X-RAY INDUCTION OF 2;3 TRANSLOCATIONS IN MATURE AND IMMATURE OOCYTES OF *DROSOPHILA MELANOGASTER*

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ONLY very low frequencies of autosomal translocations (i.e. translocations between chromosomes 2 and 3) induced by X rays in oocytes of *Drosophila melanogaster* have been reported until now, although relatively high doses were used (see Table 1). On the other hand, detachments of the attached X chromosome, which may be considered as a special kind of translocation, are readily induced (MULLER and HERSKOWITZ 1954; PARKER 1954).

When, as has usually been done, females several days old are irradiated and are allowed to lay eggs for a few days, the offspring analysed are mostly derived from oocytes which were treated in prophase I (stage 7 and possibly earlier stages). It is true that also stage-14 oocytes (late prophase I and possibly metaphase I) have been irradiated, but owing to the high doses used so far (see Table 1) and because stage-14 oocytes are extremely sensitive as regards the induction of dominant lethals, oocytes of this stage will practically not have been analysed. It might, however, be that only stage-7 oocytes (and earlier stages) are resistant to the induction of autosomal translocations, and that in stage 14, translocations can be readily induced. This would not have been observable in the work performed so far, since the doses used were so high that practically all stage-14 oocytes have been eliminated, and the frequency of possibly surviving eggs carrying translocations induced in stage 14 would have been "diluted" by eggs derived from resistant stage-7 oocytes. The detachment frequencies reported by PARKER and McCRONE (1958) are much higher in stage 14 than in stage 7. This finding suggested to the authors that autosomal translocations also might be more readily induced in stage 14 than in stage 7, provided that the high sensitivity of stage 14 to the induction of dominant lethal factors is considered.

TABLE 1

*The frequencies of autosomal translocations induced by X rays in oocytes of Drosophila melanogaster**

Dose (r)	Autosomal translocation frequency (%)	Reference
2000	0.07 (1/1342)	GLASS (1955)
≈ 4000	0.18 (1/550)	SHAPIRO and NEUHAUS (1933)
4500	0.11 (2/1813)	KANELIS and RADU (1943)

* The translocations were scored genetically, not cytologically.

Hence, in order to examine whether autosomal translocations can be induced more readily in stage-14 than in stage-7 oocytes, we had to use *low* instead of high doses (and apply them to females aged a few days). Further, oviposition had to be restricted to the first 24 hours after irradiation. For comparison (and partly as a repetition of the former work) the translocation frequency induced in stage-7 oocytes was determined.

MATERIALS AND METHODS

Translocations between autosomes 2 and 3 were scored genetically in the usual way with the aid of the markers *bw* (brown; chromosome 2) and *st* (scarlet; chromosome 3). Normal (Berlin wild) females were irradiated and mated to γ *sc⁸¹ In49 sc⁸; bw; st* males. (The markers of the paternal X are of no significance in this context.) The P and F₁ generations were maintained at $25 \pm 1^\circ\text{C}$, the F₂ generation at room temperature. 150 kv X-rays were used at a dose rate of 900r/min. The h.v.l. was 6 mm aluminium.

The following series were performed, each consisting of several experiments, the results of which were combined to a single value after the homogeneity χ^2 test had been performed:

- (1) 500r were applied to 5 to 7 day old females. Oviposition was restricted to $24 \pm \frac{1}{2}$ hours after irradiation in order to ensure the scoring of stage-14 oocytes only.
- (2) Like series 1, but 1000r were applied. P females were on the average one day older than in series 1, probably without any influence on the results.
- (3) Controls to series 1 and 2.
- (4) 500r were applied to 0 to 1.5 hour old females. After irradiation the females were aged one day and then (together with the P males) transferred in daily intervals to fresh food, until three one-day broods were obtained. Though with this technique earlier stages were possibly analysed in addition to stage 7 oocytes, for simplicity the oocytes of this series will be referred to as stage-7 oocytes.
- (5) Controls to series 4.
- (6) 4000r were applied to 7 ± 1 day old females. The P generation was removed after 7 days. Since the high dose used eliminates practically all stage-14 oocytes, only stage-7 oocytes (and possibly earlier stages) were analyzed. This series was performed both to repeat the earlier work (see Table 1) and to have additional material similar to that obtained from series 4.
- (7) Controls to series 6.

