMPLETE LOSS OF XY^S OR OF scY^L.

		TREATMENT		
		4000 r.	1000 r.	CONTROL
1. Exceptional ♀♀ tested for the fertility genes in both arms of the Y:		43	35	4
a) carrying the whole set of fertility genes of Y^L only:	9		3	2
b) carrying the whole set of fertility genes of Y^S only:	3		5	-
c) carrying both the sets of fertility genes of Y^L & Y^S:	-		-	-
2. Total carrying the whole sets of fertility genes of one or both arms of Y:	12	12	8	2
3. Remainder, carrying neither whole Y^L, nor whole Y^S:		31	27	2
4. Exceptional flies (under 3) tested for the presence of bb^+ locus:		19	12	2
5. Carrying a deleted XY^S or scY^L with bb^+ :		2	1	-
6. Remainder, not carrying bb^+ :		17	11	2
7. Percentage of exceptional ♀♀ carrying no whole arm of Y (3: 1):	$\frac{31}{43}$ =	72±7%	$\frac{27}{35}$ = 77 ±7%	$\frac{2}{4}$ = 50±25%
8. Percentage of preceding carrying no deletion with bb^+ locus (6:4):	$\frac{17}{19}$ =	89±6%	$\frac{11}{12}$ = 92 ±8%	$\frac{2}{2}$ =100± 7%
9. Percentage of exceptional ♀♀ representing complete loss of XY^S or of scY^L (7 x 8):		64±7%	71±9%	50 ± 25%

Before the progeny from one type of mating is hatched, the female must already have been mated again, and sometimes the first progeny proves not to be sufficiently numerous, or the female fails to accept the new male and continues to give offspring of the first kind, etc. Difficulties of this kind require the repetition of some of the matings but often the fly has died before the need for repetition is known, as the whole series of progeny has not yet been obtained. In the table on the preceding page the results of these tests are given. Only those flies are recorded which gave at least the two first kinds of progeny whereby the presence of the fertility genes of either or both arms of the Y is detected. The third kind of progeny - from bb-def. males - was obtained with about half of the flies which had already passed the other two tests.

The results indicate that the majority of the exceptions - roughly 2/3 in the treated series - are nullo-Y females. In addition the proportion of exceptions which are nullo-Y is, in first approximation, of the same order for the two dosages. The difference between the two series, although far from being significant, is however in the direction expected, namely a higher proportion, at the higher dose, of deletions of the X or Y, simulating loss, among the exceptional

flies.

The conclusion can be drawn that losses of the X and Y chromosomes are in fact induced by irradiation of the mature sperm and that they are not lethal to the zygote. Whether the latter deduction holds for all the induced losses of X and Y chromosomes, or only for a part of them - those which can be detected by the method adopted - will be discussed later.

Dosage-frequency relationship.

The data summarized on page 46 give a frequency of 1.68 ±0.18% exceptions at the higher dosage, of 0.54±0.07 at the lower dosage, and 0.07±0.03 in the controls, the dosage ratios between the three series being as 4:1:0. The tests of the exceptional flies have shown that the proportion of losses of the whole XY^S, or scY^L, chromosome among them is 64±7%, 71±9% and 50±25%, respectively, for the three series. We have then three points of the curve relating the dosage of irradiation with the frequency of losses, namely those corresponding to the abscissae 0r, 1000r and 4000 r. This makes it possible to test whether or not, at a first approximation, this relationship can be represented by a linear (straight line) equation.

The experimental evidence in Drosophila shows that the frequency of induced "point" mutations and

minute rearrangements increases, within the dosages here used, approximately in linear manner, that is, as the first power of the dosage. The frequency of gross rearrangements on the other hand increases, within the same range, as an exponent of the dosage higher than the first and lower than the second power: approximately as the 1.5 power (see review of the matter by Timofeef-Ressovsky for "point" mutations and by Muller for rearrangements at the 7th International Congress of Genetics).

The present data on the frequency of exceptional flies at the three above mentioned dosages can be interpolated by a linear equation as shown by the following table:

DOSAGE	Exceptional flies found %	Cases of loss of whole XY^S (or scY^L) among exceptional flies %	Frequency of losses (x) Found %	Expected %
0	0.07±0.03	50±25	0.035±0.023	0.077
1000r	0.54±0.07	71±9	0.384±0.034	0.330
4000r	1.68±0.18	64±7	1.075±0.163	1.088

(x) Interpolating equation $Y_d = \bar{y} + 0.498\ (d - \bar{d})$, where Y_d = % frequency at dosage d (in 1000r).

The agreement between the data found and those expected is very good, being $\chi^2 = 0.03$ a value which, for one degree of freedom, is exceeded by chance in more than 80% of cases.

On the other hand the possibility must also be tested that an exponential curve, with exponent 1.5 in analogy wtth that of gross rearrangements, would fit the data. The expected value for 1000r can be calculated, on this assumption, from the 4000r value, as the latter is affected by a proportionally lesser error. Allowing for the controls, this expected value for 1000r is 0.13 ± 0.02%, as compared with the obtained value of 0.35 ± 0.08. The difference, 0.22 ± 0.08, is almost three times its standard error. Thus the frequency-dosage relationship is suitably expressed by a linear equation and cannot be expressed by an exponential equation with the exponent 1.5. It may be concluded that, within the range of X-ray dosages used in this experiment, the induction of losses appears to be, in first approximation, a linear function of the dosage of irradiation.(x)

(x) The results found here are in agreement with those of a preliminary experiment made by Muller & Singh (Muller, 1940).

E) EXPERIMENT ON THE FREQUENCY OF LOSSES OF X AND Y CHROMOSOMES HAVING DIFFERENT SHAPES AND LENGTHS.

The preceding experiment has given evidence:

a) that losses of X and Y chromosomes are induced by X-rays;

b) that these losses are, in part at least, non-lethal to the zygote and

c) that within the range of 0 to 4000 r, the frequency of their occurrence is roughly proportional to the first power of the dosage of irradiation.

The mechanism whereby the losses are produced called for further investigation however. It was desirable, in the first place, to obtain evidence for or against the possibility that the losses are the consequence of some kind of action on the centromere or on achromatic constitutents of the mitotic apparatus, directly or indirectly affected by irradiation of the sperm, reducing the efficienty with which the mitotic movements are carried out. Conversely, it was desirable to get evidence, from some other angle of approach than the frequency-dosage, that breakages of chromosomes - of the same nature as those occurring in all kinds of large rearrangements - when followed by a particular type of rejoining of broken ends, are the

actual cause of the losses.

With these points in view, the following experiment was planned to assess the frequency of losses for combinations of X and Y chromosomes of different total length and for X chromosomes of different structural types (V and ring-shaped).

Experimental set.up

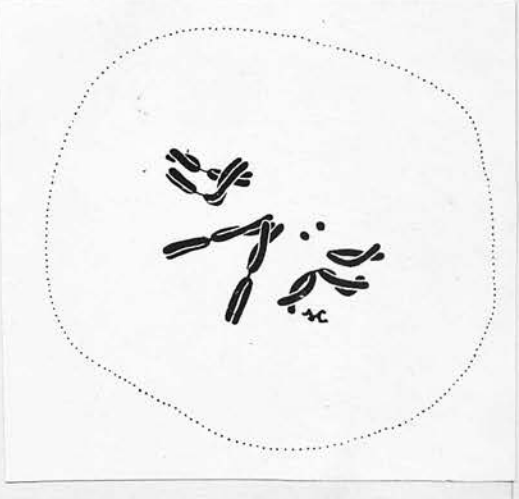
Four types of males, two from the already described stocks fXY^{S}/scY^{L} and $B^{S}Y^{S}/scY^{L}$ and the other two of constitution XY^{L}/Y and Xc^{2}/Y were X-rayed simultaneously with a dose of 4000 r and then mated individually to y v f/Y; bw^{VA}/L^{2} females (one male and 4-5 females per vial.) The technique of X-raying was that already described. A suitable number of control cultures (of 1 male and 2 females) were carried on at the same time. Normal and exceptional females were counted in F_1 and the latter submitted to the series of tests necessary to determine whether they carried some part of the paternal X or Y chromosomes.

The above types of males were chosen for the following reasons:

FIRSTLY - The first three groups of males - $B^{S}Y^{S}/scY^{L}$, fXY^{S}/scY^{L}, XY^{L}/Y - were thought, at the start of this experiment, to afford a comparison between three total breakage lengths which were distinctly different from one another. However, cytological and genetical

a

b



c

Fig. 3.- Metaphase plates showing that the scY^L is V- shaped.

a),male carrying a normal X and a scY^L.

b) female homozygous for an X-chromosome to which the long arm (sc) of the scY^L has become attached as a result of crossing over between X and scY^L

c) female carrying one X-chromosome as in b) and a normal X-chromosome.

The sc-containing arm of the scY^L is longer than the Y^L arm and it appears to be equal or perhaps longer than that of a normal X (Camera lucida drawings, all at same scale about X3050).

investigations (Pontecorvo, 1940) soon showed it to be rather different from the original description given by Crew & Lamy (1940), and this upset the original estimate of the relative breakage frequencies of the three types very considerably. The scY^L chromosome proved to be, in metaphase plates, not a rod the length of Y^L but a V-shaped chromosome, onearm - the shorter ! - of which is the long arm of the Y (Y^L). The other - longer arm - carrying distally its sc-component, is somewhat longer than an X-chromosome. The gene bb^+ is carried in this sc-arm proximally to sc. It is probable that there are two bb^+ genes in this arm, both proximally located in respect of sc, at least one of which is far from sc.

Fig. 3 shows how the cytological examination was checked by means of three different comparisons.

Now, taking the metaphase length of an X as 1, that of a Y^L as 0.75, that of a Y^S as 0.33 and that of a scY^L as 2 and remembering that (Muller & Painter, 1932) the $4RB^S$ chromosome was measured as having half the metaphase length of a whole X (i.e. 0.5) the following reckoning gives the relative metaphase lengths of the X and Y components, considered together, in the three stocks: fXY^S/scY^L 1+0.33+2.00 = 3.33; XY^L/Y 1+0.75 + 1.08 = 2.83; B^SY^S/scY^L 0.50+0.33+2.00 = 2.83. Of course only the length of the $4RB^SY^S$ chromosome is considered

as representing the X, in the case of the stock containing it, since as a rule this segregates from the scY^L while the remainder of the X, i.e. XL.4L, segregates independently, and gives, with XX eggs, inviable zygotes. Therefore losses induced in the XL.4L chromosome are not detectable as they merely cause an otherwise inviable (triplo-X) zygote to develop into either an XX/scY or an X.X/$4RB^SY^S$ female, i.e. of one of the two expected types of females.

Now with these metaphase lengths (considered from the point of view of losses of X and Y) of 3.33 for the fXY^S/sc^{YL}, 2.83 for XY^L/Y and of 2.83 for the B^SY^S/scY^L, it was thought that it should be possible to detect a) whether the frequency of losses for the first type of males is higher than for the other two; b) whether the other two, estimated to be about equal in metaphase lengths, give an almost equal frequency of losses. The above considerations were based on the idea that a fairly good parallelism would exist between metaphase length and breakage frequency, as it had appeared to be true in the relatively few cases involving heterochromatic regions that had previously been studied.

Only towards the end of the present experiment was it sufficiently realised that, on the contrary, the behaviour of the proximal heterochromatic regions of the X and Y in respect to breakage is extremely puzzling

and cannot be explained on simple assumptions. Small qualitative variations, and length variations so small as to be undetectable in metaphase, can probably be associated with great variations in breakage frequency. Investigations on this point are in progress following the general idea of Muller and co-workers (Muller & Gershenson 1935; Muller & Prokovyeva, 1935) that the proximal heterochromatic regions of the X and Y as seen in metaphase plates are composed of large blocks, within which no breakage occurs, alternating with minute regions of very high breakability.

