THE EFFECT OF X-RAY DOSAGE UPON THE FREQUENCY OF INDUCED STRUCTURAL CHANGES IN THE CHROMOSOMES OF *DROSOPHILA MELANOGASTER*

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THE induction of structural changes in chromosomes by radiation has been variously ascribed to direct effects upon the chromosomes and to indirect ones. The present study is part of an attempt to discover whether the changes produced in the various chromosome regions involved are due to one stimulus or to several separately acting stimuli.

The changes observed are the loss (deficiency) of part of a chromosome or translocations between different chromosomes or between parts of the same chromosome. Except in the case of chromosome deficiency, the changes are observably reciprocal. We cannot establish this characteristic for deficiencies, since the form of the lost fragment (rod or ring shape) is unknown.

Two general hypotheses have been inferred to explain the mechanism of induced change. According to one of them, breakages are separately induced in the chromosomes, the breakage ends persist for a time and may fuse with other breakage ends after a process of attraction or chance groping for one another in the nucleus. The refusion might occur between the breakage ends that were originally parted, in which case no structural change would result. Alternatively, the breakage ends might fuse in new ways and give a structural change. Such a change requires that at least two chromosome breaks must have occurred in the same nucleus, giving at least four breakage ends. The hypothesis explains reciprocity since, if two non-sister breakage ends fuse, each of their sister breakage ends are obliged to fuse with a non-sister end if they fuse at all. Stadler (1930) has supposed that fragments may persist through several cell generations, through their chance inclusion in a daughter nucleus at each division, and that the breakage ends may retain their capacity for refusion during this period and sooner or later reunite. But it is difficult to understand how a molecular complex like a chromosome could retain for long an unstable structure like the hypothetical breakage end.

According to the second hypothesis, elaborated especially by Muller (1932), one break acts to induce another one, or, perhaps more likely, both, or all, breaks associated with one translocation are due to a common cause. This localization of the effect almost requires that structural changes should occur only when two or more chromosomes, or parts of one chromosome, are broken at points lying in propinquity within the nucleus. In all essentials this hypothesis closely resembles the contact mechanism of Serebrovsky (1929) and Dubinin (1930). According to their interpretation, translocations would follow when chromosomes stick together and are broken at the point of contact. Accepting these principles, the rearrangements would occur by a process that is virtually crossing-over except that it occurs between non-homologous regions at a time other than synapsis.

The chief distinction between the two hypotheses lies in their initial assumptions that the chromosome breaks taking part in a structural rearrangement are (1) separately induced by separate acts or (2) induced together by a single act of the incident energy of the radiation. These hypotheses may be referred to respectively as the "breakage" and the "contact" hypothesis. There are several methods of testing them. One involves reasoning from the observed consequences of varying the magnitude, quality or duration of the irradiation dose to which the chromosomes are subjected. Another is reasoning from the relative frequencies shown by different kinds of structural change induced at the same dose under identical conditions.

Several studies of the dosage-effect relation have already been made. Khvostova & Gavrilova (1935) have shown that there is a linear relationship between dosage and the frequency of fourth chromosome interchanges having a position effect on *cubitus interruptus*. Another study suggesting a linear relation is that of Muller & Altenburg (1930). Oliver's (1932) data on chromosomal aberrations, detected by genetical methods, seem to show at low dosages a non-linear relationship between dosage and effect.

The experiment here reported is an attempt to obtain data upon the dosage-structural change relation for all chromosomes irradiated together in one gamete. The method used was cytological, based upon studies of the salivary gland chromosomes of F_1 *Drosophila melanogaster* female larvae, whose fathers' sperm had been X-rayed.

METHODS

Oregon-R males, 1–4 days old, were X-rayed at three doses, respectively 1000, 2000 and 4000 r.u., and mated to virgin white-eyed females. Ten cultures were established for each dosage, with six males and ten females per culture. The cultures were kept at 25° C. for 2 days, after which the flies were discarded. Extra yeast was added to each culture, and the latter were then maintained at 19° C.

