INDUCED CHANGES IN FEMALE GERM CELLS OF DROSOPHILA. I.
DEPENDENCE OF HALF-TRANSLOCATION FREQUENCY
UPON X-RAY DELIVERY RATE¹

IRWIN H. HERSKOWITZ AND S. ABRAHAMSON²

Department of Zoology,³ Indiana University, Bloomington, Indiana

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IT HAS BEEN definitely established for X-ray-induced mutations in plants that lowering the intensity of treatment causes a decrease in the number of chromosomal rearrangements obtained (SAX 1939a,b, 1940, 1941; SAX and BRUMFIELD 1943). This observation could result from either or both a necessity for several "units" of X-rays to accumulate in order to produce a break (and as the rate of delivery decreases some units are lost before others are produced), and a decrease in rejoinability of breaks with time (by the restitution of some breaks before others are produced, and/or by the movement of broken ends so far apart from others that their chance of ever combining is reduced). Study of *Drosophila* sperm for gross rearrangement frequency after X-ray treatment of inseminated females revealed no effect of extremely different rates of treatment (MULLER 1938, 1939a,b, 1940; RAY-CHAUDHURI 1939, 1944; MAKHIJANI 1945), supporting none of the hypotheses mentioned for sperm known to be mature. Recently, HAAS, DUDGEON, CLAYTON and STONE (1954), using delivery rates higher than those used earlier, have reported that when sperm were treated in males the higher rate of delivery gave more translocations than the lower rate. One would want this intensity effect repeated with samples of sperm of greater uniformity, for example with sperm treated in inseminated females, however, before accepting it without reservation, since it has been shown that the translocation frequency from sperm delivered by treated males on one day can be twice what it is on a successive day (and possibly in successive copulations on the same day) (ABRAHAMSON and TELFER 1954).

While a study of breakage dependence upon X-ray intensity can decide whether break production requires the accumulation of "units", and the best evidence is that no such accumulation is required for breakage itself, a study of restitution and rearrangement can tell whether breaks are temporarily unjoinable and accumulate until a specific time or whether broken ends can join to other broken ends at any time. The infrequency of gross deficiencies and inversions and of reciprocal translocations in the germ cells of female *Drosophila* has prevented the latter type of study in this organism until recently, although MULLER, VALENCIA and VALENCIA (1949) inferred union between fragments to occur fairly promptly, i.e. prior to the movements of the meiotic divisions. Such a study became possible once it was shown (HERSKOWITZ and MULLER 1953; MULLER and HERSKOWITZ 1954; HERSKOWITZ

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² Eigenmann Fellow in Zoology.

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1954a,b; ABRAHAMSON, HERSKOWITZ and MULLER 1954, 1956; PARKER 1954a,b) that a certain type of multi-break rearrangement does occur frequently in oocytes after X-ray treatment. This type of mutation was termed a "half-translocation" because it involved, of the four pieces produced by breaking two non-homologous chromosomes, only two pieces which joined eucentrically (making up one half of a reciprocal translocation) and were retained in the fertilized egg, the other centric piece having been cast into a polar body joined or unjoined (what the situation usually is has not yet been proven) to the other acentric fragment.

The type of half-translocation studied here was one in which an attached-X chromosome was broken into two arms, only one of which was retained in the egg after joining eucentrically to a piece of another broken chromosome, the other, reciprocal, pieces going into a polar body. All the present experiments were designed to detect whether there was any difference in percentage of offspring with detached-X's (detected whenever these became viable half-translocations) following different X-ray delivery rates. Part of the results presented here were reported by HERSKOWITZ and ABRAHAMSON (1955).

