A COMPARATIVE STUDY OF INDUCED MUTATION IN THE OOCYTES AND SPERMATOZOA OF DROSOPHILA MELANOGASTER

II. Deficiencies and Minutes[1]

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IN THE previous paper of this series (Glass 1955) it has been shown that in mature oocytes of *Drosophila melanogaster* translocations are scarcely ever induced by X-ray treatment, whereas inversions are induced, by the same dose and in the same genetic strain, at about one-fourth the frequency with which they are induced in spermatozoa. It consequently appeared probable that the difference between the responses of the mature male and female germ cells to ionizing radiation is not a matter of a different frequency of chromosome breakage, but rather is attributable to a different probability of recombination vs. restitution. This, it was pointed out, might be tested by studying the frequency of chromosomal rearrangements in which the two breaks involved in the rearrangement occurred in very close proximity to each other; for in that case the "non-random factor" responsible for the higher probability of recombination for breaks close together and in the same chromosome might be more nearly equal in the male and female germ cells. Small, two-break rearrangements can either be recovered as deficiencies or as inversions; but, inasmuch as very short inversions are difficult to detect genetically, a comparison of the frequencies of deficiencies in oocytes and spermatozoa was selected as the method of choice.

When the breaks in the chromosome are more than a very short distance—that is, more than a few salivary chromosome bands—apart, the individual breaks are the result of distinct "hits" by the ionizing radiation on the chromosome. But when the breaks are extremely close together, the deficiencies produced increase as the first power of the dose; that is to say, in such instances two chromosome breaks appear to result from a single "hit" (see Muller 1940; Lea 1947, pp. 233–234; Catcheside 1948, p. 308; Kaufmann 1954, p. 668). It is the latter type of deficiency that might be expected to provide the answer to the question posed here. The genetic methods of detection, however, offer no simple way of separating the 2-hit deficiencies from the 1-hit deficiencies.

Initially, an experiment was undertaken to determine the relative frequencies of homozygous-viable mutations and of deficiencies which in both cases involved the loci of certain recessive marker genes in chromosome 2. The largest deficiencies might be readily recognized, because they would involve a simultaneous loss of adjacent marker loci. Smaller deficiencies might be recognized by the lethality of the "mutation" when, by appropriate crosses, it was made homozygous. This of course assumes on the one hand that all point mutations are viable when homozygous, and on the

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lethals, and visibles from treated females to be associated with sectional dislocations that reduced crossing over, whereas 2 out of 12 mutations from treated males were associated with chromosomal aberrations.

In discussing the significance of these rather preliminary results, PATTERSON and MULLER advanced the suggestion that point mutations might be due to changes within a gene that would still leave it to the power to multiply, whereas chromosomal alterations might result from destruction of the gene and loss of its power to multiply, thus bringing about a break in the continuity of the chromosome. From this they reasoned further that either restitution or reattachment to other points might follow breakage; and the process of reattachment might well be subject to different influences than those which affect point mutation. Either the physiological condition of the chromosomes, or the spatial relations between chromosomes tightly packed together as in the sperm head, or loosely dispersed as in other cells, might alter the relative proportion of recombination and restitution. Thus the high frequency of chromosome aberrations found to be associated with mutations induced in spermatozoa might be accounted for, "though the number of 'point mutations' need not be correspondingly more frequent." They wisely refrained from further speculation at the time, but, as we shall see, this suggestion of theirs came very close to actuality.

SHAPIRO and NEUHAUS (1933) obtained 30 translocations from 434 tested spermatozoa (6.91%) following X-ray treatment, but only 1 translocation in 550 X-rayed female germ cells (0.18%). This ratio, of about 35 times as many translocations in the male germ cells as in the female, they found to be about the same as the ratio of translocations induced in mature spermatozoa to those induced in immature male germ cells. OLIVER (1935), on the other hand, reported that one-third to one-half as many translocations involving the X chromosome were induced in treated female germ cells as in similarly treated male germ cells. However, in his abstract no figures were given, and the full data have never been published. If these chromosome aberrations were detected solely by means of their alteration of crossing over frequencies in the X chromosome, as in OLIVER'S other studies, it is not clear what proportion of them may have been deficiencies or inversions rather than translocations.