This feeding and temperature treatment secures better growth of the larvae and fewer individuals with unsatisfactory salivary glands. Mature female larvae were taken from each culture, their salivary glands dissected out, stained in aceto-carmine, smeared, dehydrated in 95% alcohol and afterwards mounted in euparal according to the methods developed by Baur and by Bridges (Drosophila Information Service, 6).

Chance non-virginity of the female parents was detected by the occurrence of female larvae with white malpighian tubules. Such larvae were kept separate from the rest. A check upon them was available since the Oregon R stock was homozygous for a deficiency [*Df*(2)*Or*] of the tip of 2*R*, with the break between the halves of the doublet of 60*F* (Bridges, Drosophila Information Service, 7). The white stock had the full complement of bands. Larvae giving such corroborative evidence of non-virgin origin were discarded.

OBSERVATIONS

A summary of the results is given in Table I. It should be stated that no serious attempt was made to observe small deficiencies. Their positive identification in the salivary glands of a single individual is too precarious to be of value for the present purpose. Only interchanges, inversions and more or less complex translocations have been noted. There are also no observations of another class of translocation; namely, that in which all breaks would be in heterochromatic regions of the chromosomes. The formation of a chromocentre in *Drosophila melanogaster* makes such translocations very difficult to observe cytologically.

The observed structural changes were of the following kinds:

(1) Interchanges which were always of the symmetrical type with each product having only one centromere. In Table I these are written as *X*–3*R*, 2*L*–3*R*, etc.

(2) Inversions, in which both breaks lay in the same arm of the chromosome. In Table I these are referred to by the number of the chromosome arm they occupied, i.e. *X*, 2*L*, 2*R*, 3*L* or 3*R*.

TABLE I

Dose r.u.	Culture	Individuals analysed	Individuals discarded	Interchanges	Inversions	Eucentric inversions	Doubtful*	Intercalations	Complex translocations
1000	281	6	0	*X*–3*R*					
	282	14	4	2*L*–3*R*			2*R*		
	283	23	2		3*L*				
	284	23	2	*X*–2*L*; 2*R*–3*L*			*X*		
	285	23	2						
	286	20	2						
	287	20	3	2*R*–3*L*; 3*R*–3*R*			3*L*		
	288	27	1		3*R*		3*L*		
	289	18	1	2*L*–3*R*	2*R*				
	290	22	2			2*L*–2*R*; 3*L*–3*R*	3*L*		*X*–2*L* + 2*L* inv.
	Totals	196	19	7	3	2	5	0	1
2000	291	16	3	2*R*–3*L*			2*L*; 3*R*		
	292	20	2	*X*–2*R*	3*L*; 3*R*		3*R*	2*R* dys. in 3*L*	
	293	20	0	2*L*–3*R*	2*R*				
	294	20	0	*X*–2*L*	3*L*		*X*; 3*R*		
	295	10	3				3*L*; 3*L*		
	296	28	4	*X*–2*R*; *X*–3*R*; 2*L*–3*R*					
	297	14	2	*X*–2*R*; 3*R*–4	3*R*				
	298	15	0	*X*–3*R* + 3*L*–3*R* inv.					*X*–3*L* + 3*L* inv.
	299	25	1	2*L*–3*R*; 2*L*–3*R*		3*L*–3*R*	2*R*		
	300	13	2	*X*–2*L*; 2*L*–3*L* + 3*R* inv.	3*L*				3*L*–3*R* + 3*R* inv.
	Totals	190	17	14	7	2	8	1	2