The results of series 3 and part of the results of series 1 have been published briefly (TRAUT 1967).

RESULTS AND DISCUSSION

Table 2 represents our results. They demonstrate that in stage-14 oocytes 2;3 translocations can be readily induced. Hence the very low translocation frequencies obtained from other authors (see Table 1) can be explained by assuming that the high doses used by them eliminated practically all stage-14 oocytes from the oocyte sample, leaving only the resistant stage-7 oocytes. Therefore, the extreme resistance of stage 7 as regards the induction of autosomal translocations cannot be extended to stage-14 oocytes. (Note that ideally—as a result of meiotic segregation—only 1/16 of all eucentric translocations induced in oocytes are transmitted to the egg, if we—plausibly—assume a chromatid instead of a chromosomal origin of radiation induced translocations.)

1. *Stage dependence:* The dose of 500r induces in stage 14 about 100 times as

TABLE 2

The frequencies of autosomal translocations induced by X rays in mature and immature oocytes

Series	Experiment	2:3 translocation frequency (%)	Series	Experiment	2:3 translocation frequency (%)
1 (500r, stage 14)	1	0.22 (1/454)	3 (control to 1 and 2)	1-3	0.00 (0/3283)
	2	0.69 (3/432)			
	3	0.27 (2/731)			
	4	0.60 (2/331)	4 (500r, stage 7)	1-5	0.00 (0/9585)
	5	0.57 (4/705)			
	6	0.33 (1/300)			
	7	0.00 (0/403)	5 (control to 4)	1-2	0.00 (0/2869)
	8	0.17 (1/579)			
	average	0.36 (14/3935)			
	P* = 0.64				
2 (1000r, stage 14)	1	0.00 (0/308)	6 (4000r, stage 7)	1	0.14 (3/2110)
	2	0.00 (0/105)		2	0.00 (0/1500)
	3	0.00 (0/306)		3	0.08 (4/5267)
	4	0.43 (1/232)		average	0.08 (7/8877)
	5	0.14 (1/712)		P* = 0.32	
	6	0.53 (3/563)	7 (control to 6)	1-2	0.00 (0/2083)
	7	0.33 (1/306)			
	8	0.00 (0/167)			
	average	0.22 (6/2699)			
	P* = 0.64				

* χ^2 test for homogeneity.

many 2;3 translocations as in stage 7. This is shown by the following calculation: After irradiation with 4000r from stage 7, a translocation frequency of 0.079% is obtained (series 6). Provided that in stage 7 for the dose range of 500r to 4000r MULLER's 3/2 power rule (see MULLER 1954) can be applied, a dose of 500r would induce a frequency of 0.0035% in stage 7. This value agrees very well with the frequency of 0/9585 obtained in our 500r experiments with stage-7 oocytes (series 4). After irradiation of stage 14 with 500r a frequency of 0.36% (series 1) is obtained; $0.36/0.0035 \approx 100$.

There are two main possibilities (which may not be mutually exclusive) for interpreting the different frequencies of autosomal translocations obtained for stage-14 and for stage-7 oocytes. Either more chromatid breaks are induced in stage 14 than in stage 7 or the breakage frequencies are equal in both stages, but in stage 14 more of the chromatid fragments take part in a translocational exchange.

Though we cannot exclude the first possibility, we consider the second possibility to be more likely: According to the target hypothesis, which principally seems applicable to radiation mutagenesis, very different target sizes, i.e. different chromatid volumes would have to be postulated for the two oocyte stages investigated. However, only the *shape* and not the *volume* of the chromatid changes appreciably when the oocyte develops from stage 7 to stage 14 (owing to the higher spiralization of the chromosome in stage 14). This does not exclude that

the chromatid volume may increase somewhat when the oocyte develops from stage 7 to stage 14, but this increase does not seem to be great enough to explain the difference in translocation frequencies observed.

Some information on the fate of chromatid breaks induced by ionizing radiation in *Drosophila* oocytes can be obtained from studies on radiation induced detachments, dominant lethals, and inversions.