Other workers (HANSON and HEYS 1929; HARRIS 1929; TIMOFÉEFF-RESSOVSKY 1930) reported that a given dose of X-rays produces more mutations in mature spermatozoa than in immature male germ cells. Although it was recognized that this might mean that the latter are much less susceptible to X-rays than those which are mature, TIMOFÉEFF-RESSOVSKY (1934) expressed his opinion that germinal selection was a better explanation. Evidence in support of this was seen in SIDOROFF'S finding (1931) that the frequency of autosomal recessive lethals does not diminish in successive broods of the progeny of treated males, although translocations, according to SHAPIRO (1931), do so. The former would not be subject to germinal selection because of their recessiveness, whereas most translocations might be supposed to be eliminated by aneuploid segregation during meiosis.

In Drosophila, fertilization takes place when the oocytes have reached the first meiotic metaphase (see SONNENBLICK 1950). Oogonia and oocytes, like spermatogonia, are immature, i.e., pre-meiotic, germ cells, and would therefore be expected to yield lower frequencies of mutation than spermatozoa. Even in mature oocytes, any

induced aberrations must still pass through meiotic segregation; in other words, they are conceivably subject to germinal selection by means of the elimination of aneuploid combinations. The emphasis on germinal selection as the explanation for the lower frequency of sex-linked lethal mutations and chromosome aberrations in the immature male and all the female germ cells tended in subsequent years to obscure the value of the alternative theory proposed by PATTERSON and MULLER. Thus SCHULTZ (1936), after reviewing the subject, concluded: "On the whole, it appears that the existing data can best be understood in terms of similar rates of mutation in all cells."

Later on, it was demonstrated that dosages measured by different observers under different conditions and with different instruments, or even by the same observer at different times, cannot be reliably equated. Moreover, different strains of the same species may exhibit different mutation rates when exposed to the same dose of X-rays (DUBOVSKIJ 1935; DEMEREC 1938). It is consequently important to note that the comparative method utilizes the analysis of simultaneously treated male and female germ cells of the same genetic strain. In this way variations in the measurement of dosage or of subsidiary conditions of treatment, as well as variations in the sensitivity of different genetic strains, become of little account, since the series to be compared are identical in genotype and were treated identically.

TRANSLOCATIONS

Studies along these lines were made jointly by L. J. STADLER and the author in 1936–40, in order to see whether X-rays produce translocations by simultaneous chromosome breakage and reunion, or whether, on the contrary, chromosome breakage is followed by a reunion of broken ends only after a certain interval has transpired. The method used was to irradiate impregnated females so as to expose the chromosomes in both male and female germ cells to X-rays, and then to make a genetic test of all induced translocations to see whether any of them might involve a reunion between maternal and paternal chromosomes, which of course were in separate germ cells at the time of treatment. The method of detection was the standard genetic test for translocations. Wild type males [in series 12–15, y^2 *v f* males; in series 16, *al Sp b* L^{34}*/Cy*; *Ly Dl Pr/In(3LR)Cx, D*] were mated with virgin *pr cn*; *by*; *ci* ey^R females.[2] In series 1–15 (table 1) the female was X-rayed after insemination; in series 16 (table 1) the males and females were irradiated simultaneously before mating. In all the following experiments, except where otherwise stated, the treated parents were removed from the culture bottles 5 days after the treatment. The progenies were thus composed of single 5-day broods. F_1 males were then mated with *pr cn*; *by*; *ci* ey^R females, and the progeny was examined for the non-occurrence of any of the 8 classes of males and 8 classes of females to be expected on the basis of independent assortment. Whenever a translocation was found to have occurred, both comple-

[2] y^2, yellow-2, 1–0.0; *v*, vermilion, 1–33.0; *f*, forked, 1–56.7. *al*, aristaless, 2–0.0; *Sp*, Sternopleural, 2–22.0; *b*, black, 2–48.5; *pr*, purple, 2–54.5; *cn*, cinnabar, 2–57.5; L^{34}, Lobe-34, 2–72.0; *Cy*, Curly, located in *In(2L)Cy*. *Ly*, Lyra, 3–40.5; *by*, blistery, 3–48.7; *Dl*, Delta, 3–66.2; *Pr*, Prickly, 3–90.0; *In(3LR)Cx*, *D*, inversion involving both arms of chromosome 3, used as suppressor of crossing over. *ci*, cubitus interruptus, 4–0.0++; ey^R, eyeless-Russian, 4–0.2.