MATERIAL AND METHODS

Using *Drosophila melanogaster*, virgin females were obtained, carrying the "snoc" attached-X chromosome (*sc clⁿ oc ptg car·sn^{x2} In49 y*) and either no Y chromosome or a modified Y (*y⁺ sc^{v1}·Y⁸*), and were aged for at least 3 days under excellent nutritional conditions. The virgins were then irradiated and mated in one half pint bottles to males carrying an X (*y sc^{s1} B In49 v*) and a normal Y chromosome. When 1000r were delivered the number of parents per bottle was 30 ♀ ♀ and 60 ♂ ♂; for treatments of (or about) 2000r, the number of parents per bottle was approximately doubled. From eggs oviposited within the first 4 days after the completion of irradiation (the parents having been transferred to fresh food bottles once or twice within this period, except in Experiment I where eggs deposited only during the first two days after treatment were used), the F₁ flies were scored for detached-X half-translocations. Whether or not the irradiated mothers carried the modified Y chromosome, daughters carrying detached-X half-translocations were phenotypically non-vermilion and "heterozygous" Bar-eyed while triplo-X daughters also would be non-*v* and (wide) Bar, but sterile. The unexceptional daughters were round-eyed. Occasionally, by paternal nondisjunction, vermilion "homozygous" Bar-eyed females were produced. F₁ females due to paternal nondisjunction are not included in the tables. Sons carrying a detached-X were round-eyed, whereas unexceptional sons were vermilion and Bar-eyed (and, incidentally, sterile). The irradiations were administered to non-narcotized, well-aerated flies, at a peak kilovoltage of 200 and at 20 ma through a 1 mm thick Al filter; a Victoreen dosimeter was used to check the machine's output, just before the first and sometimes also between and after treatments in each experiment. The delivery rate to the flies was varied either by changing the target distance or by stopping the X-ray machine for different periods of time before the total dose was delivered, or both. The details of these procedures are given with the results of the specific experiments.

In experiments where the dilute treatments required more than 8 hours to de-

TABLE 1

Percentages of half-translocations in the F_1 from different experiments. (The totals of F_1 scored are given in parentheses. The exposure factors for Y-less mothers were identical to those for the corresponding treatment for Y-bearers in the same experiment.)

Experiment	Total dose	Modified Y-bearing mothers		Y-less mothers	
		Concentrated	Dispersed or dilute	Concentrated	Dispersed or dilute
I	2000r	600r/min continuously 5.4 (336)	30r/min in 2 parts 8 hrs. apart 1.7 (534)		
II	2000r	600r/min continuously 1.9 (1935)	600r/min in 4 parts 8 hrs. apart 0.9 (2836)	1.3 (549)	0.8 (877)
III	1000r	1100r/min continuously 1.6 (2905)	30r/min continuously 0.7 (3306)	1.2 (1567)	0.5 (1477)
Controls		0.05 (2025)		0 (691)	

liver, all the treated flies for that experiment were handled alike, in special plastic containers, one side of which was covered with dacron netting, which could be placed on food to permit the virgins to feed between the parts of the irradiation procedure.

All experiments were carried out at room temperature.

RESULTS AND DISCUSSION OF INDIVIDUAL EXPERIMENTS

In the first type of experiment (Exp. I, table 1) a total dose of 2000r was given either continuously at 600r/min. (taking only 3'20" for delivery) or at 30r/min. in 2 parts 8 hours apart (requiring 33'20" to deliver each of the two parts). Thus the females subjected to the dilute treatment had X-rays delivered at a rate 20 $\times$ slower than females given the intense treatment and had in addition an intermission of 8 hours midway in the irradiation. The half-translocation rate obtained was 5.4% for the intense and 1.5% for the dilute treatments ($P < .005$) in oocytes of the modified Y-bearing females. This result proves that the breaks produced in oocytes which enter into gross rearrangements do not always remain unjoined, as they do in the mature sperm (until after fertilization), for 8 hours after being produced. The amount of decrease in half-translocation frequency with protracted treatment permits the estimation that about two thirds of the breaks which, in the intense treatment, were present in cells having a second break with which they half-translocated, were, in the protracted treatment, in cells where this second break had already either restituted or become "unjoinable".

In the second type of experiment (Exp. II, table 1) 2000r were again delivered, in the intense treatment continuously at 600r/min., as in Exp. I, and in the "dilute" also at 600r/min. but in 4 equal parts 8 hours apart. The intense and dilute percentages were respectively 1.9 and 0.9 for the modified Y-bearing females ($P < .005$). In this experiment, since the rate of output of the X-ray machine was the same in the contrasting treatments, the difference noted must be due to breaks becoming unavailable for interchange in the interval between the successive irradiations.

tions. If in the dilute treatment all breaks produced in the first one quarter of the irradiation which were ever to reconstitute or rearrange did so before any breaks in the second one quarter of the treatment were produced, etc., then the total half-translocation frequency for the dilute would equal $4\times$ the rate induced by one quarter of the total dose. Since it was found (HERSKOWITZ 1954b) that half-translocations increase, in this dose range, as the $\frac{3}{2}$ power of the dose (however, see General Discussion also), then for "total dose/4" one would expect $\frac{1}{8}$ the half-translocations produced by the total dose, and since there were 4 such one quarter doses given in the dilute one would expect from the dilute $4 \times \frac{1}{8}$ or $\frac{1}{2}$ the number of half-translocations obtained from the intense treatment. However, the rate for the dilute would be more than $\frac{1}{2}$ that for the intense if there were an appreciable number of breaks open and rejoinable 8 hours after treatment. The mutation percentage obtained for the dilute being just about one half that for the intense strongly supports the view that almost all if not all breakages which are ever going to join do so within 8 hours following treatment.