SECONDLY - The fourth group of males, Xc^2/Y (i.e. carrying the ring-shaped X chromosome found by Beadle, and a normal Y) was used on the consideration that a comparison between the frequency of losses in this and in the other X-chromosomes might afford a crucial test of the loss mechanism itself. If losses are caused by broken sister chromatids, fusing with each other in such a way as to give a bilaterally symmetrical dicentric chromosome, subject to immediate loss or to repeated breakage leading eventually to loss, there is only one way in which this type of fusion can be achieved in a rod- or V-shaped chromosome, in comparison with at least two in a ring-shaped one. For in the former case only lateral fusion of the four broken ends, two by two, gives a dicentric chromosome (and

an acentric fragment). In a ring-chromosome, on the other hand, both lateral and diagonal fusion (see diagram No. 2) lead to a large double-sized dicentric ring. And even longitudinal fusion, under certain circumstances, leads to loss of a ring, as will be explained later. Therefore a ring chromosome should give a higher frequency of losses than a V- or rod-shaped one of the same breakage length if losses are really due to breakage.

In brief the experiment was planned to see whether or not the frequency of losses is correlated with the different breakage frequencies (at first supposed to be represented by metaphase lengths) of the X and Y chromosomes used, and whether or not, with the same breakage frequency, the frequency of losses is higher in a ring-shaped than in a non-ring chromosome.

Results.

Three series, each with all the four types of males ($B^{S}Y^{S}/scY^{L}$; fXY^{S}/scY^{L}; XY^{L}/Y; $X\mathbf{r}^{2}/Y$) and an additional series with the second and third types only were carried on with the conditions of treatment kept as constant as possible. After the first series, the system of culturing all the males in individual matings was abandoned because, as already mentioned, the advantage of avoiding the very rare cases of secondary

non-disjunction was found not to compensate for the difficulties of getting good cultures from single males X-rayed with 4000 r. Another change made was that in the 2nd and 3rd series, females of type y;bw;e;ey were substituted for the $\underline{y\ v\ f}\ \frac{bw^{VA}}{L^2}$ in the crosses with the males of type $B^{S}Y^{S}/scY^{L}$. This was done because some difficulty had been encountered in distinguishing regular F_1 females carrying $B^{S}Y^{S}$ and L^2 from exceptional ones carrying only L^2, both types having in some cases an extreme reduction of the size of the eye, verging on eyelessness. The autosomal recessives (bw;e;ey) in this stock serve here only as safeguards against non-virginity of the females. In the following table the crosses and the different types of regular and exceptional F_1 females are indicated.

	Composition of P_1 MALES (mated to $\underline{y\ v\ f}/Y;bw^{VA}/L^2$ females)			
	$B^{S}Y^{S}/scY^{L}$	fXY^{S}/scY^{L}	XX^{L}/Y	Xc^{2}/Y
F_1 FEMALES REGULAR	$\underline{yvf}/B^{S}Y^{S};bw^{VA}/+$ $\underline{yvf}/B^{S}Y^{S};\ L^2/+$ $\underline{yvf}/scY^{L};bw^{VA}/+$ $\underline{yvf}/scY^{L};\ L^2/+$	$\underline{yvf}/scY^{L};bw^{VA}/+$ $\underline{yvf}/scY^{L};L^2/+$	$\underline{yvf}/Y;bw^{VA}/+$ $\underline{yvf}/Y;\ L^2/+$	$\underline{yvf}/Y;bw^{VA}/+$ $\underline{yvf}/Y;\ L^2/+$
EXCEPTIONAL	$\underline{yvf}/0;bw^{VA}/+$ $\underline{yvf}/0;\ L^2/+$	$\underline{yvf}/0;bw^{VA}/+$ $\underline{yvf}/0;\ L^2/+$	$\underline{yvf}/0;bw^{VA}/+$ (x) $\underline{yvf}/0;\ L^2/+$	$\underline{yvf}/0;bw^{VA}/+$ (x) $\underline{yvf}/0;\ L^2/+$

(x) non detectable.

Whem after the first series, $B^{S}Y^{S}/scY^{L}$ males were crossed to y;bw;e;ey females, only two types of regular females - y/scY^{L}; bw/+ ; e/+ ;ey/+and $y/B^{S}Y^{S}$;bw/+ ;e/+ ;ey/+ - and one type of exceptions - y/0; bw/+ ;e/+ ;ey/+ - were produced. While in the crosses with the males of the two stocks having the scY^{L}, the exceptional females can be detected by means of the body colour, no matter whether they carry the bw^{VA} or the L^{2} second chromosome, in the two other crosses the exceptions must be detected by means of the effect on variegation. Thus in the latter crosses only half of the female progeny, namely, those carrying the bw^{VA} chromosome (recognisable as non-lobe), is utilizable. In other words, the exceptional females from the crosses of the first two lots of males can be detected in all the female progeny by the method involving scY^{L} and in half by both methods at once: that based on the suppression of variegation and that based on the "marker" y^{+} carried by the scY^{L}. However, in view of the fact that for the second and third series a different kind of female was used for crossing to $B^{S}Y^{S}/scY^{L}$ males, among the progeny of which the exceptions can be detected only by means of the scY^{L} method, it was decided for uniformity to base the detection of exceptions from both the crosses with $B^{S}Y^{S}/scY^{L}$ and fXY^{S}/scY^{L} males on this criterion alone, while the

detection of exceptions from the other two crosses (XY^L/Y and Xc^2/Y males was based on the criterion of variagation alone. The exceptions detected by the two methods do not form exactly parallel groups, as will be explained later. The results of the three series, and of the additional one in which only two lots of males (fXY^S/scY^L and XY^L/Y) were used, are reported in detail in the Table on the opposite page.

Tests of homogenity were carried out on these data, with the following results:

a) between the frequencies of the two types of exceptional flies from each of the two crosses with males of compositions B^SY^S/scY^L and fXY^S/scY^L, namely the L^2 and the bw^{VA} exceptions: $\chi^2 = 1.21$ for the first cross (one series) and $\chi^2 = 4.25$ for the second cross (four series). This gives $.30>P>.20$ (one degree of freedom) for the former and $.50>P>.30$ (four degrees of freedom) for the latter.

b) between the frequencies of exceptional flies from crosses of all four types in three different series (four series for the crosses of fXY^S/scY^L): $\chi^2 = 3.998$ and $.95>P>.90$ for 9 degrees of freedom (4 lots, of which there were 3 with 3 series, and one with four series).

From these tests the conclusions can safely be drawn that the experimental conditions have been

F_1 females from crosses of males carrying X and Y chromosomes of different length and shape.

P_1 MALES

	F_1 Females	$B^{S}Y^{S}/scY^{L}$			$fXYS/scY^{L}$									XY^{L}/Y			Xc^{2}/Y		
					bw^{VA}/			L^{2}/			Both			bw^{VA}/			bw^{VA}/		
		$\frac{yvf}{scY^{L}}$	Exceptions		$\frac{yvf}{scY^{L}}$	Exceptions		$\frac{yvf}{scY^{L}}$	Exceptions		$\frac{yvf}{scY^{L}}$	Exceptions		yvf/y	Exceptions		yvf/Y	Exceptions	
				%			%			%			%			%			%
TREATED	1st series	462	16	3.47	1505	41+½	2.76	1662	39+½	2.38	3167	80+2½	2.56	1832	24	1.31	2064	133+½	6.46
	2nd series	$\frac{y}{scY^{L}}$ 2498	73+5½	2.54	806	24+½	2.66	908	21+2½	2.42	1714	42+3½	2.54	2815	49	1.74	1720	117 (x)	6.80
	3rd series	1375	41+2½	3.07	344	9	2.62	345	4	1.16	689	13	1.89		——		217	12	5.53
	4th series	—	—		834	23+3½	2.94	1215	26+2½	2.22	2049	49+5½	2.52	567	11+½	2.03	-	-	-
	TOTALS	4335	130+7½	3.09	3489	94+5½	2.76	4130	90+5½	2.24	7619	184+10½	2.48	5214	84+½	1.62	4001	262+½	6.56
	CONTROLS	5220	8	0.183							7977	6	0.075	4065	6	0.148	2672	67	2.51

(x) 1 Haplo-4th.

kept very uniform, the differences between successive series being well within the limits of random sampling and that the two types of exceptions in each of the two crosses with scY^L-bearing males occur in frequencies that do not differ significantly from each other.

The summarised results of the experiment are as follows :

Exceptional females obtained from crosses with males carrying different X and Y chromosomes.

	P_1 MALES							
	B^SY^S/scY^L		fX^YS/scY^L		XY^L/Y		Xc^2/Y	
	Reg. No.	Except. No. %	Reg. No.	Except. No. %	Reg. No.	Except. No. %	Reg. No.	Except. No. %
TREATED (4000 r)	4,335	133½ 3.09 ±.26	7,619	189 2.48 ±.18	5,214	84½ 1.62 ±.17	4,000	262½ 6.56 ±.39
CONTROLS	5,220	8 0.153 ±.054	7,977	6 .075 ±.031	4,065	6 .148 ±.060	2,672	67 2.51 ±.30
TREATED corrected for CONTROLS		2.94 ±.26		2.40 ±.18		1.47 ±.18		4.05 ±.49

The tests of significance of the differences between the frequencies of exceptions (corrected for those found in the controls) in the four different crosses of that treated series, taken two by two, are given in the following table.

Comparison between frequencies of exceptions in crosses:		Difference	Difference / error
$B^{S}Y^{S}/scY^{L}$	& fXY^{S}/scY^{L}	0.54 ± 0.32	1.7
$B^{S}Y^{S}/scY^{L}$	& XY^{L}/Y	1.47 ± 0.32	4.6
fXY^{S}/scY^{L}	& XY^{L}/Y	0.93 ± 0.25	3.7
Xc^{2}/Y	& $BSYS/scY^{L}$	1.11 ± 0.55	2.-
Xc^{2}/Y	& fXY^{S}/scY^{L}	1.65 ± 0.52	3.2
Xc^{2}/Y	& XY^{L}/Y	2.58 ± 0.52	4.9

Thus the crosses with Xc^{2}/Y, $B^{S}Y^{S}/scY^{L}$, fXY^{S}/scY^{L} and XY^{L}/Y males give exceptional flies with frequencies decreading in the order here given. The differences between any two are significant, except for the comparison of fXY^{S}/scY^{L} with $B^{S}Y^{S}/scY^{L}$, in which case the probability of such a difference arising by chance is something more than 5%

Tests of the exceptional flies.

To distinguish between cases of flies in which the X or Y chromosome is wholly lost and those of partial loss giving the same phenotypical effect, three tests were carried on with each exception fly.

Slight modifications of the technique used in the preceding experiment were introduced, some with the aim of making the present tests more significant even in the very frequent cases in which the exceptional fly died before the completion of the tests, and some others required by the differences between the crosses in the two experiments.

One modification of technique was suggested by the observation that in almost all cases in which a partial loss, giving a yellow or variegated exception, resulted in a deleted chromosome carrying the whole set of fertility genes of the Y^S, the gene bb^+ also was present. This supports Neuhaus's (1936,36,39) findings that one gene bb^+ is certainly present in the Y^S, while its presence in the long arm is still doubtful. Thus, for our exceptions, a negative result of the test for bb^+ implies a negative one for the fertility genes of Y^S. The order of the tests was therefore changed, putting first that for the presence of Y^L, then that for bb^+ and finally that for Y^S. Even when the test fly died before the last test, a negative result of the second made it extremely likely that no part of the Y^S was present, i.e. that the loss was complete, whereas of course a positive result of the second was sufficient to prove an incomplete loss.