4000	301	8	1			3*L*–3*R*	3*R*; 3*R*			
	302	43	2	2*R*–4	*X*; 2*L*; 3*L*; 3*L*+2*L**	2*L*–2*R*; 3*L*–3*R*; 2*L*–2*R*+3*L**	2*R*; 2*R*; 3*R*			
	303	27	1	*X*–2*L*; 2*L*–3*R*; 2*R*–3*L*; 2*R*–3*L*	2*R*; 2*R*; 3*R*; 3*R*		2*R*	*X* in 2*R*		
	304	42	1	*X*–2*L*; *X*–3*L*; *X*–3*R*	3*L*; 3*R*	2*L*–2*R*	*X*; 2*L*; 2*R*; 3*L*	2*R* enc. in 3*L*+*X* inv.* 3*L* enc. in 2*R*+*X* inv.* 3*L* in 3*R*; 2*R* in *X*+2*R*–4 inv.	*X*–2*L*+2*L* inv. *X*–3*L*+*X* inv.	
	305	19	0	3*R*–4+2*R* inv.*	2*R*; 3*L*+*X* inv.*					
	306	35	2	*X*–4	2*L*		3*R*; 3*L*; 2*R*+3*R*		2*R*–3*L*–3*R*; *X*–2*R*–3*L*+3*R* inv.*	
	307	9	0	*X*–2*R*; 3*L*–4; 2*L*–3*R*+2*R*–3*L*	2*L*	3*L*–3*R*			*X*–2*R*+2*R* inv.+3*R* inv.*	
	308	8	0	2*R*–3*L*		3*L*–3*R*				
	309	13	0	2*R*–3*L*	2*L*		*X*; 2*L*; 3*R*		2*L*–2*R*–3*L*–3*R*+*X* inv.+3*L* inv.	
	310	20	6	*X*–2*R*; *X*–3*R*; *X*–2*L*+*X* inv.*		2*L*–2*R*; 3*L*–3*R*; 3*L*–3*R*	3*L*; 3*R*			
	Totals	224	13	19	15+2 17	10	19+9 28	5	6	

* These inversions are doubtful, since one break is in heterochromatin; they could be interchanges.

NOTE. Each individual, which may have one or more structural changes, is listed once only in the detailed analysis in Table I. Therefore, in making up the individual totals of the different kinds of structural change in this Table, additions have to be made in several cases from other columns of the same Table.

(3) Eucentric inversions, i.e. an inversion in which the two breaks were on opposite sides of the centromere and in which, therefore, all parts of a given segment have the same arrangement, relative to the centromere, as in the original chromosome. These are referred to as $2L$–$2R$ or $3L$–$3R$.

(4) Intercalations, in which a segment of a chromosome was inserted into another part of the same chromosome (internal) or into a different chromosome (external). The intercalated segment retained the original order of its parts relative to the new centromere (eucentric) or had an inverted order (dyscentric). The example in culture 292, referred to as $2R$ dys. in $3L$, had a segment of $2R$ inserted into $3L$ in an inverted order.

(5) Complex translocations, in which two or more of the above kinds of translocation occurred together and had one or more breaks in common. There were only nine cases belonging to this class. In culture 300, for instance, one individual had an inversion in $3R$ and a eucentric inversion between $3L$ and $3R$, the break in which was in common with one of those delimiting the $3R$ inversion. In cultures 290, 298, 304 and 307 there is a total of five cases in which an inversion is combined with an interchange, so that the two have one break in common. Culture 306 has one case of a eucentric inversion combined with an interchange and another case combining two interchanges, each with one break in common. Each of these eight examples, together with the six examples of intercalations, have three breaks, and on the contact theory in its most literal sense would require that the three regions had been precisely in contact at one point. Finally, in culture 309, there is one example of a complex interchange-inversion translocation involving four breaks and requiring, on the contact theory, four threads in contact at one point.

In a number of cases, in which one break lay in heterochromatin, it was not possible to determine whether an inversion or an interchange was present. These are tabulated separately in the "doubtful" column.