Because the percentage for the dilute treatment in Experiment I is less than $\frac{1}{2}$ the rate of the intense (actually $1/3.6$, with treatment having been given in only two parts 8 hours apart), there is good reason to believe that breaks were becoming unavailable for interchange during the extra 63'20" the X-ray machine was operating in the dilute treatment. Thus the first two experiments strongly suggest that within an hour after breaks are produced an appreciable number of broken pieces can reconstitute or interchange and that if they are to do either they must do so within 8 hours of their production.

The third experiment (Exp. III, table 1) required giving 1000r in continuous treatments either at a rate of 1100r/min. (54" delivery time) or at 30r/min. (33'20" delivery time). The incidences of half-translocations for intense and dilute treatments were, respectively, 1.6% and 0.7% for modified Y-bearing mothers ($P < .005$). Since an average of about 16 minutes elapsed between the production of successive breaks in the dilute treatments but averaged less than one half minute in the intense, the reduction in half-translocation percentage for the dilute to about one half the value for the intense is strong evidence that under these experimental conditions at least one half the breaks that are to undergo restitution or interchange do so within about 16 minutes of their production.

Whenever, as in Experiments II and III, Y-less females were also treated at the times modified Y bearers were, a drop in half-translocation frequency with dosage dilution was obtained which was similar in magnitude to that for modified Y bearers. Although this drop was not statistically significant, its indecisiveness was probably caused only by its small sample size.

The purpose of a fourth experiment was to determine whether evidence could be obtained for a change in joinability of breaks in periods of varying lengths. To do this it was decided to deliver a total dose of 2200r at the rate of 1100r/min. in 8 equal periods with 0, $\frac{1}{4}$, $\frac{1}{2}$, 1, 4, 16, or 32 minutes between successive irradiations. (For example, the total period covered by the treatment when 1 min. intervals were used was 9 minutes.) In accord with the preceding discussion it can be reckoned that at this total dose the half-translocation rate for the most dispersed treatment in this

TABLE 2

Percentages of half-translocations produced by 2200r delivered at 1100r/min. in 8 equal periods with the various time intervals indicated between successive exposures. (The totals of F_1 scored are given in parentheses.)

Minutes between exposures	Total period of treatment	Brood 1	Brood 2	Brood 3	Total
0	2 min.	4.5 (198)	8.5 (658)	5.1 (938)	6.3 (1794)
$\frac{1}{4}$	$3\frac{3}{4}$ min.	9.9 (132)	5.2 (232)	7.3 (359)	7.1 (723)
$\frac{1}{2}$	$5\frac{1}{2}$ min.	8.0 (88)	6.7 (233)	5.9 (405)	7.3 (726)
1	9 min.	8.4 (95)	8.4 (220)	7.0 (366)	7.6 (681)
2	16 min.	4.9 (61)	11.2 (187)	6.0 (317)	7.6 (565)
4	30 min.	6.4 (109)	8.9 (273)	7.1 (383)	7.6 (765)
$\Sigma 0-4$		6.9 (683)	8.8 (1803)	6.1 (2768)	7.1 ± 0.35 (5254)
16	1 hr. 54 min.	1.9 (54)	6.2 (517)	4.8 (645)	5.3 ± 0.64 (1216)
32	$3\frac{3}{4}$ hrs.	1.9 (158)	4.7 (796)	2.7 (1020)	3.4 ± 0.41 (1974)
Unirradiated (Control)		0 (202)	0 (1318)	0 (661)	0 (2181)