Another change of technique was necessary for the crosses with XY^L/Y males. In this case, the exceptional flies, when collected, were already inseminated by their XY^L/Y brothers. Thus their sons were available for the test of the Y^S. In this case then the series of tests was accomplished in the following order: 1st) Test of Y^S; 2nd) Test of Y^L; 3rd) Test of bb^+ locus. The scheme of the crosses and tests made on each exceptional fly will be better understood from the diagram below:

Each exceptional female X/? from the cross with males of type :

	$B^{S}Y^{S}/scY^{L}$	fXY^{S}/scY^{L}	XY^{L}/Y	Xc^{2}/Y
1) When collected, fertilized by brothers:	$B^{S}Y^{S}/Y$	fXY^{S}/Y	XY^{L}/Y	Xc^{2}/Y
gave sons :	$B^{S}Y^{S}/?$	$fXY^{S}/?$	$XY^{L}/?$	$Xc^{2}/?$
which, if fertile, proved presence in the mother of :	Y^{L}	Y^{L}	Y^{S}	whole Y
2) mated to males :	$ysc^{4}sc^{S1}$; (bb-def)	$ysc^{4}sc^{S1}$; (bb-def)	$yvfY^{S}/scY^{L}$	$yvfY^{S}/scY^{L}$
gave sons :	$ysc^{4}sc^{S1}/?$	$ysc^{4}sc^{S1}/?$	$yvfY^{S}/?$	$yvfY^{S}/?$
which, if fertile, proved :	non-disjunc. in father	non-disjunc. Y^{L} in mother		Y^{L} in mother
if sterile proved :	bb^{+} (outside X) in the mother	bb^{+} (outside X) in the mother	no Y^{L} in the mother	no Y^{L} in the mother
if no sterile sons appeared:	no bb^{+} (outside X) in the mother	no bb^{+} (outside X) in the mother	___	___
3) mated to males :	XY^{L}/Y^{S}	XY^{L}/Y^{S}	$ysc^{4}sc^{S1}$ (bb-def)	$ysc^{4}sc^{S1}$ (bb-def)
gave sons:	$XY^{L}/?$	$XY^{L}/?$	$ysc^{4}sc^{S1}/?$	$ysc^{4}sc^{S1}/?$
if fertile proved :	Y^{S} in the mother	Y^{S} in the mother	non-disjunc. in father	non-disjunc. in father
if sterile proved:	no Y^{S} in the mother	no Y^{S} in the mother	bb^{+} (outside X) in the mother	bb^{+} (outside X) in the mother
if no sterile sons appeared :	___	___	no bb^{+} (outside X) in the mother	no bb^{+} (outside X) in the mother
4) mated to males :	___	___	___	XY^{L}/Y^{S}
gave sons :	___	___	___	$XY^{L}/?$
if fertile proved :	___	___	___	Y^{S} in the mother
if sterile proved :	___	___	___	bb^{+} (outside X) in the mother
if no sterile sons appeared:	___	___	___	no bb^{+} (outside X) in the mother.

As already pointed out, the difficulty of obtaining from <u>each</u> exceptional fly three (and in the case of the Xc^2/Y lot, four) different kinds of progeny accounts for the fact that only part of the total number of the exceptions could be thoroughly tested. The results of the tests are here summarized :

Exceptions from the crosses with males:	Flies tested for Y^L		Flies negative for Y^L tested for bb		Calculated percentage of exceptions which gave no positive test.
	Total	Positive	Total	Positive	
B^SY^S/scY^L	87	7 (2)	50	20	55.2 ± 8.5%
fXY^S/scY^L	117	18 (3)	90	18	67.6 ± 4.5%
XY^L/Y	47	10 (6)	20	8	47.2 ± 14.7%
Xc^2/Y	138	5 (2)	38	4	86.2 ± 4.8%

In brackets, cases of presence of a whole Y.

The exceptional flies which carry some part of the X or Y chromosome, expressed as a percentage of the total regular F_1 females, give the following figures:

<u>Crosses</u>:	Total Regular females	Frequency of exceptions (corrected for controls) %	Calculated frequency of exceptions carrying parts of X or Y that do not contain known loci of euchromatic regions %
B^SY^S/scY^L	4335	2.94 ± .26	1.32 ± .28
fXY^S/scY^L	7619	2.40 ± .18	.78 ± .12
XY^L/Y	5214	1.47 ± .18	.78 ± .24
Xc^2/Y	4000	4.05 ± .49	.56 ± .21

The data are affected by considerable errors, but it is interesting to note that, quite unexpectedly, the frequency of partial X's or Y's is higher in the $B^{S}Y^{S}/scY^{L}$ cross than in any of the others. As the difference from the results of the fXY^{S}/scY^{L} cross cannot be attributed to anything else than to a difference between the paternal fXY^{S} and 4R $B^{S}Y^{S}$ chromosomes, the rather astonishing conclusion must be drawn that the latter chromosome has a breakage frequency higher than the former. This contrasts strikingly with what would have been expected from the relative mitotic metaphase lengths, and still more with expectation based on salivary sizes, and therefore disagrees with the anticipation based on the assumption of parellelism between breakage frequencies and metaphase lengths.

Summarizing, the great majority of exceptions are nullo-Y in the Xc^{2}/Y cross and something between $\frac{1}{2}$ and $2/3$ of them are of this kind in the other cases.

It must be pointed out that the detection of the exceptions by means of the two methods, namely, that based on the scY^{L} and that based on the suppression of variegation, is not exactly equivalent. In fact, all deletions of any part of the scY^{L} bearing its sc-component unaffected are undetected; a part at least of these deletions would, however, affect variegation,

if this method of detection were adopted instead. On the other hand, minute rearrangements in any part of the Y chromosome presumably do not affect variegation but if they occur in the sc-component of the scY^L, they give a yellow "mutation" which is detected as an exception.

A preliminary gauge of the amount of discrepancy between the two methods can be obtained from the following data on the cross with fXY^S/scY^L males:

number of yellow exceptions (fractional excluded) carrying the bw^{VA} chromosome	92
number of the above <u>not</u> having variegated eyes - - -	16

Proportion 17.4 ± 3.90%

Relatively to the regular flies, the yellow <u>non</u> variegated exceptions have a frequency of 0.43%. Out of 13 of these yellow non-variegated exceptions which it was possible to test, three showed the presence of a Y^L, and one of a whole Y. The remainder, on being tested for the presence of Y^S or of a deletion carrying bb^+, all showed the presence of bb^+ or of both bb^+ and Y^S. It can thus be concluded with sufficient confidence that practically none of the yellow non-variegated flies constitutes a case of loss

of the whole X or Y chromosome.

The possibility is thereby excluded that with the method of variegation many cases of complete loss will be missed, although no doubt a part (17.4±3,9%) of the incomplete loss exceptions detected with the scY^L method would escape detection as such on the method of variegation.

On the other hand there must be cases of deletions of the Y which are classified as exceptions with the method of variegation and not with the other method: namely, all cases in which a sufficiently large intercalary portion of heterochromatin in the Y^L arm is deleted while the other arm remains unaffected, so that variegation is no longer suppressed/- but in the case of a scY^L chromosome - yellow is still covered. This kind of deletions of the Y gives y v f variegated exceptions in the XY^L/Y and Xc^2/Y crosses and non-yellow, v f variegated exceptions in the crosses with the fXY^S/scY^L. This second type of exceptions is not phenotypically distinguishable from that of deletions occurring in the X chromosome with their left break to the right of y^+ and their right break between v^+ and the centromere.

An idea of the relative importance of the two types of non-yellow variegated exceptions arising in the fXY^S/scY^L cross, produced by deletion in the scY^L and in the fXY^S, respectively, may be obtained by comparing the frequency of this type

of exceptions in this cross with that of deleted XY^L - with left break right of y^+ and right break right of $\overset{+}{v}$ - arising in the XY^L/Y cross.

	CROSS with MALES:	
	fXY^S/scY^L	XY^L/Y
Regular F_1 females: phenotype	vf non var.	yvf non variegated
No.	3489	4065
Exceptional F_1 females: (phenotype)		
a) vf, variegated	37(x)	13
b) vf, non var.	--	13
c) v, variegated	(indistinguishable from vf var.)	6
d) yv, variegated	(indistinguishable from yvf var.)	2
e) yv, non var.	--	1
a) b) c) No.	37	32
%	1.06 ±0.17	0.79±0.14

(x) As the Y^S plus the whole proximal region of the X does not suppress variegation (see page 35) it seems reasonable to assume that no case of deletion of the fXY^S could give non-variegated vf females.

Although the numbers are too small for an accurate determination, they show that the difference between cases of deleted scY^L, giving variegated but not yellow flies, and the corresponding cases of deleted

Y, giving variegated and yellow flies, cannot be very great. Taking the figures at their face value, the difference is 0.25%

Summarizing, we have a frequency of 0.25% for incomplete-loss exceptions detectable with the method of variegation but not with that of the scY^L, and of 0.43% for incomplete-loss exceptions detectable with the method of the scY^L but not with that of variegation. On the whole, when allowance is also made for the large errors affecting these estimates, it seems reasonable to consider that with the scY^L method more flies are recorded as exceptions than with the other.

Another point to be considered is that a detected partial or total loss of the X has made a viable exception out of a zygote which would otherwise have been an inviable triplo-X. The triplo-X zygotes are of course produced in equal numbers as the X/Y zygotes. Therefore in calculating the frequency of exceptions, the number of the latter should be divided by the number of attached -X flies of the normal viable type - indicated throughout this paper, for sake of simplicity, as "Regular" flies - plus the exceptions produced by loss of the Y (or scY^L) chromosome, were their number determinable. The error made by disregarding this point is irrelevant: the figures given would be modified by some 0.01 to 0.03%,

thus by an amount smaller by one order of magnitude than the experimental error.

It is to be noted, however, that the simple percentage of exceptional to non-exceptional females is the percentage which the combined losses of the X and the Y form in relation only to the Y-bearing sperms, and hence does not represent the average frequency of a loss of a sex chromosome (X or Y) per sperm. The latter frequency, better expressing the real frequency of losses, is only half of the percentage above mentioned. This must be borne in mind in the interpretation of the figures given here, which are of the already mentioned empirical kind.

Relationship between the length and the shape of the X and Y chromosomes and the frequency of losses.

Going back to the data on page 70-71, a calculation of the percentage of complete loss gives the following results :

P_1 Males	Frequency of induced exceptions %	Percentage of exceptions constituting loss %	Frequency of losses %
B^SY^S/scY^L	2.94±.26	55.2±8.5	1.63±.29
fXY^S/scY^L	2.40±.18	67.6±4.5	1.63±.16
XY^L/Y	1.47±.18	47.2±14.7	0.69±.23
Xc^2/Y	4.05±.49	86.2±4.8	3.49±.43

Now, disregarding for a moment the Xc^2 cross, it was

expected on the basis of metaphase length that the frequencies of losses should be higher with the fXY^S/scY^L males and lower with the B^SY^S/scY^L and XY^L/Y. On the other hand, on the basis of the rough indications concerning the breakage frequency, obtainable from the data on the frequency of partial losses (page 15) the B^SY^S/scY^L males should have given a higher frequency of losses than the other two groups. At first approximation, the above data do not contradict the latter assumption. ¶ Finally, the frequency of induced losses with Xc^2/Y males is much higher than with any of the others, as expected if the assumed mechanism for losses is true. The calculation of how much higher the frequency of losses is for the Xc^2 chromosome alone, in comparison with that for a rod-shaped X-chromosome, would require a knowledge of the separate contributions of a normal X and of a normal Y to the frequency of losses. This is not obtainable from our data because of the already discussed discrepancies between breakage frequencies and cytological length. If, however, we compare the frequencies of losses with XY^L/Y and with Xc^2/Y males, the difference between the two can only be attributed to the Xc^2 chromosomes as compared with the XY^L chromosome. Thus, even supposing that the Y did not contribute at all to the losses, the Xc^2 would have

undergone loss $\frac{3.49}{0.69}$ = 5.- ± 1.8 times as frequently as an XY^L and this despite the additional length of the latter. As this estimate is obviously ultra conservative, it may be safely concluded in the sense not only that induced losses of an Xc^2 chromosome are more frequent than those of a normal X, but that they are more than twice as frequent as the latter.

In conclusion, the results of this experiment seem to show that: a) some positive correlation does exist between breakage frequency and frequency of losses, and b) the frequency of losses of an Xc^2 chromosome is higher, probably considerably more than twice as high, as that of a normal X-chromosome.

F) "FRACTIONAL" (Mosaic) LOSSES AND REARRANGEMENTS.

In both experiments described, a certain number of the exceptional flies were of the "fractional" type. In the crosses with scY^{L}-bearing males, the loss of the XY^{S} or of the scY^{L} chromosomes - or their structural change simulating a loss - was observable in these "fractionals" in some 1/4 to 3/4 of the body of the exceptional fly, the exceptional part showing the yellow colour while the remainder was grey. In the other crosses, mosaics affecting the body colour were, in the exceptional portion of the body, hyperploid either for a whole or for a deleted X, as the case might be, while those (very rare) mosaics showing variegation in only one eye originated from loss or deletion in one chromatid of the Y and in other cases from a very large deletion of one chromatid of the XY^{L} or of the Xc^{2}.