TABLE 1

Translocations induced in Drosophila melanogaster spermatozoa and oocytes treated simultaneously

Series	Dose, r	No. gametes tested (per sex)	Trans. in ♂	Trans. in ♀
1	1800	50	5	0
2	1080	49	0	0
3	900	28	0	0
4	1800	28	2	0
5	2700	4	0	0
6	2000	15	2	0
7	3000	24	4	0
8	(not recorded)	38	6	0
9	(not recorded)	141	11	0
10	(not recorded)	59	5	0
11	(not recorded)	65	0	0
12	(not recorded)	12	0 + (1?)	0
13	(not recorded)	30	1 + (1?)	0
14	600	277	6	0
15	2400	95	14	0
16	1500	342	17	0
Total		1257	73 + (2?)	0

mentary types of individuals were tested to determine whether the translocation had occurred in the treated male gamete or the treated female gamete. For example, in the case of a translocation between chromosomes 2 and 3, the commonest type observed, only wild type and purple cinnabar blistery phenotypes would occur (irrespective of sex and the fourth chromosome segregants). Both (a) wild type and (b) purple cinnabar blistery males would then be tested. The wild type males were tested by backcrossing to *pr cn*; *by*; *ci* ey^R females. If the translocation had been induced in the treated male gamete, the wild type males would yield only the two previously found types of offspring; if not, they would yield both (c) purple cinnabar and (d) blistery types as well. It required a somewhat more elaborate procedure to test the purple cinnabar blistery males of the original test progeny. They were crossed to wild type females, and 7 to 10 of their sons were then crossed singly to *pr cn*; *by*; *ci* ey^R virgin females. If a translocation had been induced in the original treated female germ cell, some of these progenies should show only two classes, (a) wild type and (b) purple cinnabar blistery, instead of four classes. In 16 series given various doses (table 1), a total of 73 translocations was induced in 1257 tested progeny, representing equal numbers of treated spermatozoa and mature oocytes. All 73 were found to have been induced in the male germ cells. The experiment therefore failed to answer the question originally posed, but it brought to light the interesting fact that translocations were apparently not inducible in female germ cells.

This finding throws some doubt on the interpretation given by SIDKY (1940) of a remarkable 3,Y translocation found in the progeny of an X-rayed male mated to an untreated attached-X ($\widehat{yy}$; *bw*; *e*; *ey*) female.[3] Among various possible explanations,

[3] $\widehat{yy}$, yellow in attached-X chromosomes; *bw*, brown, 2–104.5; *e*, ebony, 3–70.7; *ey*, eyeless, 4–0.2.

SIDKY came to the conclusion that a translocation between a paternal third chromosome and a maternal Y chromosome furnished the best explanation. In the light of the great rarity of translocations in female germ cells, even when X-rayed, any involvement of the Y chromosome of the $\widehat{XX}Y$ female seems very doubtful. Instead, a translocation between the paternal, X-rayed Y and third chromosomes in a spermatozoon seems more likely. SIDKY felt that this was ruled out by the relative scarcity of non-ebony females in the progeny of the F_1 males, which would presumably in such a case have two Y chromosomes, the paternal one involved in the translocation and the maternal normal Y. He says: "But that would mean that the ring-X-chromosome would segregate freely with either of the Y chromosomes, resulting in a larger number of exceptional—non-ebony—as well as numerous ebony females." Random segregation of the ring-X and the two Y's would yield one-third non-ebony females. The actual number of non-ebony females was not given in SIDKY's paper, and from his ambiguous remark it is not clear how scarce the non-ebony females were, although they apparently did occur. The possible interpretation of a purely paternal translocation is therefore not ruled out, particularly if it can be supposed that the segregation was non-random. Alternatively, one can suppose that in oocytes the Y chromosome is more breakable by X-rays than any other chromosome; or that a break remains open longer, so as to have permitted an exchange between a broken maternal Y chromosome and a broken paternal third chromosome. This hypothesis may readily be tested by X-raying $\widehat{XX}Y$ females and mating them to treated or untreated males.

In further studies male and female Drosophila were irradiated simultaneously in 4 series at a dosage of 2000 r units (60 kvp, 15 ma., about 160 r/min.). In six of these series, a check was made for the occurrence of translocations involving the X-chromosome in treated spermatozoa, in order to have a basis of comparison for the frequency of X-to-autosome translocations in female germ cells. The breeding plan was to irradiate *f B* ♂♂ and then to cross them to *Cy/Pm*; *H/Sb-C* ♀ ♀.[4] The progenies of the F_1 Bar Curly Stubble females were then examined for failure of free recombination. The inversions present in chromosomes 2 and 3 (*Cy* and *Sb-C*) effectively prevented crossing over in those chromosomes, and the customary reduction of crossing over in translocation chromosomes was relied upon to prevent much recombination between the locus of *B* and any X chromosome break. The method, although not perfect, is seemingly quite efficient, for no borderline cases were found that upon retesting failed to prove to be translocations.

