THE PRODUCTION OF TRANSLOCATIONS IN *DROSOPHILA* OOCYTES¹

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THE availability of a great variety of stocks of *Drosophila melanogaster* has made it possible to carry out many experiments of elegant design. However, the study of chromosome breakage and rejoining in this organism has been limited by the failure of many breaks to rejoin in mature sperm until after sperm entrance. Furthermore, while breaks may rejoin rapidly in earlier stages of spermiogenesis (OSTER 1955) there is difficulty in making exact identification of stages involved in irradiation, and obtaining replicable samples of sperm is correspondingly difficult.

The finding (PARKER 1953) that radiation-induced detachment of attached-X chromosomes in *Drosophila* is a translocation process has given another method for the study of breakage and rejoining in this organism. To recover viable detachments, the attached-X must be broken in the proximal heterochromatin. PARKER and McCRONE (1958) believe that the recovered detached X is usually the centric X, the broken end of which has rejoined with the acentric tip of another broken chromosome. The reciprocal product is occasionally recovered, the acentric X combining with the centric fragment of another chromosome. The recovery of a detachment depends upon subsequent disjunction of the two X's involved in the rearrangement. For this reason, this type of process has been called, perhaps unfortunately, "half-translocation," which implies failure of the other half of the translocation to be generated (HERSKOWITZ and ABRAHAMSON 1956; ABRAHAMSON, HERSKOWITZ and MULLER 1956). Since these exchanges in all likelihood are chromatid translocations (PARKER and McCRONE 1958), non randomness of second division segregation together with the possibility of separation at the first division make it quite unlikely that both products might be included in the egg nucleus. Because of the way these translocations are recovered, we know only that the other chromosome fragments either have been lost or have been included in polar nuclei. The writers prefer simply to use the term translocation to describe the processes involved.

Analysis of a number of the detachments obtained has shown that in most cases the new end of the broken X may be identified. PARKER (1954a,b) found that if a Y chromosome be present, it most often supplies the other end for the detached X; in the absence of a Y, translocations with chromosome 4 are most

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frequent. There is, somewhat infrequently, involvement of breaks near the tips of the major autosomes, and occasionally deletion of nearly an entire X (HERSKOWITZ and MULLER 1953; ABRAHAMSON, HERSKOWITZ and MULLER 1956).

SONNENBLICK (1950) states that meiosis is arrested, and does not proceed beyond Metaphase I until sperm entrance. From this, it may be inferred that all breaks rejoin prior to the completion of meiosis, otherwise the acentric fragments would not be expected to be included in the egg nucleus, and two-break events would not be recoverable. This should allow measurement of the time required for breaks to rejoin, using variation in dose rate, or fractionation of the treatment as has been done with *Tradescantia* and other organisms (SAX 1939; and others).

The sensitivity of oocytes to ionizing radiations was found by PATTERSON, BREWSTER and WINCHESTER (1932) to increase with age. This has been confirmed by PARKER (1955) for detachment of attached-X and for dominant lethals, and by KING, DARROW and KAYE (1957) for recessive and dominant lethals. KING, RUBINSON and SMITH (1956) have described oogenesis in adult *Drosophila* and have designated 14 stages. It seems clear from their description that the cells being treated in the newly emerged females are in stage 7 and those in females three days old or older are in stage 14.

This change in sensitivity with age raises a number of questions relevant to the translocation processes. The experiments here reported are attempts to see if dose response is "two hit" in oocytes of various ages, how long breaks remain open, and if the increase in sensitivity of the oocyte is associated with any change in the rate of rejoining of breaks. PARKER and HAMMOND (1957) have reported these results in abstract form.