About 8–10% of the larvae gave glands that were too poor to analyse. These were small glands, presumably from immature larvae, or glands that had been damaged in smearing or in which the chromosomes had not spread out satisfactorily. The frequency of unanalysed glands was independent of dosage, being respectively 9·7, 8·9 and 5·9% at the low, middle and high doses. There is no indication that greater doses of X-rays induce more unanalysable glands.

Table II summarizes for each dosage the percentage of affected individuals, the percentage of contacts inferred on the contact theory, and the percentage of breaks inferred. For all three of these criteria the

curve is not noticeably divergent from a straight line passing through the origin.

TABLE II

Dose (r.u.)	No. of individuals	% individuals with structural changes	% contacts inferred	% of breaks inferred
1000	196	9·2	9·2	18·9
2000	190	16·8	17·9	37·4
4000	224	32·6	38·4	81·2

DISCUSSION

There appears to be a direct linear relationship between dosage and the observed frequency of induced structural changes. To test whether the breakage or contact hypothesis fits this fact we need to know the shape of the dosage-chromosome structural change curve expected on each hypothesis. It has usually been assumed that the breakage hypothesis would demand an exponential curve and the contact hypothesis a straight line. Indeed Muller (1932) discarded the breakage hypothesis on this ground. However, Stadler (1936) has pointed out that with fairly high frequencies of breakage per cell the curve would closely approximate to a straight line. In these circumstances the form of the curve at low dosages would be highly significant.

We know that many of the induced changes yield chromosomes which are mechanically unfit to survive many cell generations and nuclei which have more or less extensive deficiencies acting as dominant lethals. All the dosage data, of the kind described in this paper, are taken from samples of individuals from which those with unfit chromosomes have been eliminated. The samples are therefore a residue of survivors from original samples of unknown size. We have no data from which the number of inviable individuals may be estimated, but we can determine in a general way what the effect of their elimination would be (*a*) on the breakage, and (*b*) on the contact hypotheses. It should be pointed out that individuals with one unfit chromosome would be eliminated no less effectively than those with two or more. On any set of assumptions it is clear that individuals with more than one unfit chromosome will become more frequent at higher dosages. Hence the rate of production of new inviable individuals in the sample will fall off with rising dosage.

In what follows we shall assume that there is a direct proportionality between dosage and the primary effect resulting in a structural change. According to the breakage hypothesis, the primary effect would be

breakage of one chromosome region; while on the contact hypothesis, the primary effect would be that condition capable of leading to a structural change (viable or inviable).

The breakage hypothesis

The relative frequencies of nuclei with 0, 1, 2, ..., n breaks each can be calculated for any sample from an infinite population with a given mean number of breaks per nucleus, by means of the Poisson distribution. These frequencies are given in percentages in Table III for different mean numbers of breaks per nucleus; they have been taken from the tables of Soper (1914).

TABLE III

Mean no. of breaks per nucleus (prop. to dosage)	% of nuclei with 0 ... 6+ breaks						
	0	1	2	3	4	5	6+
0·1	90·5	9·0	0·5	—	—	—	—
0·2	81·9	16·4	1·6	0·1	—	—	—
0·4	67·0	26·8	5·4	0·7	0·1	—	—
0·8	44·9	35·9	14·4	3·8	0·8	0·1	—
1·6	20·2	32·3	25·8	13·7	5·5	1·8	0·6
3·2	4·0	13·0	20·9	22·3	17·8	11·4	10·5
6·4	0·2	1·0	3·4	7·3	11·6	14·8	61·3

TABLE IV

Breaks per nucleus	Proportional contributions to		
	Original chromosomes	Inviable chromosomes	Translocated chromosomes
2	0·33	0·33	0·33
3	0·07	0·60	0·33
4	0·01	0·77	0·22
5	0·001	0·88	0·12
6	Nearly 0	0·93	0·07
7	—	0·96	0·04
7+	—	Nearly 1·0	Nearly 0

The next problem is to determine the proportions of nuclei, having a given number of breaks, that would (*a*) return to the original state, (*b*) give inviable chromosomes, and (*c*) give mechanically fit translocated chromosomes. These values would be different for each breakage number. Any attempt towards calculating values for them is handicapped by not knowing whether all breaks rejoin and whether they rejoin at random or preferentially according to spatial disposition. For the purposes of calculation we shall assume (1) that all breakage ends rejoin, (2) that they rejoin at random, and (3) that breaks occur one in each chromosome so

that we are dealing only with interchanges. The effect of variation from these assumptions will be examined later.