Table 2 gives the results from these experiments, together with the foregoing, and reveals that the frequency of translocations induced in spermatozoa by doses of X-rays averaging about 2000 r is as much as 150 times greater than that in mature oocytes. KANELLIS and RADU (1943) reported obtaining 2 translocations in 1813 oocytes given 4500 r, a frequency of 0.11 percent. This may be compared to the frequency of 0.07 percent obtained in the present series, or the frequency of 0.04 percent if all the series reported in table 2 are combined.

What are the causes of this observed difference in the frequency of induced translocations in male and female germ cells? It might be supposed, first of all, that because

[4] *B*, Bar, 1–57.0; *Pm*, Plum, 2–104.5, inseparable from *In(2LR)Pm*; *Sb-C*, Stubble, 3–58.2, accompanied by *In(3R)C*; *H*, Hairless, 3–69.5.

TABLE 2

Translocations induced by X-rays in spermatozoa and oocytes treated simultaneously

Dose	♂♂			♀♀		
	Gametes tested	Trans-locations	Percentage	Gametes tested	Trans-locations	Percentage
Various doses (600r–3000r)	1257	73	5.81	1257	0	0.00
2000r *f B*	92	3	3.26	198	0	0.0
2000r Ore-R	264	19	7.20	286	0	0.0
	337	26	7.72	392	1	0.26
	437	29	6.64	466	0	0.0
Total	1130	77	6.81	1342	1	0.07
Grand total	2387	150	6.28	2599	1	0.04

the mature oocytes treated in the females are halted in the first metaphase of meiosis, any translocations that were induced might be selectively eliminated during meiosis, or would fail to give viable offspring because of aneuploidy resulting from segregation. Chromosome or chromatid breakage and exchange of an asymmetrical type would result in a dicentric chromosome and an acentric fragment; but any such translocation would be eliminated, whether it occurred in sperm cell or oocyte. The differential probability of transmission is then restricted to symmetrical translocations. A chromosome exchange induced in a sperm cell would be transmitted through mitosis without elimination; a chromatid exchange would presumably be lost in one half of the cases, because of failure of the two chromatids involved to enter the same daughter cell at the first subsequent mitosis. As for female germ cells, it can be calculated that one fourth of the cases of chromosome breakage and reunion (occurring in mature oocytes) would be transmitted; for in Drosophila one half of the metaphase I orientations would result in aneuploid combinations, and one half of the orthoploid gametes do not carry the translocation because it is heterozygous. For chromatid exchanges the reasoning is the same, except that at the second meiotic division there is a further reduction in transmission of the translocation to one fourth. Hence the probability of transmission of a chromatid exchange is $\frac{1}{4} \times \frac{1}{4}$, or $\frac{1}{16}$. Consequently, the probability of transmission of a chromosome exchange is 4 times as great for spermatozoa as for oocytes; and for a chromatid exchange, it is 8 times as great. It therefore seems that the great reduction of translocations in the tested female germ cells must be due either (a) to a different frangibility of the chromosomes in male and female germ cells, or (b) to a different probability that broken chromosomes in male and female germ cells will reunite so as to produce translocations. It seemed possible to test this alternative. If the latter reason were the true one, and if in oocytes the low probability of reunion to yield translocations is due to distance between the chromosomes or to their relative immobility prior to the time at which the broken ends heal, then agents that would stimulate greater movement on the part of the chromosomes in the oocytes ought to produce an increase in the frequency of translocations in the female germ cells.

The influence of supplementary infrared radiation on the induction of translocations by X-rays

The first such agent to be tried was near infrared radiation (GLASS 1949), because of the fact that KAUFMANN, HOLLAENDER, and GAY (1946) had already shown that a treatment with infrared radiation of about 10,000 Å before giving a dose of X-rays greatly increases the frequency of translocations and other chromosome aberrations induced in Drosophila spermatozoa. KAUFMANN's results had been obtained from a cytological analysis of salivary gland chromosomes in the larval offspring of treated males. For our purposes it seemed desirable to stick to the method of genetic detection, so as to keep all our series of data comparable and to permit detection of the numerous translocations with breaks confined to the heterochromatic regions of the chromosomes, such translocations being very difficult, or impossible, to detect by cytological means.