EXPERIMENTAL METHODS

In preliminary experiments, cited in Table 1, attached-X chromosomes marked with *br ec* or with γ were used; in all other experiments an attached-X marked with $\gamma^2 su-w^a w^a bb$ was used. The attached-X females also carried a modified Y, *sc³*.Y. This is a "complete" Y chromosome in that it supplies all Y chromosome requirements for male fertility; in addition it carries the normal alleles of yellow and achaete, allowing ready identification of a number of translocations by linkage of yellow⁺ with the detached X.

Virgin attached-X females, raised at a temperature of 23.5-24°C, and differing in age by no more than 12 hours were irradiated either shortly after emergence, or were aged from three to six days at a temperature of 25°C before being irradiated. In the experiments given in Table 1, these females were mated individually with Muller-5 males immediately after irradiation, being removed three days later. In the other experiments, mass matings of 25 females with 25 Muller-5; *ci ey^R* males in half pint culture bottles were used; young females were aged two days after irradiation before mating. In view of the great differences in sensitivities of oocytes of stages 7 and 14, females were allowed to lay eggs for no more than 24 hours before being removed. Under these conditions, an average of about 20 eggs per female will be laid.

TABLE 1
Frequency of detachment in young females (stage 7 oocytes)

Females irradiated	Dose, r	<i>br ec</i> , or <i>y</i> ♀♀	<i>w^a B</i> ♂♂	<i>B</i> ♀♀	<i>br ec</i> , or <i>y</i> ♂♂	Percentage detachment
<i>br ec</i> /Y	250	2300	2461	1	1	0.04± .04
<i>br ec</i> /Y	500	1190	1309	3	1	0.16± .08
<i>br ec</i> /Y	1000	746	788	7	9	1.03± .26
<i>y</i> /Y	250	2133	1306	1	2	0.09± .05
<i>y</i> /Y	500	1444	974	5	2	0.29± .11
<i>y</i> /Y	1000	586	316	6	7	1.42± .40

The irradiations referred to in Table 1 were made using an air-cooled Coolidge tube operated at 55 KVP with 1 mm of Aluminum filtration. Treatments indicated in Table 2 were given using similar equipment operated at 60 KVP. The dose rates were 100r/minute in each of these. Later work was carried out using a G. E. Maximar-100 machine operated at 100 KVP, with 1 mm of Aluminum filtration, giving a HVL of 1.3 mm of Aluminum. The dose rate was approximately 290r/minute at a target distance of 15 cm. All dosimetry was with a Victoreen r-meter with a 100-r thimble chamber; in all work except that shown in Table 1, the instrument used had been calibrated against a Cobalt-60 source in the Biology Division of Oak Ridge National Laboratory.

Unetherized flies were placed in acrylic plastic containers during irradiation. In the case of interrupted treatments, if the time interval between irradiations exceeded five minutes, flies were returned to culture vials without etherization. All irradiations were carried out at temperatures between 23° and 26°C.

All frequencies were calculated on the basis of total flies counted, and are crude percentages differing in this respect from the method previously used (PARKER 1954a,b). In previous publications, due to the variation in viability of Muller-5 males depending on the type of Y chromosome carried, regular male classes were disregarded and the frequency determination was based on the number of regular females and detachments, with correction for the estimated numbers of inviable or poorly viable classes.

TABLE 2
Frequency of detachment in females aged five days before irradiation (stage 14 oocytes)

Dose, r	<i>su-w^a w^a</i> ♀♀	<i>y² su-w^a w^a</i> ♀♀	<i>w^a B</i> ♂♂	<i>w^a B</i> ♀♀	<i>y² su-w^a w^a</i> ♂♂	<i>su-w^a w^a</i> ♂♂	Percentage detachment
0	4	2878	2664	1	0	1	0.04± .03
100	6	2685	2684	9	0	1	0.19± .06
200	5	2185	2162	18	9	0	0.62± .12
250	1	2581	2654	31	8	3	0.80± .12
400	2	1214	1273	30	14	3	1.85± .27
500	3	1691	1864	58	27	3	2.41± .25
800	0	362	420	23	6	0	3.58± .65
1000	0	269	306	11	6	4	3.52± .75

RESULTS

Dose response in stage 7 oocytes

PARKER (1953) irradiated newly emerged females (aged 0–12 hours), and immediately mated them individually in culture vials, where they remained three days before being discarded. Two different attached-X lines were used, *br ec/Y* and γ/Y . Table 1 gives the results of these irradiations. In the range of 250–1000r, the frequency of detachment seemed to vary approximately as the square of the dose. PARKER (1954a) found the increase with dose somewhat less in the range 1000–2000r.