As has been noted in earlier work, the head, in the great majority of cases, was not mosaic, in other words, it was either all "mutated" or all non-"mutated", a circumstance caused by the fact that all of the head is usually derived from but one of the two nuclei formed at the first mitosis after fertilization. When a fractional fly carried the bw^{VA} chromosome, the eyes were in a condition to

show the effect on variegation: however, this occurred in only 4 cases (1 from the fXY^S/scY^L, 2 from the Xc^2/Y and 1 from the XY^L/Y crosses), owing to the above mentioned rarity of cases in which the head is mosaic. Incidentally, these mosaics of variegated eye prove that the effect of extra heterochromatin in suppressing variegation is to a considerable extent a localized one and does not act to a noticeable extent by means of some substance released into circulation throughout the body.

Particular attention was paid to the fractionals originating in the two crosses with scY^L males (fXY^S/scY^L and B^SY^S/scY^L) because it was thought that none of those originating from partial loss or structural change of the scY^L would show that sterility (or failure to produce viable progeny) found in mosaics produced by partial loss (or structural change) of the X-chromosome (Moore, 1934).

"Fractional" exceptions in offspring of the B^SY^S/scY^L and fXY^S/scY^L crosses.

P_1 males	Regular flies	Exceptional flies					Ratio fractionals / all exceptional
		"Total"	"Fractional"				
			1/4	1/2	3/4	Sum	
B^SY^S/scY^L	8158	134	1	2	4	7	1:20
fXY^S/scY^L	7619	184	-	7	3	10	1:19
	15777	318	1	9	7	17	

The ratio fractionals found here is less than one third of all except that - 1:6 - found by Patterson (1933) in his classical work. This is to be expected (1) because, unlike Patterson's, a large number of our cases concern parts of the Y, the presence or absence of which is not lethal even in a "total" exception, and (2) because our treated males were crossed to attached X females rendering more of the "total" exceptions involving the X viable as well. In Patterson's case, the treated males were crossed to females with free X's, so that a great number of his "totals", involving as they did the X in all cases, must have been lethal, while the corresponding "fractionals" could in many cases have survived, the smaller the "mutated" fraction, the higher the chance of a milder deleterious effect on the zygote as a whole.

Out of 17 fractionals produced in the fXY^{S}/scY^{L} and $B^{S}Y^{S}/scY^{L}$ crosses, 14 gave enough progeny to furnish a genetic test. In these 14, the gonads were mosaic in only one case. 9 bred with the yellow part (including the one which bred with both parts) and these were thoroughly tested. 7 of them proved to contain one or other part of the X or Y, while only 2 gave all tests for X or Y parts negative. Thus $\frac{7}{9} = 78 \pm 14\%$ of the yellow part of the body in "fractionals"

originate not by a loss of a whole chromatid, but by one of the several types of minute or gross structural changes which simulate a loss. Pooling together the results given on page 75 for the two crosses, the proportion of non-fractional exceptions which contain a part of X or Y chromosome turns out to be 36.1±3.7%. The difference between this result and that for the fractionals is significant (41.9±14.4%) and shows that losses of whole chromatids among the "fractionals" are definitely less frequent than losses of whole chromosomes among the "totals". The bearing of these findings is of particular interestin the interpretation of the mechanism of production of losses.

G) BREAKABILITY OF THE TIP OF THE X CHROMOSOME.

A certain number of the exceptional flies obtained in the preceding experiments were produced by:

a) large deletion of the fXY^S and XY^L chromosomes respectively from the fXY^S/scY^L and XY^L/Y treated males. In all cases, one break was distal to the locus of y^+ (0.0) or so near to y^+ as to give the effect of mutation to y.

b) minute rearrangement in the y^+ region of the scY^L, giving a "mutation" to y, or large deletion of the same arm of the scY^L with distal break to the left of the y^+ locus or at any rate causing it to "mutate" to y.

Two points are of some interest in connection with breaks occurring in the very terminal region of the X, namely, the frequency of minute rearrangements occurring in it, and the location of the distal break, the second matter having a bearing on the problem of the so-called terminal deletions.

As for the first point, the scY^L chromosome, in which the small active portion carrying sc^{Sl} is located between two heterochromatic regions, affords an opportunity of measuring the occurrence of minute rearrangements involving the y^+ locus.

Occurrence of minute rearrangements ("mutations" to y) in scY^L (fXY^S/scY^L P males treated with 4000 r).

Frequency of exceptions corrected for controls	Proportion of exceptions produced by minute rearr. in scY^L	Frequency of minute rearrangement in scY^L
2.41±.18%	12.8±3.1%	0.308±0.077%

The frequency found is in very good agreement with that -0.318±0.038 - obtained by Muller and Makki (1939,

unpublished) for "mutations" to yellow in the $sc^{8}X$-chromosome. In this chromosome the y^{+} locus is situated, as in the scY^{L}, between the two heterochromatic regions respectively of the tip of the X and of its inverted proximal region. As Muller, Prokofyeva & Raffel (1937), Belgovsky & Muller (1937) and Belgovsky (1938) very clearly proved, this situation increases the liability of genes lying nearby to be affected by minute rearrangements.

The other point which could be tested with our material was in how many cases a break in the very distal region of the normal X, on the one hand, or of the scY^{L} on the other hand, leaves the locus of lJ^{1}, which is still nearer the end than is y^{+}, still covered.

For this purpose a certain number of "mutated" scY^{L} and of deleted fXY^{S} chromosomes were tested for this locus. The test consisted in crossing a female with attached X's and the mutated chromosome to males of composition $scJ^{1}\underline{l}$/Del.14/Y, i;e; males in which the lethal inseparable from the minute rearrangement which gives the "mutation" scJ^{1} was covered by Del.14 (Muller, 1932). The appearance in F_1 of males showing the sc^{Jl} character was a proof that the break had occurred to the left of yellow (locus 0.0), but proximal to $\underline{l}J^{1}$ (locus 0.0).

The results are as follows :

Type of chromosome tested	Tested N°	"Covering" $1J^1$ N°
"mutated" scY^L	26	5
deleted fX^S	10	-
	36	5

Raffel (1938) in analyzing 15 different sc^8 X-chromosomes in which a break left of yellow had been produced, found that in 5 $1J^1$ was still covered. The proportion 5/26 found here for the scY^L chromosome, -in which the terminal region of the X is, as in sc^8, juxtaposed to a heterochromatic, previously proximal, region-is of the same order as that found by Raffel. It is interesting to note on the other hand that out of 10 tested cases of X-chromosomes derived from X's of normal structure, no cases occurred of $1J^1$ being covered. Perhaps in an X chromosome with normal arrangement breaks in the distal end occur more easily in the very terminal bands (left of 1J1) which Prokovyeva (1937) has shown to be heterochromatic. All this gives further support to Muller and co-workers' (1938) ideas, that the heterochromatic regions are especially predisposed not only to breakage, but to compound breakage with minute rearrangement.

H) EXPERIMENT ON THE INDUCTION OF LOSSES OF THE 2nd AND 3rd CHROMOSOMES.

The present experiment was planned to investigate whether by X-raying mature sperms, losses of the two major autosomes also can be induced which, under suitable conditions, will be non-lethal to the zygote. As a secondary objective, it was expected to be able to detect those cases of translocations (of type X-Autosome, Y-Autosome or II-III) which are lost with the usual techniques, namely, cases of reciprocal translocations in which a dicentric chromosome and an acentric fragment are produced. It was thought that the direct detection of these translocations might open a promising new way of investigating - by direct inspection of F_1 - problems of chromosome rearrangements in general, and particularly in some details of the frequency-dosage relationship. Finally it was believed that the possibility of producing viable zygotes in which the "dose" of the paternal autosomes could be varied, with all intermediate steps from 2/4 to 0/4, might be of technical use in studies of interspecific hybrids. The positive results concerning the production of losses of the autosomes, to be described in what follows, are in fact now being used in an investigation of this kind into the causes of sterility

of D. melanogaster x simulans hybrids.

Experimental set up.

Males of composition v; $\frac{Cy\ L^4}{+}$; $\frac{D\ CXF}{+}$, i.e., carrying a normal X marked with the recessive vermilion (v;eye bright red, 33.-), a normal Y, and heterozygous for 2nd and 3rd chromosomes containing a series of crossover suppressors and dominant markers (see page 42) were X-rayed with a dose of 4000 r with the technique already described and immediately afterwards crossed to virgin triploids of composition y/sc^{Sl};bw;e. Four days after this mating the males were discarded and the females transferred so as to give a second "brood", derived from the eggs layed for the next 6-7 days. The F_1 flies of the two broods were recorded separately as the possibility was not excluded that the proportion of the different kinds of gametes produced by triploids changes with age. Two series were carried out with treated males and a control series with untreated males.

Results of main experiment and correlative tests.

The results are given in detail in Table A. They show that:

a) losses of either of the two autosomes actually occur and, in part at least, give viable zygotes, the corresponding exceptional flies having a frequency of the order of 2-2.5% at the dose here used (4000 r);

b) losses of both autosomes together caused by dicentric-acentric translocations appear always to give inviable zygotes as the few exceptions showing both losses at once are not significantly more than expected as a result of the mere coincidence of two independently produced losses of an autosome;

c) the exceptions spontaneously occurring in the control series have a very low frequency, some two orders of magnitude lower than in the treated series;

d) a high proportion of triploid females of this stock carry an extra Y as shown by the appearance among their progeny of numerous fertile patroclinous (vermilion) sons.

A sample of the regular sc^{SI} and v males heterozygous for both bw and e obtained in F_1 of the treated series were tested for translocations between the second and third chromosomes by crossing them

TABLE A

F_1 from v; $\frac{Cy\ L^4}{+}$; $\frac{DCXF}{+}$ males, crossed to y/sc^{Sl}; bw; e triploid females.

		First Brood						Second Brood						Both Broods					
		Regular	Exceptional					Regular	Exceptional					Regular	Exceptional				
			bw	e	bw e	N°	All %		bw	e	bw e	N°	All %		bw	e	bw e	N°	All %
Treated P Males 1st Series	Triploid ♀♀	438	(2)					322						760	(2)				
	Diploid v/sc^{Sl} ♀♀	604	5	5		10		444	2	7		9		1048	7	12		19	
	Diploid y ♀♀	671	4	6		10		394	2	4		6		1065	6	10		16	
	Males sc^{Sl}	431	5	2		7		270	3	1	1	5		701	8	3	1	12	
	Males v	105	3	8		11		78	2			2		183	5	8		13	
	Intersexes y	247	(1)	(1)				109						356	(1)	(1)			
	Intersexes v/sc^{Sl}	488						225						713					
	All Diploids	1811	17	21		38	2.10	1186	9	12	1	22	1.86	2997	26	33	1	60	2.01
	All F_1 Flies	2984						1842						4826					
Treated P Males 2nd Series	Triploid ♀♀	260	(3)					191	(2)					451	(5)				
	Diploid v/sc^{Sl} ♀♀	396	5	5		10		448	2	6		8		844	7	11		18	
	Diploid y ♀♀	377	5	6		11		328	4	1		5		705	9	7		16	
	Males sc^{Sl}	173	5	3	1	9		191	3	2		5		364	8	5	1	14	
	Males v	136	1	1		2		121	3	1		4		257	4	2		6	
	Intersexes y	104						117						221					
	Intersexes v/sc^{Sl}	177		(1)				261						438		(1)			
	All Diploids	1082	16	15	1	32	2.96	1088	12	10		22	2.02	2170	28	25	1	54	2.49
	All F_1 Flies	1623						1657						3280					
Treated P males Both Series	All Diploids	2893	33	36	1	70	2.42	2274	21	22	1	44	1.94	5167	54	58	2	114	2.21
	All Flies	4607						3499						8106					

( ) Cases of deleted 2nd or 3rd chromosome.

Controls.

	1st brood	2nd brood	Both broods
Total flies	4362	2340	6702
Total diploids	2833	1570	4303
Exceptions	2(*)		2
%			0.046

(*) 1 male v; bw.
1 female y; bw.

individually to homozygous bw;e;females. The translocations were identified by the apparent linkage of bw and e among the F_2.