The general method used successfully by KAUFMANN *et al.* (1946), and also by SWANSON (SWANSON and HOLLAENDER 1946; SWANSON 1949; SWANSON and YOST 1951) with Tradescantia, was applied. A commercial infrared lamp (250 watts) was used with a cooling system, a pair of plano-convex condensing lenses, and a glass filter. The cooling system used at the start (series 1–3) was like that used by KAUFMANN *et al.*, and consisted of a flat-sided white glass battery jar filled with a saturated solution of iodine in carbon tetrachloride, 4 cm. in thickness, and cooled by means of a U-shaped glass coil with circulating tap water. A cooling system that proved after some trial to be more convenient and entirely adequate was composed of two units: (a) a glass absorption cell 10 x 10 x 2 cm. (series 4–8) or 21.5 x 14 x 1 cm. (series 9–12) inside measure through which tap water was circulated from a low intake opening to a high outlet; and (b) a copper coil ¼ in. in diameter through which cold tap water circulated and which made 3 loops around the bottom and 3 loops around the top of the shell vial containing the flies. The vial was so placed that the focal point of the infrared beam was in the middle of the volume of air within the vial. However, a sharp focus was avoided, since it was feared that the flies, even though confined to a space 3 cm. high between two corks covered with moistened filter paper, would be able to escape from a sharply focused beam. The beam was therefore regulated to cover the space occupied by the flies ($30 \times \pi(4.5)^2$ mm.) as uniformly as possible. For this purpose the condensing lenses proved to be unnecessary, and in series 9–12 were discarded. A large electric fan was also used to blow cool air over the infrared lamp and the side of the cooling cell exposed directly to the lamp. The temperature inside the shell vial never rose to more than 28°C. during the course of any of the exposures, which lasted up to 11½ hours duration.

In the first eight series with infrared supplementary treatments, the 2 cm. thick glass cell was used as a heat filter. When it was realized that this might be absorbing a significant portion of the infrared at 10,000 Å, the thinner cell was substituted (series 9–12). In this final arrangement the cooling cell, 8 cm. from the infrared lamp, was mounted over an aperture in one side of a wooden box 16 cm. deep. The glass infrared filter, described below, was mounted over a smaller aperture, 6 x 6 cm., on the opposite side of the box, and the cooled vial containing the flies was placed 3 cm. from the filter. Furthermore, when SWANSON's results with Tradescantia indi-

cated that elevated, or even normal, temperatures may nullify the effect of infrared radiation (SWANSON and YOST 1951), the infrared treatments were administered at low ambient temperatures (9°C. for series 10 and 11; room temperature of 18°C. for series 12).

The infrared filter used was a Corning filter 2550, color specification 7–57 (Sextant Red), 3⅛ in. square and 2 mm. thick. This filter is very similar to the one used by KAUFMANN *et al.*, but cuts off more sharply at 27,500 Å and transmits more energy between 7500 and 10,000 Å. It transmits 7% of the radiation at 7500 Å and more than 73.2% at 10,000 Å. One cm. of water cuts off one third of the infrared at 10,000, about two thirds at 12,500, and all at 4,000 Å. The importance of a very thin water filter is therefore obvious. The use of water as a filter in combination with the Corning filter 2550 limited transmission of the radiation chiefly to the wave-lengths between 8000 Å and 13,000 Å, with a maximum at 10,000 Å.

The interval between completion of an infrared pretreatment and commencement of the X-ray treatment was limited to the time required to convey the flies from the place of treatment to the X-ray machine. In no instance was the interval more than 2 minutes, and often it was a matter of seconds. The same applies for the interval between completion of an X-ray treatment and commencement of a supplementary infrared posttreatment.

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Table 3 summarizes the results of the infrared series, when the infrared was given as a supplementary treatment either before or after the treatment with X-rays. No statistically significant difference between the series treated with infrared and the controls treated with X-rays alone was found, although these experiments were repeated twelve times in the course of four years. There may be a slight effect of the infrared posttreatment, contrary to the findings of KAUFMANN and WILSON (1949); for the frequency of translocations induced in spermatozoa by 2000 r units of X-rays averaged just one percent higher with an infrared posttreatment than without; but χ^2 is only 1.33 for two degrees of freedom, and P = .20–.30. That there is some effect of an infrared posttreatment is substantiated by the results for the treatment of the oocytes. Six of the seven translocations so far induced in female germ cells have come from the series given an infrared posttreatment. Although such a result might also be obtained by chance alone, the concurrence of the results in the males and females does indicate a positive, though slight, effect of the infrared radiation in increasing the frequency of translocations.