experiment can be as little as (but probably no less than) $1/3.5$ of the rate for the most intense treatment, at which there was no interval between the irradiations, provided that the breaks produced in different one eighths of the most dispersed irradiation are unable to join with each other. The shorter the intervals between irradiations the more likely it would become that some breaks from earlier one eighths would still be available for rearrangement with breaks produced in later one eighths, causing the percentage of detachments obtained to approach that for the most intense treatment. The results from eggs oviposited the first day after irradiation, the next two days, and the fourth day, are presented as broods 1, 2, and 3, respectively, separately and also combined, in table 2. As expected, the intervals of 32 minutes between irradiations gave half-translocation percentages which were lower than the values in corresponding broods for all other, less dispersed treatments, in 20 of 21 possible comparisons, although they were equal in value in the 21st. Also as expected, the 16-min. intervals between irradiations gave half-translocation percentages which were lower than the values in corresponding broods for other, less dispersed treatments in 17 of 18 possible comparisons. This might be taken to mean that more unjoined but joinable breaks overlapped the different fractions of the treatment in the 16-min. than in the 32-min. intervals. However, for the 4-min. intervals, in which the total treatment period covered 30 minutes, the values were lower than in corresponding broods of less dispersed treatments in only 5 of 15 possible comparisons. It is clear from the frequencies for different broods separately and combined that no difference has been detected between the treatments having intervals in the range of 0-4 minutes. The half-translocation rate when the periods between irradiations were 4 minutes or less (the treatments requiring 30 minutes or less) averaged $7.1 \pm 0.35\%$. They decreased from this value to $5.3 \pm 0.64\%$ ($P = .025$) with the 16-min. inter-

vals (where the treatment took almost 2 hours) and to $3.4 \pm 0.14\%$ ($P < .0001$) with the 32-min. intervals (which required nearly 4 hours to deliver). The frequency for the 32-min. intervals is only $\frac{1}{2}$ that for intervals of 0-4 minutes. However, since it appears significantly higher than $1/3.5$ of the latter value, it is probable that, under these latter experimental conditions, some breaks that are produced 32 or more minutes apart cooperate to produce half-translocations, although most of the break-ages which join do so within 4 hours of their production.

GENERAL DISCUSSION

The present establishment that the number of detached-X half-translocations depends upon the amount of dispersal of the X-ray dose, is at the same time an independent demonstration that half-translocations have an origin that is multi-event and not single (such as the production of a break which is at the same time "healed" to form a new telomere), supporting the other evidences for multi-event origin obtained from X-ray dosage studies (HERSKOWITZ and MULLER 1953; HERSKOWITZ 1954a,b; and, independently, PARKER 1954a) and from genetic studies for identification of half-translocations (ABRAHAMSON, HERSKOWITZ and MULLER 1954, 1956; PARKER 1954b).

The higher rate of half-translocation in oocytes containing the modified Y chromosome ($y^+ sc^{v1} \cdot Y^s$) than in Y-less oocytes, discovered by PARKER (1954b), has been confirmed in all 4 possible comparisons in the present experiments (table 1). At least a part of the reason for this difference, it may be proposed here, lies in the fact that, in an oocyte, where the chromosomes are assumed to be in tetrad condition, when a half-translocation between the X and an autosome enters the mature egg there is about one chance in four that the reciprocal centromere-bearing piece will also enter, thereby preventing the detection of a viable half-translocation either because it produces a full translocation or (when this centromere-bearing piece has not joined to an acentric fragment) death due to dicentric formation. However, when a half-translocation between the X and Y enters the egg the reciprocal, homologous, centromere-bearing piece will almost always enter a polar body since homologous centromeres disjoin during chromosome reduction.

It should be noted that among the possible sources of heterogeneity in different experiments, other than the irradiation itself, are variations in daily temperature, humidity, nutrition, and age of the females used, requiring one to proceed with caution when comparing results of different experiments. All of these factors were carefully randomized, however, within each experiment.

A possible source of error in some experiments lies in the production of triplo-X females that might be mistaken for cases of half-translocation. In the controls there was only one detachment, a male of type $sc cl^n oc car$, obtained among a total of 4897 offspring, and no exceptional females, hence no triplo-X's. Since culture conditions for the treated flies were made as similar to the controls as possible (food, temperature, and crowding among developing offspring), it is extremely likely that few or no triplo-X female offspring survived in the treated cultures either. In support of this is the fact that when exceptional females of $y^+ sc^+$ "heterozygous" Bar phenotype, which would include all cases of triplo-X, were subtracted from the exceptional off-

spring of all irradiated series, in no case did the direction of change, in one treatment as compared with another, differ from what is given in the tables, or produce a change which would require modification of the conclusions drawn from the results as presented. For these reasons it is concluded that the number of triplo-X flies scored erroneously as half-translocations is negligible. Accordingly we have not listed separately in the tables the phenotypes, as regards y , sc or sex, of the exceptional flies.