Dose response in stage 14 oocytes

The conclusion of SONNENBLICK (1940) that dominant lethals induced in oocytes of females aged five days resulted from the failure of breaks to rejoin raised the possibility that no recoverable two-hit detachments could be produced in this age, or that dominant lethals were produced in some manner other than the one postulated. It has been found that dominant lethals in young oocytes are produced in some "two hit" manner, while in aged cells their origin is "one hit" (PARKER 1955; KING, DARROW and KAYE 1957). Obviously, the technique of detachment of attached-X's gives a method of answering the question of the ability of breaks induced in these older (stage 14) cells to rejoin.

The range of dosages used was from 100 to 1000r; as the fraction of eggs surviving is about $e^{-1.77D}$ (where D is measured in Kr) it is not feasible to use dosages above 1000r, and the recovery of offspring is very poor even at that dose. Table 2 gives the results obtained with females aged five days before treatment. A good "two hit" relationship is found at lower doses; it is interesting to see a possible levelling of the detachment frequency at higher doses. Possibly oocytes of two different sensitivities are included in the sample, with the more sensitive stage 14 which is more likely to yield detachments also being much more readily killed. The apparent "break" in the otherwise linear survival curve of SONNENBLICK (1940) perhaps indicates the presence of a few cells of much lower sensitivity (stage 7?) in his samples.

Dose-fractionation experiments with stage 7 oocytes

Attached-X females were given a total dose of 2000r, either in one uninterrupted dose, or in two fractions of 1000r each, with a measured interval between the two treatments. The results of two series of irradiations are given in Table 3. It will be seen that all treatments involving intervals of ten minutes or more give frequencies which are significantly lower than the continuous-irradiation controls. The values obtained vary around about 70 percent of the control frequency.

There is a marked difference between frequencies obtained in the first and in the second series of experiments, these being carried out about two months apart. A similar difference is to be seen in experiments in the aged series, carried out at about the same time (Table 4). Methods of treatment were as nearly similar as

TABLE 3
Effects of dose-fractionation in young females (stage 7 oocytes)
Total dose 2000r, divided into two 1000r fractions separated by measured time interval

Time interval	$su-w^a w^a$ ♀ ♀	$y^2 su-w^a w^a$ ♀ ♀	$w^a B$ ♂ ♂	$w^a B$ ♀ ♀	$y^2 su-w^a w^a$ ♂ ♂	$su-w^a w^a$ ♂ ♂	Percentage detachment	Difference	D/s.e.
0	59	5053	4739	225	71	72	$3.60 \pm .18$	—	—
15 minutes	10	1149	1165	30	19	13	$2.55 \pm .32$	$-1.05 \pm .37$	2.84
30 minutes	28	2964	2638	55	33	27	$2.00 \pm .20$	$-1.60 \pm .27$	5.93
45 minutes	28	2443	2162	74	22	22	$2.69 \pm .23$	$-0.91 \pm .29$	3.14
60 minutes	39	3043	2889	102	40	30	$2.80 \pm .21$	$-0.80 \pm .28$	2.86
0	34	5249	5021	144	65	53	$2.48 \pm .15$	—	—
5 minutes	31	4393	4230	114	46	38	$2.24 \pm .16$	$-0.24 \pm .22$	1.09
10 minutes	41	5202	4875	109	46	28	$1.78 \pm .13$	$-0.70 \pm .20$	3.50*

* Difference 5-10 minutes is $0.46 \pm .21$, which is 2.2 times its standard error.