The reason for the marked conflict between these results and those reported by KAUFMANN *et al.* (1946, 1949) must be considered. Every precaution was taken in the present studies to ensure that the flies received an adequate dose of infrared radiation. In Series No. 6 the time of infrared treatment was extended to 11½ hours, although KAUFMANN had reported that a maximum effect was obtained after no more than two hours. Exposure of the flies to infrared was given with the same apparatus and set-up that was used on the same day for treating Tradescantia buds with the usual

TABLE 3

The effect of supplementary infrared radiation on the induction of translocations by X-rays

Series	Dose	X-rays only			Infrared pretreatment			Infrared posttreatment		
		N.	T.	%	N.	T.	%	N.	T.	%
A. Spermatozoa										
1, 5	2574r	114	5	4.4				114	7	6.14
2, 5	2480r	232	15	6.5				232	21	9.05
3	2000r	92	3	3.26	95	7	7.38			
4	2009r	260	11	4.26	268	11	4.1	237	19	8.02
6	1990r	156	10	6.4				125	4	3.2
7	1991r	166	10	6.03				156	9	5.77
8	2000r	199	9	4.5				237	21	8.86
9	1950r	264	19	7.2				257	14	5.5
11	1978r	337	26	7.7	355	27	7.6	337	29	8.6
Total	2000r	1474	88	5.97	718	45	6.27	1349	96	7.12
Total	2500r	346	20	5.78				346	28	8.1
12	4000r	79	9	11.4				142	15	10.6
B. Oocytes										
1	2574r							169	1	
2	2480r							243	0	
3	2000r							454	0	
9	1950r	286	0					275	4	1.5
10	1978r	392	1		389	0		393	1	
Total	2000r	678	1	0.15	389	0		1122	5	0.54
	2500r							412	1	0.24
								1534	6	0.45

N, gametes tested. T, translocations found.

Duration of infrared treatment: series 1, 4 hr.; series 2–4, 6 hr.; series 6, 11½ hr.; series 7–9, 5 hr.; series 10, 3¼ hr.; series 11, 3 hr.; series 12, 3 hr. pretreatment + 3 hr. posttreatment.

positive results. After SWANSON and YOST (1951) had found that a high circumambient temperature could nullify the effects of the infrared radiation, Series Nos. 10 and 11 were run at 9°C. and Series 12 at 18°C., with the same results as before, namely, no difference when the infrared treatment was given before the X-ray dose, and about a 1% increase when the infrared was given afterwards. The divergence in results being so complete, additional work must be done to clarify the matter. It may be suggested, however, that perhaps the frequency of translocations detectable by salivary chromosome analysis is much higher than that detectable by genetic analysis. In other words, most of the aberrations detectable in the salivary gland chromosomes are perhaps of a non-viable type that would seem to be eliminated between the third instar of larval life and the formation of germ cells in the adult male. If this were so, the discrepancy in the reported effects of infrared supplementary treatments in

Drosophila might be explainable by postulating that the infrared particularly increases the frequency of non-viable chromosome aberrations, but that it does not greatly alter the frequency of viable ones.

Frequency with which particular chromosomes are involved in translocations

Table 4 gives the distribution of the translocations according to the chromosomes involved. The small fourth chromosome was involved only 8 percent of the time. Chromosomes 2 and 3 were involved almost exactly equally often, about 80 to 85 percent of the time. The X chromosome was involved rather less than half as frequently as chromosomes 2 or 3; and the Y chromosome was involved somewhat less frequently than the X, and about one third as often as either large autosome. These frequencies may be compared with those found for breakage and recombination in the salivary gland chromosomes. They agree very closely with those which may be calculated from the data of BAUER, DEMEREC, and KAUFMANN (1938, table 2) by halving the frequency of breakage per chromosome in their data, and comparing the results with the frequencies of involvement of particular chromosomes given here in table 4. (This comparison assumes that the frequencies of triple and higher multiple-break rearrangements are so low that they may safely be neglected.) In comparison to the findings of BAUER *et al.*, the present data reveal a slightly higher frequency for involvement of chromosomes 2 and 3 (+2% and +3.6%, respectively), a rather lower frequency for the X and Y chromosomes (−2.3% and −1.35%, respectively), and a higher frequency for chromosome 4 (+2.5%). The present data agree with those based on the salivary gland chromosomes in indicating that chromosome 2 is slightly more often involved than chromosome 3, and the X about 3.0% more than often the