The contributions to the three possible classes, with normal, inviable and translocated chromosomes, have been calculated (Table IV) for nuclei with two or more breaks on the assumption that each breakage end must rejoin to another one at random. As we have assumed that all breakage ends must rejoin, nuclei with one break would contribute wholly to the normal class. The formulae used for calculating these figures are as follows, where n is the number of breaks per nucleus:

Proportion original = 1 in $1.3.5.7. \ldots . 2(n-1)$.
Proportion translocated = $(n!-1)$ in $1.3.5.7. \ldots . 2(n-1)$.
Proportion inviable = remainder.

TABLE V

No. of breaks per nucleus	% of nuclei	Contributions to nuclei with		
		Normal chromosomes	Inviable chromosomes	Translocated chromosomes
0	44·9	44·9	0	0
1	35·9	35·9	0	0
2	14·4	4·8	4·8	4·8
3	3·8	0·2	2·3	1·3
4	0·8	0	0·6	0·2
5	0·1	0	0·1	0
Totals	99·9	85·8	7·8	6·3

TABLE VI

Mean breaks per nucleus	Normal chromo-somes	Inviable chromo-somes	Trans-located chromo-somes	% individuals surviving	% of survivors translocated
0·1	99·7	0·2	0·2	99·8	0·2
0·2	98·8	0·6	0·6	99·45	0·6
0·4	95·6	2·5	2·0	97·6	2·1
0·8	85·8	7·8	6·3	92·1	6·7
1·6	62·2	23·2	14·5	76·8	18·8
3·2	25·8	53·9	20·3	46·1	44·0
6·4	2·8	87·6	9·6	12·4	77·4

The contributions to the three classes of nuclei, amongst samples of irradiated nuclei having different mean numbers of breaks per nucleus, have been calculated from the figures in Tables III and IV. The process is shown in detail (Table V) for 0·8 breaks per nucleus. The collected values for different numbers of mean breaks per nucleus are given in Table VI. The percentage of individuals surviving and the percentage of these that are translocated is also given. The theoretical curve is therefore sigmoid with the middle region, between 0·8 and 3·2 breaks per nucleus, approximately linear.

Divergences from the conditions presumed by the three assumptions used in arriving at this result will be of importance only so far as they affect the proportions of the classes with normal and with translocated chromosomes. If some breakage ends do not rejoin and if the others rejoined more frequently to give normal chromosomes, the net effect would be to reduce the survival rate and the relative frequency of translocated individuals amongst the survivors without materially altering the shape of the curve. A study of the effects of radiation upon a ring *X*-chromosome in *Drosophila* has shown that if single breaks occur the two breakage ends do not fail to rejoin. Distortion of the curve from failure of breakage ends to rejoin therefore seems unlikely.

The third of our assumptions is the simplest for which to make allowances. If we supposed that the breaks occurred at random amongst the chromosomes, inversions could be recovered from nuclei having two or more breaks in a chromosome. When one chromosome had sustained three or more breaks, the contribution to the translocation class would rise slightly at the expense of the inviable class. Since such events (more than two breaks in one chromosome) would be rare, the alteration to our calculations would be slight. In the case of three breaks per nucleus distributed at random in the chromosomes of *Drosophila melanogaster* sperm, about 32% would have one break in each of three chromosomes, 54% two breaks in one and one in another chromosome and 14% all three breaks in one chromosome.