Radiation induced detachments of the attached-X are based on translocational exchanges involving the proximal part of the X. Detailed studies (see PARKER 1963 for literature) have revealed that the proximity of the breaks is an important requirement for exchange, i.e. for the process resulting in detachment. This finding is in line with the general experience and has led WOLFF to formulate his site concept (WOLFF 1962). Further, the detachment studies have made it likely that breaks induced in stage 14 stay open (i.e. they neither reconstitute nor recombine) until the time of fertilization, i.e. the beginning of anaphase I. (When chromatid fragments resulting from the same break join, we speak of "restitution", when fragments from different breaks join, we speak of "recombination". In both processes the breaks "rejoin".) In stage 7, on the other hand, the breaks usually rejoin (i.e. either reconstitute or recombine) after a few minutes, probably because metabolic activity is higher in stage 7 than in stage 14 (see also WOLFF and LUIPPOLD 1955). This conclusion is supported by the dominant-lethal studies of PARKER (1959). Only when anaphase I is initiated (by fertilization) are breaks induced in stage 14 able to rejoin, apparently because metabolic activity increases.

Dose-effect studies on dominant lethals induced in stage-14 and stage-7 oocytes (SONNENBLICK 1940; KING *et al.* 1956; PARKER 1959) have been interpreted as evidence that in stage 7 rejoining of chromatid breaks (mostly as restitution, sometimes as sister-union) is a very efficient process (TRAUT 1966).

In stage 7 not only the frequency of X-ray induced autosomal translocations but also that of inversions seems to be very low (THOMAS and ROBERTS 1966), contrary to what had been reported before (GLASS 1955). This observation is in line with the assumption that the chromatid breaks rejoining in stage 7 mostly reconstitute instead of recombining.

Applying the information obtained from these studies to our results on translocations induced in stages 14 and 7, respectively, we propose the following interpretation of the great difference in translocation frequencies observed for these two oocyte stages.

In stage 7 probably most of the breaks rejoin, apparently because of the relatively high metabolic activity of this stage. This rejoining will much more likely be restitution than recombination, because the chance is low that fragments from different breaks will contact each other. Hence the translocation frequency is low in stage 7.

In stage 14, breaks do not rejoin, apparently owing to the low metabolic activity of this stage. When fertilization initiates the anaphase I movement, rejoining is possible. Though the movement of the fragments renders rejoining more unlikely than it is in stage 7 (i.e. many breaks stay open resulting in dominant lethal factors) those breaks rejoining will be more likely than in stage 7 to form a trans-

location instead of restituting. Hence a relatively high translocation frequency is obtained for stage 14.

When male imagoes of *Drosophila melanogaster* are irradiated, the brood pattern technique reveals a sharp decrease from the maximal translocation frequency found about a week after irradiation to a value of practically 0% at the eighth day after irradiation. This has been regarded as strong evidence that the stages highly resistant to the induction of translocations in both sexes are homologous ones, i.e. primary spermatocytes and primary oocytes (TRAUT 1962). Since our experiments have shown that in stage-14 (late prophase I and possibly metaphase I) oocytes translocations can be readily induced, it is likely that translocations can also be obtained from the corresponding spermatocyte stages, provided that the dose is sufficiently low (to prevent elimination *via* dominant lethal induction). Hence prophase I (except late prophase) is the stage resistant to the induction of translocations both in spermatocytes and oocytes. However, it would be difficult for methodological reasons to separate the translocations probably induced in the stages of spermatogenesis corresponding to stage 14 both from the higher frequency of the immediately following stages (i.e. early spermatids) and the very low frequency of the preceding prophase I stage.