TABLE 4

Distribution of translocations according to chromosomes involved

	Total	Y,2	X,2	Y,3	X,3	Y,4	X,4	Y,2,3	X,2,3	2,3	2,4	3,4	2,3,4
Induced in spermatozoa													
Tests for Y,2,3,4	75	9		11		0		1		46	3	2	1
Tests for Y,2,3	284	41		43				6		194			
Tests for X,2,3	59		13		5				2	39			
Induced in oocytes													
Tests for X,2,3	10		1		4					5			

Percentage involving a particular chromosome				
Y	X	2	3	4
21/75 (28.0%)		60/75 (80.0%)	61/75 (81.3%)	6/75 (8.0%)
90/284 (31.7%)		241/284 (85.0%)	243/284 (85.6%)	
	25/69 (36.2%)	60/69 (87.0%)	55/69 (79.7%)	
111/359 (30.9%)	25/69 (36.2%)	361/428 (84.4%)	359/428 (83.8%)	6/75 (8.0%)

Y chromosome. The data of BAUER (1939, table 25) are not in quite so good agreement, for he lists translocations that total 50 X-2, 55 X-3, and 102 2–3 exchanges. This result gives a ratio of involvement of 50.7%, 73.4%, and 75.8% for X, 2, and 3 respectively, in comparison to 36.2%, 87.0%, and 79.7% in the present tests (last row above totals, table 4). Not only do BAUER's data in the 1939 papers give a higher frequency of involvement of the X chromosome, but chromosome 3 also slightly outranks chromosome 2. It is perhaps worth remarking that in the present data chromosome 3 was in fact more often involved than chromosome 2 in the more extensive series of tests of Y-bearing spermatozoa, whereas chromosome 2 outranked chromosome 3 in the tests of X-bearing sperms and of oocytes. This relationship agrees with BAUER's results, but no substantiation can be found in the data of BAUER, DEMEREC, and KAUFMANN. Probably none of these minor differences are therefore real.

Frequency of translocations in gametes of different ages

In those series in which broods were tested separately (series 9–11), it was found that the frequency of translocations induced by X-rays in spermatozoa is lower in the first four-day brood and the next three-day brood than in the final four-day brood (table 5). These data show that for X-rays, just as for nitrogen mustard (KAUFMANN, GAY, and ROTHBERG 1949), there is an increase in the frequency of induced translocations in spermatozoa utilized from the 8th or 9th day after treatment through the 11th or 12th day. AUERBACH (1951) has attributed this rise to the maturation of germ cells that were immature at the time of treatment, for increasing the duration of storage of mature, treated spermatozoa does not result in any increase in the frequency of mutations. On the other hand, these data do not agree with those of CATSCH and RADU (1943), who found that there was at first a very high rate in the most mature spermatozoa, namely, those used on the first day after treatment; next, a drop to a level at about 50 percent of the initial frequency, the new level being maintained through the 13th day; and finally, a marked reduction to very low levels. If the present data are valid, it follows that translocations, like dominant lethals, sex-linked recessive lethals, and autosomal recessive lethals (AUERBACH 1951; BONNIER and LÜNING 1950; LÜNING 1952 a, b), show an increase in sensitivity in male germ cells beginning about six or seven days following treatment either with X-rays or with one of the mustards, although only in the case of translocations has this been demonstrated for both the ionizing radiation (present experiments) and the chemical mutagen (KAUFMANN, GAY, and ROTHBERG 1949).

As for the female germ cells, it is noteworthy that all translocations obtained to

TABLE 5

Frequency of translocations in male and female germ cells used at various intervals after treatment