The contact hypothesis

The irradiation is presumed to act at spatially independent regions in the nucleus and to induce one or more breaks in propinquity which may give a structural change. It follows that the contributions to normal, inviable and translocation nuclei would be constant proportions per unit of energy absorbed independently of the total amount absorbed in each nucleus. Let us assume that for each X-ray hit there is a mean proportion x contributed to normal, y to inviable and z to translocation nuclei: $x+y+z$ being unity. The contribution to each class for n X-ray hits in a nucleus is given by $(x+y+z)^n$. Those terms with y entering into them will represent relative frequencies of inviable, those with z but no y translocation nuclei and those with x alone (x^n) normal nuclei. If values are assigned to x, y and z, the contributions to normal, inviable and translocation nuclei respectively can be calculated for $1 \ldots n$ units of energy. If each is given the value one-third, the values given in Table VII would be obtained.

The values assigned to x, y and z, though arbitrary, are the most plausible. Although there might be preferential reconstruction to give chromosomes of the original type, the relative chances y and z of new fusions at a contact giving mechanically unfit and fit chromosomes should be equal. A small alteration in the values could be introduced to make allowance for the effects of three or more regions in contact at a point.

TABLE VII

Hits in one nucleus	Proportional contributions to: Normal chromosomes	Inviable chromosomes	Translocation chromosomes
1	0·33	0·33	0·33
2	0·11	0·55	0·33
3	0·04	0·70	0·26
4	0·01	0·84	0·15
5	Nearly 0	0·90	0·10
6	—	0·93	0·07

From the values in Tables III and VII, the contributions to normal, inviable and translocation class may be calculated for means of 0·1 to 6·4 X-ray hits per nucleus. These values are given in Table VIII, together with the percentage survival and the per cent of survivors that have translocations.

TABLE VIII*

Mean no. of hits per nucleus	% individuals with: Normal chromosomes	Inviable chromosomes	Translocated chromosomes	% survivors	% translocation individuals among survivors
0·1	93·5	3·3	3·2	96·7	3·3
0·2	87·6	6·5	6·2	93·8	6·6
0·4	76·5	12·0	11·5	88·0	15·0
0·8	58·6	23·4	17·9	76·5	23·4
1·6	34·0	42·5	23·4	57·4	41·0
3·2	11·6	67·2	21·1	32·7	64·5
6·4	1·3	88·7	9·5	10·8	88·0

* Dr D. E. Lea has shown me that these probabilities can be obtained more simply by applying the Poisson distribution algebraically to the expression in x, y and z before proceeding to use numerical values of the Poisson coefficients. The probabilities thus obtained are:

$$\text{Normal} = e^{-n(y+z)};\ \text{Lethal} = 1 - e^{-ny};\ \text{Translocation} = e^{-ny} - e^{-n(y+z)}.$$

The form of the curve on the contact hypothesis is therefore slightly sigmoid, but with the lower portions nearly linear; its precise shape would depend upon the values of x, y and z.

CONCLUSIONS

The observed values of the induced rate of structural change between 1000 and 4000 r.u. could be fitted equally well to both theoretical curves

in their middle and upper regions. The only critical data that could be obtained by this method would be that upon the induced frequencies at lower dosages than those used so far. The dosage relation has not given data incompatible with either the breakage or contact hypothesis.

Significance of complex rearrangements

Muller (1932) states that the contact mechanism would necessitate the "rare coincidence of three strands meeting at exactly the same point". There are several such progressive rearrangements in the present data which on the contact hypothesis would be inferred to have come from such contacts. The numbers of them are rather high (Table IX). They amount to about 11% of all the contacts inferred. This high value is far from being a rare coincidence and would seem to invalidate the contact hypothesis. This is a strong argument for the breakage hypothesis, that has already been raised by the discovery (Dubinin & Khvostova, 1935) of cases of progressive translocation requiring on the contact hypothesis many threads or regions of threads to be in contact at one point. They have cases, in *Drosophila*, requiring up to nine coincident contacts.