TABLE 4

Effects of dose-fractionation in aged females (stage 14 oocytes)
Total dose 500r, divided into two 250r fractions separated by measured time interval

	Time interval	<i>su-w^a w^a</i> ♀♀	<i>y² su-w^a w^a</i> ♀♀	<i>w^a B</i> ♂♂	<i>w^a B</i> ♀♀	<i>y² su-w^a w^a</i> ♂♂	<i>su-w^a w^a</i> ♂♂	Percentage detachment
First series	0	4	1900	2286	95	31	8	3.10± .26
	15 min.	3	1957	2467	72	28	7	2.36± .23
	(5-day)* 30 min.	4	1682	1969	86	41	6	3.51± .30
Second series	0	4	2592	2815	85	36	5	2.28± .20
	1 hour	8	2539	2831	108	34	7	2.87± .22
	(5-day)* 2 hours	12	2875	3147	111	43	12	2.68± .21
Third† series	0 (5-day)*	7	2318	2392	89	17	9	2.38± .22
	0 (6-day)*	2	2001	2149	65	12	5	1.94± .21
	24 hours	4	2393	2566	91	25	7	2.42± .22
Fourth‡ series	0 (3-day)*	15	2607	2672	87	32	5	2.28± .20
	24 hours	5	1522	1627	51	17	2	2.17± .26

* Figures in parentheses give age at time of irradiation.

† In the third series, all females were mated when six days old.

‡ In the fourth series, all females were mated when four days old.

possible. There can be no clustering of identical translocations, since all cells were oocytes at the time of treatment. Furthermore, PARKER (1953) found that the detachments fell in a Poisson distribution in the progeny of individual treated females. Again, this difference may be the result of heterogeneity in the samples.

Dose-fractionation experiments with stage 14 oocytes

This series of experiments was carried out in essentially the same way as the preceding, except that the dosage chosen for these irradiations was 500r. As seen in Table 2, the recovery of offspring at doses of 800r or more is so low as to make it impractical to use these higher dosages. Also, the frequency obtained at 500r is roughly similar to that found at 2000r in stage 7 oocytes.

The results of these experiments are given in Table 4. With the exception of the fifteen minute interval group in the first series, in no case does the fractionated treatment differ significantly from the control, nor is there any suggestion of a lowering of the frequency as a result of the intermittent treatment. In the one case, the fifteen minute interval, the difference is greater than twice its standard error, $0.74 \pm .35$ percent. The finding of a higher frequency in the 30 minute group makes it likely that this difference is the result of some unknown experimental error, or of sampling errors, rather than representing a real reduction in translocation frequency.

The results are quite uniform for females treated at ages of from three to six days except that, as in Table 3, the first series is somewhat higher than the later ones. It seems clear that after three days of aging there is no further increase in sensitivity of oocytes.

DISCUSSION

HERSKOWITZ and ABRAHAMSON (1956) have irradiated females aged three or more days, and from various findings have concluded that the production of detachments in their material is dose-rate dependent, and that the breaks induced in this material rejoin fairly rapidly. There are certain difficulties brought about by their experimental procedures which might cause one to question the plausibility of the conclusions reached. By allowing females to oviposit over a period of several days, they have introduced a great deal of heterogeneity of sensitivity, as is shown in the results of this paper. KING, RUBINSON and SMITH (1956) state that in actively ovipositing females, there will be stage 14 oocytes in the most posterior follicle of the ovariole, followed immediately by a follicle containing a stage 7 oocyte. The differences in survival of stages 7 and 14 at 2000r (the dose most used by HERSKOWITZ and ABRAHAMSON) would allow one to recover some 80 percent of stage 7 cells, while only three percent of the more sensitive stage 14 cells would survive. It would seem that most of the F_1 flies recovered in the Indiana experiments must therefore have developed from oocytes of much less sensitivity than those dealt with in the aged females in the present paper. With this modification, their results do not differ greatly from the ones here reported.