Despite the fact that only the eggs oviposited within the first 4 days after the completion of treatment were used in these experiments, and all were "oocytes" when treated, there was a significant decrease in mutability in successive eggs oviposited. Analysis of the data shows that in every case in Experiments II and III, as was found also in earlier work (see table 1 in HERSKOWITZ 1954b), the eggs laid the first days after treatment have more mutations in them than those oviposited soon after. This is undoubtedly partially responsible for the mutation rate with the continuous treatment in Experiment II, where eggs from all 4 days were used, being lower than the rate with the same treatment in Experiment I, where only eggs from the first 2 days were used. In Experiment IV, while the mutation rate in brood 2 is lower than the rate in brood 1 in only 2 of 7 possible comparisons (probably due to chance variation in the percentages of the smaller sample sizes in brood 1), there was a lower mutation rate in brood 4 in 7 of 8 cases both when compared to the rates for brood 2 alone and for broods 1 and 2 combined.

The dilute treatment in Experiment III (table 1) and the treatment having intervals of 4 minutes in Experiment IV (table 2) gave contradictory results; whereas both were delivered in about the same period of time (33'20" and 30', respectively) the former showed a significant decrease in mutation rate from the corresponding concentrated treatment but the latter showed no decrease. In other words, the former case had a considerable amount of rejoining during the treatment period, while the latter had little or none. There are, however, two differences between the conditions of irradiation in these experiments, either or both of which may be responsible for the results. One difference is in the dose administered (one being about twice the other), the other is in the rate at which the X-rays were applied to the cells (slow and continuous in the former experiment and quick and discontinuous in the latter). WOLFF and ATWOOD (1954) obtained similar results in experiments with *Vicia*, where a lower dose produced fewer rearrangements when given at a lower rate than when given at a higher rate, but a higher dose gave the same number of rearrangements for both intense and dilute treatments. The basis for such results is still undetermined.

Since the relationship of half-translocation rate to X-ray dosage (HERSKOWITZ 1954b) was studied before the effect of concentration was known, it is desirable to re-examine this dosage relationship. The purpose of that earlier work was to determine whether one event (break) or more was required to produce the mutant type studied, to be decided from the mutant's rate of increase with dose. Whereas in the case of mature sperm there is strong reason for believing that during irradiation all breaks remain open and equally joinable (so far as those breaks are concerned which are later to rejoin), so that the time required to deliver the dose makes no difference, it has been established in the present study that, in oocytes, there can be a substantial decrease in breaks available for joining during the course of an irradiation. In the

earlier dosage study the intensity was about 250r/min. If the higher dose used (4000r), delivered in 16 minutes, did not prevent rejoining before the irradiation was completed then the exponent (observed to be the $3/2$ power of the dose) was correctly estimated, but if the higher dose prevented rejoining while the lower dose (1000r) did not, or did so to a lesser degree, the exponent was an overestimation if taken as representing the relation of rearrangement frequency to breakage frequency. Which of these situations obtained is unknown at present.

A significant observation is that while the numbers of parent females irradiated at the different intensities in Experiment IV were very nearly equal (except that for the 0 min. intervals twice this number was used) the total number of offspring obtained increased in the 16 min. as compared with the 0-4 min., and in the 32 min. as compared with the 16 min. intervals. It is also noteworthy that the dispersed or dilute has more offspring than its corresponding concentrated treatment in every case in table 1 where the numbers of parents used were the same for both treatments within an experiment (Experiment III is to be excluded, however, since more parents were used in the dilute). This statistically significant increase in fecundity with dilution of the dose has been a subject for further investigation and will be reported in the next paper of this series (ABRAHAMSON and HERSKOWITZ, in manuscript).

SUMMARY

The frequency of half-translocations obtained following detachment of attached-X chromosomes was employed as the measure of multi-break chromosomal rearrangements induced by X-rays administered at various concentrations to *Drosophila melanogaster* oocytes. It was found that higher concentrations produced more half-translocations than lower ones, proving that breaks in oocytes can join in new arrangements before fertilization, unlike what is the case for breaks in fully mature spermatozoa (those in inseminated females).

The results show that of the joinings which will take place the great majority do so within 8 hours, and that about one half do so within 4 hours. Under certain experimental conditions (as in Exp. III, see p. 423) at least one half the breaks that join can do so within $1/4$ hour, but under other conditions (Exp. IV, see pp. 423-425) joinings in this period were not detected.

The relation these results have to earlier work on the dosage-frequency relationship for half-translocations and in confirming the influence of a modified Y chromosome on increasing their frequency is discussed.

It was found again that among oocytes oviposited during the first four days following treatment there were significantly more mutations in the eggs laid in the first days than in those oviposited in later ones. There was also a significant decrease in fecundity of the parent females in this 4-day period when the treatments were more concentrated.

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