2. *Dose dependence:* As Table 2 shows, the translocation frequency obtained for 1000r (0.22%) is *lower* than that obtained for 500r (0.36%). The difference is significant ($P = 0.002$, as calculated with the χ^2 test, taking into account YATES' correction for continuity). However, according to a quadratic dose-effect relationship at 1000r four times as many translocations are expected as at 500r, i.e. a frequency of $0.36 \times 4 = 1.44\%$ instead of the observed one of 0.22%. (According to the $3/2$ power rule, the expected value would be a little lower.) In order to examine whether the low 1000r value departs significantly from this expectation, a regression analysis for linear trends in frequencies has been applied, based on the χ^2 method and described by ARMITAGE (1955). In this procedure the frequencies are weighted according to the absolute numbers of chromosomes on which they are based. In order to test the significance of both the general heterogeneity and the linear regression, χ^2 and χ^2_0 were calculated. From the difference $\chi^2 - \chi^2_0 = 11.34 - 4.30 = 7.04$ (for 1 df) $P = 0.007$ is obtained, i.e. the departure from a *linear* dose-effect relationship (in the "not expected" direction) is highly significant. Hence the departure from a quadratic dose effect relationship is more significant than ever ($P < 0.007$).

Since our dose-effect curve is based on three points only, we emphasize the preliminary nature of the following considerations. If instead of a decrease of the translocation frequency with increasing dose, i.e. the formation of a maximum, only a saturation of the dose-effect curve were to be explained, WOLFF's site concept (WOLFF 1962) could be applied. If only an increase of the translocation frequency to less than the square of the dose were to be explained, MULLER's $3/2$ power rule could be applied.

One might ask whether the dose-effect relationship obtained is caused by the fact that our stage-14 samples were to a small extent "contaminated" by stage-7

oocytes. According to PARKER (1963) this stage-7 portion probably is not larger than 5%, since oviposition has been restricted to 24 hours after irradiation. Could this possible heterogeneity be the cause of the decrease of translocation frequency when the dose is increased from 500r to 1000r? Assuming a positive correlation between radiosensitivity as regards the induction of translocations and dominant lethals, the *registered* translocation frequency at 1000r would be based to a larger extent on the resistant stage-7 oocytes than at 500r. Hence the translocation frequency obtained would be lower than the induced one. However, a simple calculation (not to be carried out here) based on plausible assumptions concerning both the portion of stage-7 oocytes and the radiosensitivity as regards the induction of translocations and dominant lethals in the two oocyte stages, does not support this possibility: Heterogeneity could at most explain an increase with a factor of about 3 (instead of 4) when the dose is doubled, and does therefore not seem to be able to account for a decrease characterized by a factor of 0.61 ($= 0.22/0.36$). Nevertheless, a final decision about this possibility will have to be postponed until more points of the dose-effect curve are available by work which is under way. The same is true for another possibility assuming that each non-restituting break in addition to those two breaks required for a translocational exchange between chromosomes 2 and 3, acts as a dominant lethal factor (probably by bridge formation in the embryo). According to this hypothesis the *registered* translocation frequency would at first rise faster than the dose and then decrease with increasing dose, provided that certain suppositions about the fate of chromatid breaks induced in stage 14 are valid. Dose-effect curves with extremes as a result of additional hits have already been postulated by DITTRICH *et al.* (1949) on the basis of their experiments of the effect of fast electrons on leaf length of *Hordeum vulgare*.

One might doubt whether the low translocation frequency obtained for stage-7 oocytes exposed to high doses (e.g. 0.08% by 4000r; series 6) is the result of the particular radioresistance of this stage instead of being the consequence of the peculiar dosage-effect relationship described: The 4000r value might fit the dose-effect curve obtained for stage 14 by lying on its declining part. However, this assumption is invalidated by our finding that the translocation frequency induced by 500r in stage 7 is much lower than that obtained for stage 14 (see Table 2).

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SUMMARY

Only very low frequencies of autosomal translocations induced by X rays in oocytes of *Drosophila melanogaster* have been reported till now. Our experiments show that this resistance to the induction of autosomal translocations is characteristic only of stage-7 oocytes (and the earlier stages), while in stage 14, translocations can be readily induced. With 500r the translocation frequency is about 100 times greater for stage 14 than for stage 7. In order to explain this great

difference in sensitivity, differential restitution of radiation induced chromatid breaks together with differences in the spatial arrangement of the chromosomes in the two oocyte stages has been assumed.—For stage 14 the frequency of translocations obtained by irradiation with 1000r is significantly *lower* than that obtained by 500r. The departure even from a linear (i.e. more than ever from a quadratic) dose-effect relationship is highly significant. It is suggested that this decrease of translocation frequency with increasing dose is caused by a close connection between the induction of autosomal translocations and dominant lethals.

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