Translocations of type II-III found among the $F_1 sc^{SI}$;bw/+;e/+ and v;bw/+;e/+ males of the treated series.

Males tested	Fertile cultures	Translocations	
	No	No	%
sc^{SI};bw/+;e/+	164	12	7.3
v;bw/+;e/+	95	9	9.5
	259	21	8.1±1.6

As there is no ground for believing that the recombination of broken ends of two chromosomes is more likely to occur in the eucentric than in the acentric-dicentric way, it can be assumed that acentric-dicentric translocations of type II-III must have been produced in the sperms with the same frequency (8.1%) as found for the eucentric translocations.

Now, 1728 intersexes were recorded in the treated series. To these, a number equal to that of the sc^{SI}/v intersexes, i.e. 1151, must be added to allow for the mostly inviable supermales originating from fertilization by a Y-bearing sperm. Should the dicentric translocations be eliminated in the early cleavage divisions so as to be non-lethal to the zygote, some 2879x.081 = 233±37 diploids showing

both bw and e would have been obtained from eggs containing a diploid set of autosomes, fertilized by sperms in which such a translocation had been induced. Instead only two such cases were found. As many as two are to be expected, as a result of the coincidence, in the same sperm, of the loss of the two autosomes.

The fact that exceptional bw or e flies occur among all types of diploid progeny is in itself a proof that the loss of an autosome can not always be connected with a dicentric translocation induced in the paternal sperm between the X- and the autosome. In fact, if this were the case, no exceptional diploid females of type sc^{SI}/v could be produced. If on the other hand losses were connected with a translocation involving the Y, the y exceptions would not carry the paternal Y chromosome and should therefore give sterile sons. Similarly the sc^{SI} exceptions (males) would not carry a Y and should therefore be sterile. Fertility tests of the sc^{SI} exceptional males and of the sons of the y exceptional females were carried out and compared with tests of the corresponding non-exceptional flies. However, firstly, the presence of an extra Y in the triploid mother, which could have transmitted it to both the exceptional y females and sc^{SI} males (though to not more than half of the two combined if the mother carried only one Y) and secondly, the low

fertility and viability of sc^{SI} males, especially when homozygous for bw or e, suggested, a priori, that these tests would not be decisive.

The following results were obtained:

	Total No	Gave fertile sons No	Died without progeny No
Exceptional y females tested for fertility of their sons	18	13	
Regular y females tested for fertility of their sons	36	32	
		Fertile No	
Exceptional sc^{SI} males tested for fertility	17	10	7
Regular sc^{SI} males tested for fertility	243	137	106

In view of the complications already described, these tests, and particularly that of the males, are not reliable, although they do suggest that the detected losses of autosomes cannot all result from dicentric translocations involving the Y and so causing the loss. Yet it was still possible that a part, perhaps the greatest part, of losses might be produced by some such mechanism.

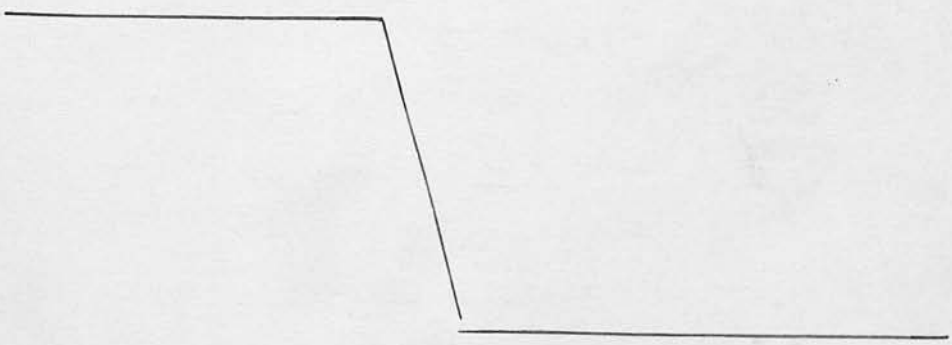
To test this point more decisively the following experiment was designed. Treated males of type fXY^{S}/scY^{L} were crossed to bw;e triploids of the type already used. In this case all the y regular progeny (both diploids and intersexes) are phenotypically non-yellow because of the y^{+} marker carried by the scY^{L}. If an egg with

the y-bearing attached -X chromosome (y) is fertilized by a sperm in which a potential loss of the fXY^S or scY^L has been induced, a phenotypically yellow fly, diploid or intersex, will appear, provided the egg had respectively a haploid or a diploid set of autosomes. If on the other hand the loss of an autosome has been induced, a homozygous brown or ebony diploid will develop in those cases in which the egg was of the complementary aneuploid type. As found by Bridges (loc. cit) the aneuploid eggs constitute more than 50% of all eggs laid by triploids. In a very small percentage of cases, the two types of losses may be induced independently in the same sperm and produce a yellow and brown (or ebony) fly. Finally, if the loss of the fXY^S or of the scY^L is linked with the loss of an autosome through a dicentric translocation, and the sperm in which it has been induced fertilizes an egg diploid for an autosome (more than 25% of the eggs being of this kind) a diploid yellow female, which was at the same time brown or ebony, should appear.

Eucentric translocations between a normal Y and one or the other major autosome, when only viable and fertile types are considered, occur at 4000 r with a frequency of at least 5%. Eucentric viable and fertile translocations between a normal X and either autosome also have a frequency of about 5% at this

dose. For the considerations already mentioned on the chance of acentric-dicentric reunion, it may thus be inferred that <u>at least</u> 10% (notably more if we consider the breakage length in our case) of dicentric translocations should occur in the treated sperms between the fXY^S and scY^L on one hand and either autosome on the other. As somewhat over 50% of these would fertilize the proper kind of aneuploid egg, the frequency of fXY^S-Autosome (or scY^L-Autosome) dicentric translocations which should have the possibility of being detected, is, at a very conservative estimate, of the order of somewhat more than 5%, that is, a frequency at least twice as high as that of the detected losses of autosomes.

The results of the special test made with the fXY^S/scY^L males (shown below) seem to prove definitely that this kind of translocations are at least in the great majority of cases, perhaps always, lethal to the zygote, and that the detected losses of the autosomes must result from a mechanism other than that of a dicentric translocation.



X & Y,
Losses of/2nd and 3rd chromosomes obtained in F_1 of treated fXY^S/scY^L males & $\hat{y}/sc^{Sl}$;bw;e triploid females.

Type of F_1 progeny	Regular flies	Exceptional flies					
		bw	e	bw;e	y	y;bw	y;e
TRIPLOID ♀♀	111	-	-	-	-	-	-
DIPLOID ♀♀ (y/scY^L or sc^{Sl}/fXY^S)	244	9	6	-	12	-	1
MALES sc^{Sl}	118	2	2	-	-	-	-
MALES fXY^S	15	1	1	1(x)	-	-	-
INTERSEXES (sc^{Sl}/fXY^S or y/sc^{YL})	133	-	-	-	4	-	-
ALL DIPLOIDS	377	12	9	1	16	-	1
ALL FLIES	621						

(x) Very abnormal aspect.

In fact, as the table shows, 12 exceptional yellow females and 15 exceptional grey females were found, in comparison with only one case of exception showing both yellow and one of the autosomal recessives. Out of the 15 grey exceptions, 13 were fertile. The inspection of their progeny showed that 6 had been of composition y/scY^L. The appearance of a single y;e exception is no more than expected as a result of mere coincidence of the two types of losses.

Although the data cannot be large enough to exclude the possibility that on rare occasions a dicentric Y-A. or X-A translocation can be eliminated and allow the zygote to survive, it can be concluded that in the great majority of cases elimination does

not occur in time or that if it does, it itself is in some way lethal.

A final observation is needed with regard to a particular type of exception recorded on the table A at page 94. Seven triploids and three intersexes are recorded that show one of the maternal recessives. Out of these, eight (7 triploids and 1 intersex) showed the character brown eye. All showed in addition the dominant Cy and half of them also the dominant L^4, both carried in one of the paternal 2nd chromosomes. Neither of the two ebony intersexes, of very abnormal appearance, did show the dominant D, carried in one of the paternal 3rd chromosomes.

All these are surely cases of small or large deletion in one of the paternal (treated) major autosomes. That this is so, is shown by the fact that in many of the bw exceptions L^2, which is located as bw in 2R, was lost together with the deficient portion.

It is interesting to observe that no such cases were found among the diploid progeny. Thus a deleted autosome appears to be almost always lethal to a diploid zygote while in many cases it is not so to a triploid one, no doubt on account of the proportionally lesser unbalance.

The formation of aneuploid eggs :-

The property of attraction and resultant segregation of chromocentral regions of non-homologous chromosomes accounts, according to Muller (unpublished), for the fact first noted by Bridges that segregation in triploids is not independent in each group of 3 homologous chromosomes, but there is a tendency to produce gametes in the formation of which nearly equal numbers of chromosomes have gone to opposite poles. Therefore in Drosophila triploids the most frequent type of segregation gives $\frac{3 \times 4}{2} = 6$ chromosomes per egg (see also: Morgan, Sturtevant & Bridges 1925; Mather 1935; Darlington 1937; Sturtevant & Beadle 1939).

Assuming that all the chromosomes have the same chance of contributing to this segregation, then, from triploids having no Y, eggs receiving two X chromosomes(as a first assumption even if the X's are attached) will more frequently have none or only one autosome in diploid condition, than both autosomes diploid, and eggs receiving one X will more frequently have one or both autosomes in diploid condition, than both haploid. In Bridge's work with detached -X triploids this proved to be the case.

Disregarding now the X and the 4th chromosome, if segregation were random for the others, 25% of the

eggs would have both large autosomes in diploid condition, 25% in haploid and 50% one in haploid and the other in diploid.

From data by Lamy & Muller (1939 in the press) on XX/X(Y?) triploids of a stock from which ours was derived are suggestive of some 40% mortality in the very early prelarval condition, and of some 75% mortality of the remainder in the larval and pupal stages: all together less tham 15% of eggs produce mature flies. As the different types of zygotes, even if produced by the same type of egg, have different viability, it is difficult to say how many of the zygotes which fail to reach the imago stage are those aneuploid for the 2nd or 3rd chromosomes. However, the attempt has been made to reach some grossly approximately conclusions.

The different types of zygotes produced in the control series in our experiment occur in the following numbers

Type of zygotes	
a) TRIPLOID FEMALES	817
b) DIPLOID sc^{Sl}/v FEMALES	1068
c) DIPLOID y FEMALES	883
d) DIPLOID sc^{Sl} MALES	492
e) DIPLOID v MALES	372
f) INTERSEXES sc^{Sl}/v	457
g) INTERSEXES y	255
	4362

From these data, disregarding for the moment the fourth chromosome, it may be seen that all but one of the four types of segregation (shown below) in which both major autosomes went to the same pole in diploid number produce at least one type of zygote of practically normal viability. There are, however, four possible types of zygotes resulting from each type of segregation. Thus from the numbers of the normally viable types the total numbers for the corresponding class of segregation can be derived :

A) Type of segregation	B) Type of zygotes of normal viability formed from A)	C) Numbers of zygotes as B) found
a) $\frac{\underline{\underline{XX}} \text{ ; 2 II ; 2 III}}{\text{X ; 1 II ; 1 III}}$	sc^{Sl}/v females	1068
b) $\frac{\underline{\underline{XX}}\text{ X ; 2 II ; 2 III}}{\text{(Y?); 1 II ; 1 III}}$	v males	372
c) $\frac{\underline{\underline{XX}} \text{ ; 1 II ; 1 III}}{\text{X ; 2 II ; 2 III}}$	$\underline{y}$ females	883
d) $\frac{\underline{\underline{XX}}\text{ X ; 1 II ; 1 III}}{\text{(Y?); 2 II ; 2 III}}$	none	none

(To make the understanding easier the symbol $\underline{\underline{XX}}$ is used for attached Xs)

Segregation d) must occur oftener than b) and less often than c). Therefore the numbers 372 (for b) and 883 (for c) can be taken as the limiting values for

any of the four classes of zygotes derived from d). The total numbers of all four possible types of zygotes from segregations a) to d), inclusive, can be worked out on the basis of each of these two limiting values for d). They turn out to be 4 (1068+372+883+372) = 10,780 and 4 (1068+372+883+883) = 12,824, respectively. Allowing for a 90% viability - as a quite usual value for the zygotes of even the "normal viability" classes - we obtain the numbers of 11,966 and 14,249 for the zygotes of these classes formed without reference to whether they developed. Of these 4362 actually developed to the imaginal stage.