The results obtained in the fractionation experiments allow the conclusion that breaks do rejoin rapidly, in about ten minutes in stage 7 oocytes but remain open for a long period of time in stage 14. A comparison of the second and third series in Table 4 shows that there is no loss of detached X's resulting from an additional day of aging after irradiation. In the second series, by the time the female is six days old (24 hours after irradiation) eggs in the sample have all been laid, hence all the rejoining which will be possible has been completed. In the third series, a comparison of the fractionated series with those given uninterrupted irradiation shows that at the time the flies become six days old, breaks induced 24 hours earlier are still open and able to interact with those induced by the second treatment. In one case, breaks have rejoined within 24 hours, while in the other these breaks have all remained open for 24 hours. The conclusion seems inescapable that breaks remain open until about the time the egg is laid; one would suspect sperm entrance as being the event which triggers rejoining, as is the case with the rejoining of breaks induced in sperm (MULLER 1940).

The discovery by WOLFF and LUIPPOLD (1955) of the role of oxidative metabolism and ATP formation in the rejoining of radiation-induced breaks in chromosomes suggests that perhaps the difference in the behavior of the two stages dealt with here, i.e., failure of breaks to rejoin in older cells, may be due to changes in oxidative metabolism during meiotic prophase. Such changes in respiratory pattern of eggs during maturation and fertilization have been reviewed by BRACHET (1950). In the sea urchin, for example, there is a decrease of oxygen consumption during maturation of the egg; the unfertilized egg's respiration is not inhibited by carbon monoxide nor by cyanide, while in the fertilized egg respiration is inhibited by these poisons. Apparently, the amount of cytochrome oxidase does not

change with fertilization, but an intermediary transport compound is formed at that time. The existence of a similar situation in the *Drosophila* oocyte would account for the differences in rejoining rates in the two stages.

STONE, HAAS, ALEXANDER and CLAYTON (1954) and HAAS, DUDGEON, CLAYTON and STONE (1954) have shown an increased production of translocations in an atmosphere containing carbon monoxide. STONE (1956) gives evidence from the difference in response of poky and of wild type *Neurospora* irradiated in an atmosphere containing carbon monoxide that cytochrome oxidase protects against radiation damage. It may be permissible to speculate that the changes in sensitivity of *Drosophila* oocytes may in part be due to changes in the activity of cytochrome oxidase, which is also reflected in ability of breaks to rejoin. Experiments are under way to test this hypothesis, using dominant lethals as a measure of radiation damage.

Another striking difference between the young and aged series is the relative difference in sizes of the three detachment classes recovered. In the young series, nearly as many males as females are obtained, and the males are about equally divided between those carrying yellow⁺ and those showing yellow². In the aged series, there is a marked deficit of males as compared to females, and only about a fifth of the males carry the marker, yellow⁺, from the *sc*^s.Y. PARKER and McCONE (1958) show these differences to be due to a relative increase in autosomal translocations and gross X deficiencies over X-Y translocations. The possible relation of this finding to radiation sensitivities of cells is still obscure, in that it might result from changes in proximity and coiling of the chromosomes, or from the delayed rejoining of the broken ends.

SUMMARY

Attached-X females of various ages were irradiated, and the frequency of detachment determined in females of various ages. There is an increase in radiation sensitivity of oocytes with aging up to three days, after which there is no evident increase. A "two hit" formation of detachments is found both in young (stage 7) and old (stage 14) oocytes. Dose fractionation experiments show breaks in stage 7 rejoin in about ten minutes, while those in stage 14 do not rejoin until fertilization. There is evidently a change in the proportions of the various kinds of exchanges obtained when females are aged before treatment. Speculations are made as to the possible role of cytochrome oxidase activity in the changing sensitivity of oocytes.

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