TABLE IX

Frequency of 2, 3 and 4 threads inferred to be in contact at one point

Dose r.u.	Total individuals	Two threads	Three threads	Four threads
1000	196	17 (8·7%)	1 (0·5%)	0
2000	190	31 (16·3%)	3 (1·6%)	0
4000	224	74 (33·0%)	10 (4·5%)	1 (0·5%)

However, if the chromosomes completely filled the treated nucleus, two or more would be in contact and one or more could be broken by the X-rays. Complete filling of the nucleus by the chromosomes would have the effect of immobilizing the chromosomes. Hence any broken ends would be maintained in the spatial relationship in which they were produced. With breakage of one chromosome there would be presumably a reconstitution of the original structure; with two or more chromosomes in contact, there would be chances for reorganization in new ways.

Hence, if the chromosomes filled the treated nucleus the frequency of rearrangements requiring contacts of two, three and more chromosome regions at a point must be regarded as a measure of the frequency of such contacts. If they did not fill the nucleus, the frequency of the more

complex rearrangements must be regarded as positive evidence against a contact mechanism and for the breakage mechanism.

In any case, this would still leave open the possibility that one hit might produce breaks in two or more regions that are in spatial propinquity without being in actual contact or in a knot (Dubinin & Khvostova, 1935).

Muller (1932, p. 218 footnote) has raised the question of whether for instance in the case of an intercalation, the apparent single break in one chromosome was not in fact two breaks close together. Close study of the intercalations in the present series has failed to disclose any evidence for this supposition. There are only three cases, in the present data, in which one chromosome arm had been involved in exchanges at two separate loci with two other chromosome regions. This markedly contrasts with the fourteen instances in which one region of a chromosome arm had exchanged with two other chromosome regions in a progressive translocation.

Spatial preference in rearrangement

Omitting cases of translocations doubtful in their assignment through having one break in heterochromatin, the proportions of interchanges, inversions and eucentric inversions are constant, within the limits of the sampling error, for all doses. Adding together interchanges and eucentric inversions, there are fifty-four translocations between different arms and twenty-seven within the same arm (inversions). On either the breakage or contact hypothesis, there are four ways in which a different chromosome arm may be chosen at random, for a break or a contact, for each way in which the same arm may be so chosen. The observed ratio of 2 to 1, instead of the expected 4 to 1 were the mechanism random in operation, indicates that exchanges within the same arm are twice as favoured as those between different arms. A similar divergence from expectation is shown by three and four point structural rearrangements. This fact argues that rejoins, on the breakage hypothesis, or contacts, on the contact hypothesis, cannot be spatially at random. In the case of the ratio of interchanges between arms of different chromosomes to eucentric inversions between opposite arms of the same chromosome, the observed value of 40 to 14 is not significantly different from the expected ratio of 4 to 1. Translocations between the two arms of one chromosome are therefore probably no more favoured than those between arms of different chromosomes. This indicates that the distance over which spatial preferment acts is fairly small.

SUMMARY

The frequency of induced structural changes of chromosomes observed in F_1 female larvae of *Drosophila melanogaster* raised from X-rayed males shows a direct linear proportionality with the X-ray dosage between 1000 and 4000 r.u. The relation of this fact to the "breakage" and "contact" hypotheses of their induction is discussed. Both hypotheses theoretically require sigmoid curves (with different characteristics) connecting dosage and effect. At intermediate doses the relation would be approximately linear on both hypotheses. The observations therefore do not provide any basis for discrimination.

The high frequency (11 %) of rearrangements, which on the contact hypothesis would require three or more threads in contact at one point, makes it unlikely that the chromosomes are ever in contact before, during or after irradiation unless they completely fill the treated nucleus.

The high frequency of inversions in one chromosome arm, relative to interchanges between different arms, demonstrates a spatial preference either in the refusion of breakage ends or in a grouping of original breaks through a contact or analogous mechanism.

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