As previously stated, egg counting by Miss Lamy showed that only 10 to 15% of the eggs laid by triploids like ours develop into images. This would make a total lying between 43,620 and 29,080 eggs for supplying our 4362 images. Subtracting the above maximum and minimum figures (for the zygotes formed from all four types of segregation in which the same pole receives both major autosomes in diploid) from the latter figures for the total eggs laid we find the limiting values of 43,620 − 11,966 = 31,654 and 29,080 − 14,249 = 14,831 for eggs derived from other types of segregation, namely, heterochromatically symmetrical types in which the two 2nd chromosomes go to one pole and the two 3rd to the other. These values represent proportions of 0,73 and 0,51: we

see then that such segregation is at least as frequent as, and probably considerably more frequent than, that of the heterochromatically asymmetrical type. In conclusion then $\frac{1}{2}$ to $\frac{3}{4}$ of the eggs are aneuploid for the major autosomes.

Now it is evident that of all the fertilized eggs from such segregation, "symmetrical" in respect to the major autosomes, only a part would be of a type able to survive if a <u>given</u> one of the autosomes, 2nd or 3rd, in a <u>given</u> type of sperm (X or Y bearing) were lost.

In fact, in the first place the sperm with a <u>given</u> autosome lost must, to give a surviving zygote, fertilize an egg having two of <u>that</u> autosome and one of the other. The chance of this event is 0.50. In the second place, if the sperm carries a Y it must fertilize an egg with one or two X's (not with three or none), and if the sperm carries an X it must fertilize an egg with no or one X (not with two or three).

Rather complex calculations, carried out by Muller (unpublished) on this same material, based on the chance of the different types of segregation occurring, lead to something between 0.25 and 0.31 as the values for the chance that, <u>in this material</u>, a given

egg, aneuploid for the major autosomes, will survive when fertilized by a treated sperm in which a given major autosome has been so affected as to get lost.

Now we have on the one hand that between 0.73 and 0.51 of the eggs are aneuploid and, on the other hand, that 0.25 to 0.31 of these eggs will survive when fertilized by a sperm in which an autosome-loss has been induced. The combination of these figures two by two gives 0.18 and 0.23 as the limiting values for the proportion of all eggs laid which are "candidates" for the detection of a loss.

From these two values and from the found frequency of exceptions it will be possible to make a roughly approximate estimate of the real frequency of losses induced in the sperm.

Incidence of the exceptional flies among the different types of diploid zygotes.

A preliminary test of homogeneity between the two broods of the same treated series and between the two treated series in the experiment previously reported was made and showed a non-significant difference in both cases (between broods: $0.50 > P > 0.30$; between series: $0.50 > P > 0.30$)

Analyzing the summed results of both broods and series taken together, the following figures result:

	Regular No.	Exceptional No.	Exceptional %
Diploid females v/sc^{Sl}	1892	37	1.95±0.32
Diploid females y	1770	32	1.81±0.31
Males sc^{Sl}	1065	26	2.45±0.47
Males v	440	19	4.32±0.92
	5167	114	

A test for homogeneity of the frequency of exceptions in the four groups gives $\chi^2 = 10.33$, $0.02 > P > 0.01$ (three degrees of freedom). It can be concluded that there are significant differences between the frequencies of exceptional flies for the different types of zygotes. Summing together the data for v/sc^{Sl} females and sc^{Sl} males, both produced by the same kind of eggs, the frequencies for each type of eggs

are

Eggs	Frequency of exceptions
$sc^{Sl}/(Y?)$	2.12 ± 0.27%
y/(Y?)	1.8 ± 0.31%
Y (or 0)	4.32 ± 0.92%

Thus the significant differences are between the Y (or 0) eggs and the others.

The higher frequency of exceptions above noted presumably means that losses induced in the sperm have a higher chance of giving a viable exception when the sperm fertilizes a Y (or 0) egg than when it fertilizes one of the other two types. This in turn implies that the proportion of eggs aneuploid for an autosome is greater among the former than among the latter.

This result seems to fit in very well with the ideas: a) that segregation is non-random in triploids but tends to give more nearly equal numbers of chromosomes at the two poles: b) that consequently the presence of an extra Y chromosome, as in the stock used, may reduce the occurrence of those types of segregation which give eggs aneuploid for the 2nd and 3rd chromosome.

In fact, among the three types of eggs - $sc^{Sl}/(Y?)$, y/(Y?) and Y only the first two will frequently have,

in addition to the sc^{Sl} or y chromosome, an extra Y. (The case of more than one extra Y in the mother is here disregarded). If now we admit that when the Y segregates together with sc^{Sl} or y the possibility for an extra autosome to segregate towards the same pole is reduced, the different frequencies above noted are to be expected. They mean that eggs receiving only a Y chromosome more often receive one of the autosomes in double dose than eggs receiving a sc^{Sl} and a Y, or a y and a Y.

The method here used could be adopted in investigations into the frequency of different types of segregation in triploids.

The contribution of each autosome.

As shown in Table A , out of 114 exceptional flies, 54 were homozygous for brown alone, and 58 for ebony alone. Thus the two autosomes contribute to the losses with almost equal frequency.

The relative metaphase length of the two autosomes is something like 1:1.25, respectively, for the 2nd and 3rd, according to Gowen and Gay (1933), although some other studies indicate more nearly equal lengths. The breakage length calculated by Bauer et al. (1938) gives values of 1:.96.

Numbers of detected losses of autosomes

	2nd	3rd
Found	54	58
Expected (metaphase length G. & G.)	50	62
Expected (breakage length)	57	55

For metaphase length $\chi^2 = 0.58$, $0.5 > P > 0.30$

For breakage length $\chi^2 = 0.32$, $0.70 > P > 0.50$

Although the data do not disagree with either, agreement is slightly better with the expectation on a basis of proportionality to breakage length.

The frequency of losses of the autosomes and of the X and Y chromosomes compared.

It has been shown that, on the whole, in the stock of triploids used, the eggs aneuploid for either autosome constitute between 0.73 and 0.51 of the total. This proportion has also been shown to be definitely higher for at least one type of eggs, i.e. the Y (or 0) eggs. It is very probable that a large scale investigation would show that the proportion found as a whole is made up of distinctive values for each type of egg. However, as the proportion obtained is a kind of weighted average for all the progeny in this material, it may be used in calculating, from the frequency of

exceptional flies found, the frequency of losses induced in the sperms.

The exceptional flies found among the 5167 F_1 regular diploids were 54 bw, 58e and 2 bw;e. This gives a percentage of $\frac{116}{5167} = 2.25 \pm 0.20$. Subtracting that found in the controls - 0.046 ± 0.033 - the percentage of induced exceptions remains of 2.20 ± 0.20. which can be increased to 2.25 ± 0.22 to allow for a 90% viability. This figure expresses the ratio of cases of/ detectable losses of either autosome to the number of eggs which are asymmetrical, in respect to segregation of 2nd and 3rd, and which give origin to diploids.

From the data given on page , it can be seen that the latter kind of eggs constitute only $\frac{2715}{0.90}$ or $\frac{3206}{0.90}$ out of 29,080 to 43,620 eggs laid, hence a percentage between 8% and 10%.

Thus the above corrected percentage of exceptions, 2.25 - 0.22, must be multiplied by these figures to give the percentage of exceptions <u>per total number of eggs</u> and then divided by 0.18 or 0.23 - the proportion of eggs "candidates" for a detectable loss - for finding the real frequency of losses induced in the sperms. When this is done two limiting values of 0.78% and 1.25% result for the frequency of losses of 2nd plus 3rd chromosomes <u>per treated sperm</u>.

Now the frequencies of losses found for the

different types of non-ring X and Y combinations (page 81) vary from 0.69 ± 0.23% for the XY^L/Y combination to 1.63 ± .29 for the fXY^S/scY^L.

These figures are directly comparable with those of losses of the autosomes because they should be divided by 2, to allow for the fact that loss of an X makes a viable exception out of an inviable triplo-X zygote, and multiplied by 2 to allow for the fact that losses of X and Y occur in separate kinds of sperm, while those of the autosomes occur in the samekind.

The breakage length of the two major autosomes, taken together, has been found by Bauer et al. to be something like twice as that of a normal X and Y together. But this is, admittedly, an overestimate owing to the technique (salivary glands) adopted, which fails to detect many breaks in chromocentral regions.

It can be seen that the agreement between the frequency of losses of X and Y and that of the 2nd and 3rd is as good as it could be expected considering a) that the breakage lengths of our X plus Y combinations are surely higher than those of normal X plus usual Y; and b) possible differences in treatment.

If the results are considered of the small additional experiment, in which losses of the autosomes and of the XY^S and scY^L were detected among the same F_1 of treated males, they are seen to be also in agreement,

as far as the small numbers allow.

In conclusion, the results obtained on the losses of the autosomes are in support of the idea of a correlation between frequency of losses and breakage frequency.

D I S C U S S I O N

The results of the series of experiments previously reported show that :

losses of entire chromosomes-X, Y and the two major autosomes - can be produced by X-raying the mature sperms and, at least in part, they are non-lethal to the zygote.

the relationship between dosage of irradiation, as measured by the amount of ionisation produced, and frequency of losses can be expressed, for the dosages of 0,1000 and 4000 r, by a linear equation and is certainly not compatible with an exponential equation having an exponent as high as 1.5.

the results with chromosomes undergoing gross rearrangements with different frequencies suggest that a positive correlation does exist between frequency of losses and breakage frequency.

the frequency of losses of ring-shaped X- chromosomes is more than twice as high as that of non-ring X's.

the loss of a whole chromatid, giving rise to a "fractional" fly in which only a part - generally 1/4 to 3/4 - of the body lacks the corresponding chromosome, has a <u>proportionally</u> much smaller chance to occur than the loss of a whole chromosome.

dicentric translocations between the major autosomes are lethal to the zygote even when - as arranged for in the present experiment - the mere elimination of the two chromosomes would have left the zygote with a normal diploid set.

the same rule holds for dicentric translocations between the X or Y and one of the large autosomes, although it is possible that there are exceptions here.

the fact that but a single case was found among 40,000 daughters of attached X flies crossed to treated males , which was lacking in both the Y (or X) and the IV chromosome, indicates that dicentric translocations between the X, or the Y, and the IV chromosome are inviable also. For we should expect one such coincidence of two independent losses, whereas the number of translocations of the type in question would have been over 40.

Let us consider what light these results throw on the mechanism of losses advocated in the introduction and more generally on the mechanism of structural changes in the chromosomes. It must be first discussed whether or not the evidence indicates that such losses merely represent a special case of a process of breakage and rejoining of chromosomes.

For our general problem, the most important point is the evidence found that the relationship between dosage and frequency of losses turns out to be very well expressed by a linear equation and surely not by an exponential one with a power as great as, or greater than 1.5 - the relation found for the frequency for viable two-breaks gross rearrangements— within the dosages here used. Although there is no significant departure from linearity, if any exists, it is in the direction of an exponent still lower than 1.

All this agrees very well with the idea that the primary cause of losses is a single break produced by a single ion or pair of ions. It is to be expected that a refinement of the experiment would show that the slight departure from linearity is real, for this would arise from a) the fact that the basically linear curve would tend to "saturation", i.e. at higher dosages the coincidence of two or more breaks, each leading to loss, becomes greater; b) the fact that, with increased number of breaks per nucleus, greater opportunity would be given for a "loss" to take part in a gross rearrangement, either lethal or not. Thus a proportionally greater elimination, through lethality, of cases of losses at higher dosages. That the effect of a) can be but slight, there is no need of discussing. This must be true also for b), as the

gross rearrangements to be considered here are, mostly, two-break translocations between the distal fragment of the chromosome involved in the loss and another chromosome. The frequency of such rearrangements involving the X and the Y, for instance at 4000 r, is something like 10%. The upper limit of the proportion of cases of losses which could be eliminated by this means is therefore 10% at this high dose thus incapable of producing a very large departure from linearity.

On the dosage frequency-relationship for losses, a most interesting preliminary report by Bauer (1939) has just appeared. Bauer attempted to study the induction of losses of the ring X-chromosome at different doses by measuring the amount of changes caused in the sex-ratio when the irradiated father, bearing the ring, was crossed to females with normal X's. For a comparison males with non-ring X's were X-rayed with one of the doses (4000 r). The difference between the sex-ratio of the F_1 from treated and untreated non-ring fathers was $2.03 \pm 0.67\%$, thus showing a small, but significant effect, actually smaller than reported by previous authors. (Gowen & Gay, 1933). It is concluded that the X and the Y chromosome contribute in not very different measure to dominant lethal gene or chromosome mutations. But in the case of the Xc^2 males, X-rayed with dosages ranging from 75 to 6000 r, the

change in the sex ratio was many times as greater as this. The results, expressed as the ratio of males minus females to males (all corrected for controls), when plotted against dosage gave "an oversaturated 1-hit curve, which results from the difference between a 1-hit and a 2-hits curve. The former suggests the proportionality to the dosage of the production of dicentric double rings, the latter corresponds to the 2-hit curve of the lethal Y-translocations." Since however this investigator did not differentiate between these two causes of change of the sex ratio, or find the relative part each played in the results, his later statement that these results give direct proof that individual breaks are caused by individual ionisations seems without foundation.

Our data which are not masked by other effects, give a calculated frequency of losses for the Xc^2 of less than 4% at 4000 r. From Bauer's graph, the value at the same dosage is about 40% (deficit of females relative to males). The frequency found in our experiment calculated in a way comparable to Bauer's, turns out to be about 6%. Supposing that the absolute values of the dosages in the two experiments are not the same and that our doses of "4000r" are really only half as large as his - an allowance which seems excessive - still only 12% of losses would occur at 4000r giving a remainder of over 28%

out of Bauer's 40% to be ascribed to dominant lethals arising from mutations and gross or minute rearrangements. Now at 4000 r some 5% of viable (mostly 2-break) translocations involving a rod-shaped X-chromosome are usually produced. With the ring-chromosome, both the translocations corresponding to these and the complementary acentric-dicentric ones would be lethal but with the Y (which gives roughly the same number) only the latter would be lethal, leaving a difference of 5%. Thus only 5% is accounted for by this type of lethality. Data for making allowance for the other types, for the moment, are not at hand.

However, it seems possible that one of the reasons for discrepancy between the two sets of results may come from a part of the losses being lethal. If this is so, Bauer's method presents from this point of view the advantage of scoring both kind of losses: the lethal and the non-lethal ones. In that case the discrepancy between the two results is less than shown by the above calculations. An experiment based on the simultaneous use of both Bauer's method and that adopted here is in progress. It is hoped that a solution of this question will be found if it will be possible to account for all the other causes of dominant lethality.

Another kind of evidence supporting the idea that the primary cause of losses is breakage comes from the results of the experiment with X and Y chromosomes of different breakage length, and from that on the losses of autosomes. Even though the results are not refined enough to allow the inference that losses are actually proportional to breakage length, they suggest that some kind of positive correlation exists.

The frequency of the losses of the two large autosomes, found in the crosses with triploids, as compared with that of X and Y, is also in support of this general conclusion, as far as the conditions of treatment allow a comparison. When identical conditions were obtained, as in the small scale additional experiment, in which both the losses of X and Y and of the autosomes were detected among the same F_1, the results were much more confirmatory. Finally the comparison between the frequency of losses of the two autosomes are also in agreement.

That frequency of losses and breakage length are correlated, is a fact which it was necessary to make sure of. Should the contrary have been true, the whole assumed mechanism for losses woulf have fallen. But the positive result, taken <u>per se</u>, is capable of another interpretation - loss through lagging, which

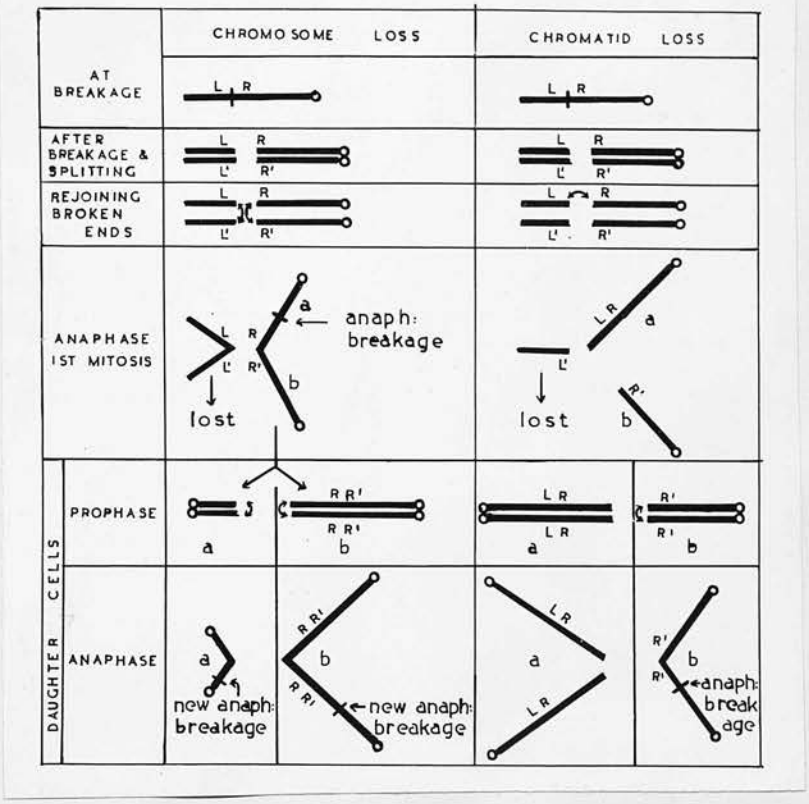


DIAGRAM 1

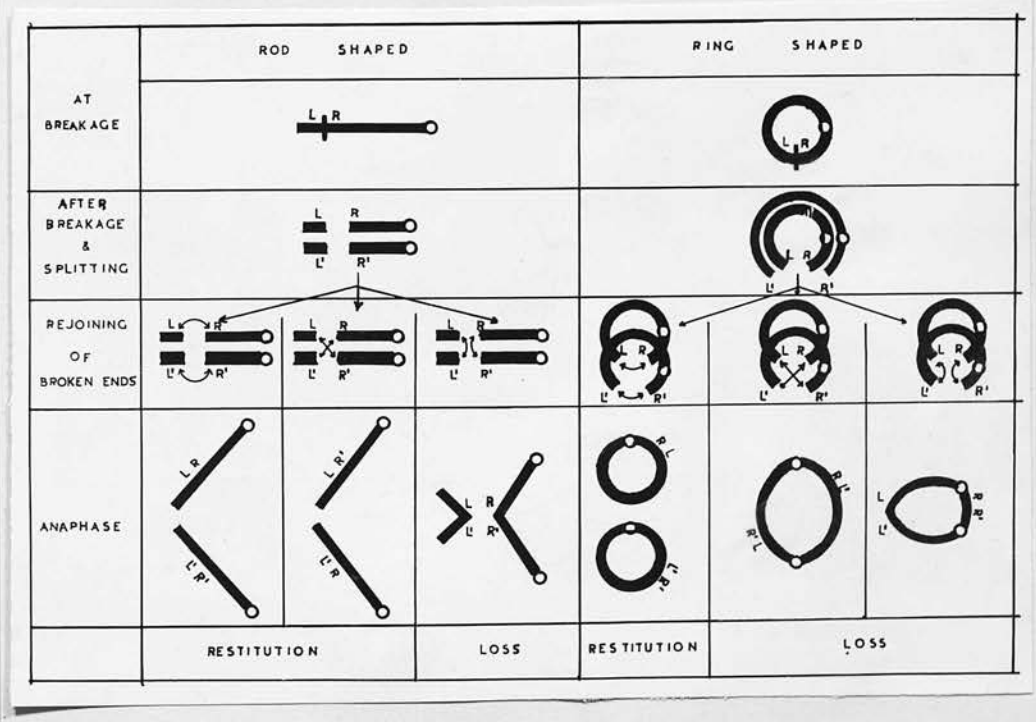


DIAGRAM 2

could be greater for longer chromosomes - until cases can be examined in which the breakage lengths do not correspond to metaphase lengths, as when certain types of inert regions (Muller's "blocks") are duplicated or deficient.

The results given by the Xc^2 chromosome are very interesting. The frequency of induced losses has been calculated to be <u>at least</u> 5. ± 1.8 times as great as for a V-shaped XY^L chromosome.

As already pointed out, for each break there is only one way - out of the three possible - whereby a rod- or a V-shaped chromosome can give a dicentric chromosome by fusion of the broken ends of the sister chromatids, while there are two ways - always out of the three - for a ring-shaped chromosome. The situation is better explained by graph No. 2 in which, for simplicity, it is assumed that rejoining occurs always after splitting of the chromosomes. (vide infra). In general, if we call R,R' the proximal broken ends of the two chromatids of a chromosome, and L,L' the distal ones, reunion can occur in three ways:

R - L) "longitudinal" fusion which gives restitution in all cases.
R'- L')

R - R') "lateral" fusion, which gives a dicentric chromosome in all cases (Double dicentric ring with a ring-shaped and a dicentric chromosome plus acentric fragment with a rod or V-shaped).
L - L')

R - L') "diagonal" fusion, which gives "restitution" in the case of a rod- or V-shaped, and a dicentric ring in the case of a ring-shaped chromosome.
L - R')

These facts are sufficient to show that, if the theory of the mechanism of losses here advocated is correct, a greater frequency of losses is - coeteris paribus - to be expected from a ring than from a V- or rod-shaped chromosome. How much greater, depends first of all on the frequencies of "diagonal" fusion as compared with "lateral".

The evidence of various kinds is strong, that of the breaks induced in non-ring chromosomes, the majority does not lead to a permanent structural change, but undergoes "restitution". Particularly striking in this direction is the observation by Faberge (1940) who merely by changing the conditions which favour rearrangements instead of restitution (low temperature and concentrated treatment) obtained with the same total dosage, and therefore presumably the same number of breaks, a number of rearrangements in Tradescantia microspores some five times as great as before.

"Restitution" in a non-ring chromosome is achieved either by what has above been termed "longitudinal" or by "diagonal" fusion. No way of ascertaining the chance of occurrence of the one as compared with the other of these modes of fusion for non-rings is for the moment at hand. Data by Offermann - reported by Muller (1940), appear to have a bearing on the question showing that minute rearrangements,

of the type of those which are responsible for a considerable proportion of induced lethal "mutations" (Sacharov 1935, Alikhanian 1937, Slizynski 1938) very seldom, if ever, are produced by what here is termed a "diagonal" type of chromatid fusion. Here then the "longitudinal" exceed the "diagonal", but the frequency of "lateral" is not known. This result must be taken in connection with the consideration that the process of fusion in the case of minute rearrangements is different than in the case of "simple breaks" or, much more likely, that the three ways of rejoining have different relative frequencies in the two cases, owing in part at least to the fact that in the case of minute rearrangements another break is very near at hand on the same chromatid.

If, as a very much simplified postulate the three ways of rejoining are taken as having the same chance, then in the case of non-ring chromosomes "restitution" would for each break have twice as much chance to occur as new, viable and inviable, arrangements including losses. The contrary would be true for a ring-chromosome, provided, of course, the very nature of the ring, with its different torsional and other strains, did not substantially alter the situation. Now the frequency of induced losses found for the Xc^2 chromosome is at least 5.- $\pm$1.8 times - and probably much more - as great as that calculated

for a non-ring XY^L chromosome. The generalisation seems to be legitimate that the frequency in the Xc^2 is surely more than 2 times that with a normal X, 2 times being the expected value on the too simplified assumption made above. Alternative explanations for this fact - perhaps part or all true - are easily found while still keeping within the general scheme:

a) diagonal - and perhaps also longitudinal - fusions are, under the present experimental conditions, much more frequent than lateral fusion, in the case of both ring- and non-ring chromosomes.

b) some mechanical peculiarity of the ring chromosome makes either or both the lateral and the diagonal rejoining easier, relatively to longitudinal rejoining, in it than in non-ring chromosomes.

c) should rejoining of broken ends occur frequently when the chromosome thread, but not necessarily the centromere, is already split (vide infra) then also longitudinal fusion could, in the case of ring-chromosomes, lead to loss when it gives origin to two monocentric interlocked daughter rings.

A discrimination between these possibilities may perhaps be obtained by comparing losses of ring-and of rod-shaped chromosomes under different conditions, for instance, temperature at the moment of rejoining, which may differentially affect the three ways of rejoining or shift the time of rejoining in respect

to the time of splitting. But whatever the answer to this question, the fact of the greater frequency of losses of the ring-chromosome as compared with the non-ring is in support of the hypothesis of losses being produced by breaks followed by fusion of chromatids such as to give a dicentric or interlocked chromosome.

A third type of evidence came, quite unexpectedly, from the examination of the fractionals. It has been shown that not all the F_1 exceptional females which show the phenotype expected as a result of a loss of the paternal X or Y, are actually XX/O females. Something more than one third, in the case of crosses with males carrying the scY^L chromosome, proved to contain a part of the X or Y which, through gross or minute rearrangements, produced the same phenotypical effect as if a complete loss had occurred. Now about one in every twenty exceptional flies were "fractional" exceptions, showing the changed phenotype only in part of the body. With those in which the ovaries bred as mosaics or bred according to the changed phenotype, the tests for establishing the proportion of complete losses were carried on as with the "total" exceptions. The results were significantly different for the two groups: in place of some 64 %

true losses, found among the "total" exceptions, only some 22% were found among the "fractional" exceptions. Thus losses far more frequently involve both chromatids, instead of only one, of the same chromosome of the treated sperm, and this disproportion in frequency is considerably greater for losses than for deletions. It must first be pointed out (see Muller, 1939, 40) that for explaining the occurrence of fractional rearrangements, it is not necessary to assume that the chromosomes are already split at the moment of treatment in some sperms, but not in all (Patterson, 1933; Mather, 1937). With the present day's better understanding of the mechanism of chromosome rearrangements, it can be plausibly assumed instead that rejoining of broken ends can occur before, at, or after the time of splitting. "Fractionals" would arise, in some of the two latter cases, when one chromatid united in a different way ~~than~~ from the other. The reason why one of the two different ways is generally(x) "restitution" is easily understandable, if on the one hand restitution has a higher chance of occurring than a new arrangement and, on the other

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(x) In the course of present experiments one case was found of a fractional which could be interpreted as a result of different rearrangements involving each of the two paternal chromatids. Bridges (1935), Offerman (1936) and Kaufmann & Bate (1938), have described some such cases.

hand, for a two-rearrangement mosaic to survive, the coincidence is required of two rearrangements both of the eucentric type and without dominant lethal effects.

How then does the finding that losses as compared with deletions occur so much easier when they involve both than one of the chromatids, fit in with the general scheme drawn ? Graph No. 1, which shows the postulated mechanism of losses in the two cases, may clear up the situation. For a non-ring chromosome loss to occur, it is necessary only that at the first splitting the two proximal ends of the broken chromosome fuse laterally. The dicentric chromosome is thus immediately produced and presumably is either lost in the first anaphase of zygotic mitosis or starts the process of repeated breakage at that time. For a non-ring chromatid loss it is necessary instead that while at the first splitting one chromatid undergoes restitution (longitudinal fusion) the proximal part of the other does not rejoin at all, enters the daughter nucleus with its broken end still unhealed, and remains with it unhealed throughout the following resting stage. Only, at the second cleavage division, after splitting, do the two broken ends of the proximal part of the sister daughter-chromatids fuse laterally. All this requires this concurrence of events: 1) that the broken distal fragment does not enter the same daughter

nucleus as the proximal, or, if it does, fusion between them, which would lead to restitution, again fails to occur; 2) that there are no other broken ends at hand with which fusion could take place.

On the other hand, in the case of fractional gross or minute rearrangements it is not necessary for any broken end to wait so long, before rejoining, as in the case of fractional loss. In these cases, in which at least two breaks have always occurred, the delay in union need only be until after the first splitting, a probably not uncommon occurrence; after that, while one chromatid undergoes restitutional fusion, the other, without need for more dealy than the first, undergoes recombination fusion.

The results from the crosses with triploids have shown without the possibility of doubt that dicentric chromosomes originating from a reciprocal translocation between the two major autosomes are practically always lethal to the zygote. Instead of the expected 233±37 exceptional flies - showing the loss of both paternal autosomes, should the dicentric translocations be eliminated - only two were found, an occurrence which is not more frequent than expected for the coincidence of losses produced independently in each autosome. That this result for translocations

of type 2-3 does not represent a special case but probably a general one for all reciprocal translocations between any two chromosomes is shown by the finding of only one case, in the whole series of experiments reported, showing simultaneous loss of the X (or the Y) and of the 4th chromosome, as well as by the similar finding about the translocations between the X (or Y) and the 2nd or 3rd chromosomes. Although the figures are too small for saying that the latter never, or practically never, survive, they are sufficient for concluding at least that their survival is not the rule.

Now, if the postulated mechanism for losses holds, why, when the dicentric chromosome has originated by fusion of sister chromatids, does its relatively early elimination take place, so often, if not always, as to allow the zygote to survive, whereas, when it has originated by fusion of two different chromosomes, the zygote cannot survive? A possible explanation might be derived from the following considerations. certain

When the dicentric chromosome is produced by sister-strand fusion, the two contromeres are sisters and therefore tend to go to opposite poles. This leads to the formation of a chromatin bridge at the very first cleavage division, and probably at each mitotic anaphase the process is repeated, so that,

if in most cases breakage follows, the elimination of the chromosome proceeds rapidly in a not very great number of cell generations. In the other case, however, the two centromeres may or may not tend to move towards opposite poles, and this situation is repeated at each anaphase. If, for a first approximation, it is supposed that the chance of their both going to one pole is equal to the alternative chance, in half the anaphase separations the bridge will not be formed, thus giving no chance for the occurrence of breakage. The result would be a great slowing down of the process of elimination.

The above argument rests on the assumption that losses must follow breakage and consequent reduction in size of the dicentric chromosome, but should this not be the case in Drosophila, there are other factors which may lead to the same effect: a) a dicentric chromosome derived from sister-chromatids is a symmetrical structure with respect to the equatorial plate, while a dicentric translocated chromosome is not; and b) the forces acting at the centromere might be somewhat different for different chromosomes or chromosome parts. Both facts would favour the inclusion of the whole dicentric translocated chromosome in one of the daughter nuclei even more often than would occur as a result of randomness of moving apart viz. in company of the two centromeres,

thus still more reducing the chance of bridge formation followed by breakage, or, on the alternative hypothesis, of loss due to lagging of the dicentric. And since, in fact, our results show that loss of a dicentric translocated chromosome in the first division practically never occurs, it seems likely that one or both these latter factors are in operation.

In conclusion the different effects upon survival of the zygote in the case of sister-chromatid dicentric chromosomes and of translocated dicentric chromosomes could be a consequence a) of the genetic unbalance affecting, for a different time and to a different extent, the embryo as a whole; and b) of some mechanical disturbance in the normal mitotic process. It is realized that a) alone would with difficulty explain completely the situation because dicentric translocations between the Y and the IV chromosome should sometimes give viable haplo-IV individuals, while apparently this is not the case. However, the very low viability of haplo-IV's may have prevented their having been found.

An alternative explanation would require the complete rejection of the scheme postulated for the losses. If for example the losses were caused not by the mechanism above depicted but by some inactivating effect of an individual successful ionization on the centromere, causing failure of the chromosome to move

on the spindle, then all losses would involve, except for rare coincidences, a single chromosome. But it would be hard to reconcile such a hypothesis as this with the results on breakage length, on the behaviour of the ring-chromosome and of the peculiarity of "fractional" losses already discussed.

There is another reason why the scheme previously advocated for the origin of losses appears to be more plausible. That is, the agreement of the results obtained here with expectation on the "breakage first" theory of structural change. As already pointed out, this theory assumes that "primary changes", each produced independently by individual ionisations interact with each other to give rearrangements. The primary changes are supposed to be actual or potential breaks, while the interaction is supposed to be the rejoining of broken ends. The evidence in favour of this theory is based mainly on four groups of results: a) those showing the independence of the effects, in Drosophila sperm, from the time distribution of treatment and from wave-length thus proving the production of the "primary changes" by single ionisations; b) those on the effect of increasing dosage on the frequency of gross rearrangements, obtained both in Drosophila and in Tradescantia. This relationship is expressed by equations with power greater than 1, while a power equal to, or smaller than 1, would be expected in case

that the single-ionisation primary change directly led to the rearrangement; c) those on the effects of the "time" factor (and of temperature, which are related to those of time) obtained in Tradescantia and showing that the interaction between two or more primary changes is limited in time; and d) those on the biparental rearrangement, described by Sidky, proving that gross rearrangements must occur in two distinct stages.

The results reported in the present paper can be explained very well on the "breakage first" theory of structural changes. They show in fact that the frequency of losses, as expected if they are produced by a single primary change, increases in proportion with the first power of the dosage.

The fact that results obtained from such a different direction fit with the general theory in the manner expected brings a strong support to the theory itself, and suggests that the mechanism of losses here proposed is, in its main lines, probably correct.

SUMMARY

1) The problem has been investigated as to whether single breaks induced by irradiation in the chromosomes of mature sperm of Drosophila may produce the loss of whole chromosomes by formation of dicentric chromosomes originated from lateral fusion of broken sister chromatids at the point of breakage.

The two methods adopted for detecting losses of X and Y chromosomes and another for detecting losses of the autosomes are described in detail.

2) A dosage experiment (0, 1000, 4000 r) showed that losses of X and Y are actually produced by irradiation of the mature sperm, that they are, in part at least, non lethal to the zygote and that they occur with a frequency proportional to the first power of the dosage.

3) An experiment in which the frequency of induced losses was investigated with X and Y chromosomes of different cytological length and of different shape (V shaped and ring shaped), showed that this frequency is correlated with the breakage frequency of the X and Y chromosomes used, and that a ring-shaped (Xc^2) X-chromosome undergoes loss with a much higher frequency than a V- or rod-shaped X-chromosome of the same length.

4) Losses of X or Y chromatids, giving origin to "fractional" (mosaic) flies, in proportion to chromatid deletions or minute rearrangements, are less frequent than the corresponding losses of chromosomes in proportion to chromosome deletions.

5) An experiment based on the use of triploids in which induced losses of the two major autosomes were investigated, showed that losses of the latter also, are, under suitable conditions, non-lethal to the zygote . Their frequency, when losses of one autosome are compared with that of the other and also with losses of X and Y, is again suggestive of a correlation with breakage frequency.

6) In the preceding experiment, and in an additional one made with the purpose of definitely settling this question, no fly developed in which the loss of both autosomes or of an X (or Y) and an autosome, could be attributed to a dicentric translocation between the chromosomes involved. The conclusion is drawn that dicentric translocations are generally, perhaps always, lethal to the zygote even when, as in the present case, the elimination of the dicentric would have left the zygote with a normal diploid set.

because in fact dicentric is not eliminat

7) The evidence found is considered as in agreement with a loss mechanism of the kind suggested in 1). It is concluded that losses are produced by single breaks induced by single ionizations along the chromonema leading to formation of a sister-chromatid dicentric which either is lost immediately at first mitotic anaphase or undergoes a process of repeated breakage which eventually leads to complete loss.

8) To explain why dicentrics of the kind described in 7) can be eliminated by the zygote in such a way as to allow /it to survive, while dicentrics formed by fusion of different chromosomes can not, it is pointed out that the symmetrical structure of the former as compared with the asymmetrical of the latter may account for a different behaviour in mitotic anaphase, perhaps facilitating, in the latter, the inclusion of the whole dicentric in one daughter nucleus instead of its immediate loss through lagging or repeated breakage.

9) It is emphasised that the results found here are in strong support of the "breakage first" theory for structural chromosome changes, as there is a direct proportionality to dosage for "single break" losses in contrast with the proportionality to about the 1.5 power of the dosage found, within the range of dosages used here, for gross rearrangements.

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