	♂♂			♀♀		
	Gametes tested	Translocations	Percentage	Gametes tested	Translocations	Percentage
Brood A (days 1–4)	540	34	6.3	773	6	0.78
Brood B (days 5–7)	529	28	5.3	777	0	0.0
Brood C (days 8–11)	481	53	11.0	184	0	0.0

date were produced in the most mature oocytes. No data of this sort seem to have been previously reported for translocations in Drosophila, although a reduction in the frequency of mutations induced in oogonia (data from eggs laid more than seven days after treatment) as contrasted with the frequency in oocytes (data from eggs laid within the first seven days following treatment) has been reported in abstracts by MULLER, VALENCIA, and VALENCIA (1950) for sex-linked visible mutants, and by GALL (1950) for sex-linked lethals. GLASS (unpub.) has shown that a similar difference applies also to dominant Minutes, other autosomal dominants, autosomal recessives and deficiencies, and sex-linked semilethals, and has confirmed the lower rate for the sex-linked lethals and visibles. These results are entirely consistent with the reported relations in Sciara and Habrobracon. In the former (cf. BOZEMAN and METZ 1949), sensitivity, as measured by the frequency of chromosome aberrations detected in the salivary gland nuclei, is zero during the long germinal vesicle stage before the prophase of the first meiotic division begins. It then rises through prophase to metaphase, and reaches a peak in mid-anaphase. Meiosis is halted at late anaphase pending fertilization. In Habrobracon, WHITING (1945) found that metaphase I was far more sensitive to X-rays than was prophase I, as gauged by egg hatchability and apparently also by chromosome fragmentation.

INVERSIONS

The data accumulated in regard to the relative frequencies of translocations in female germ cells made it of interest to test also the relative frequencies of inversions. Like the commonest class of translocations, most inversions involve two distinct breaks, but with the difference that in the inversions the two breaks fall within the limits of the same chromosome ("intrachromosomal exchanges"), whereas in translocations they are in separate chromosomes ("interchromosomal exchanges"). It is well established, for a variety of plant and animal species, that "the proportion of exchanges in which both breaks are in one chromosome, rather than in separate chromosomes, is higher than would be expected on the basis of random union between breakage ends" (CATCHESIDE 1948, p. 284). CATCHESIDE himself (1938), HELFER (1941), and KOLLER and AHMED (1942) have demonstrated this to be true for Drosophila spermatozoa. It thus becomes of interest to determine whether the same ratio between translocations and inversions, found under given conditions in the spermatozoa, also holds for oocytes under those same conditions. Said differently, is the ratio between the frequencies of translocations induced in oocytes and spermatozoa treated with the same dose of X-rays the same as the ratio between the frequencies of inversions in male and female germ cells treated simultaneously? The answer might be expected to throw light on the question whether the differences in response to ionizing radiation on the part of the oocytes and spermatozoa are due to differences in the frequency of chromosome breakage, or rather to differences in the probability of recombination between breaks. The latter in turn might be either a result of differences in the rate or probability of "healing," or else of differences in the amount of chromosome movement between the times of breakage and recombination.

Unfortunately, inversions require far more labor to detect genetically than do translocations; and, for the reasons already stated, a comparison with cytological analyses of salivary gland chromosomes scarcely appears to be valid. Four series of

TABLE 6

Comparative frequencies of inversions induced in X-rayed spermatozoa and oocytes of D. melanogaster

Dose	♂♂			♀♀		
	Gametes tested	Inversions	Percentage	Gametes tested	Inversions	Percentage
600 r						
Series 1*	254	1	0.4	254	0	0.0
2400 r						
Series 2*	91	2	2.2	91	1	1.1
1500 r						
Series 3a†	185	2	1.1	185	0	0.0
Series 3b	116	1	0.9 (1.0)	116	1	0.9 (0.3)
2000 r						
Series 4‡	138	11	8.0	166	3	1.8
Total	784	17	2.2	812	5	0.6

* In Series 1 and 2, y^2 *v f* ♂♂ were mated to + ♀♀, and the inseminated ♀♀ were then irradiated and their progeny examined for crossing over within the two marked regions of the X chromosome totalling 56.7 units in length.

† In Series 3, *al Sp b* L^{34}*/Cy*; *Ly Dl Pr/In(3LR)Cx*, *D* ♂♂ were mated to *pr cn*; *by*; *ci* ey^R ♀♀, and the inseminated ♀♀ were then X-rayed. F_1 ♀♀ that were Sternopleural and Lobe in phenotype were back-crossed to *pr cn*; *by*; *ci* ey^R ♂♂ in Series 3a, for a test of crossing over within the three marked regions of chromosome 2 totalling 50.0 units in length. Similarly, F_1 ♀♀ that were Curly Lyra Delta Prickly in phenotype were crossed to + ♂♂ in Series 3b, for a test of crossing over within the two marked regions of chromosome 3 totalling 49.5 units in length.

‡ In Series 4, Oregon-R wild-type ♂♂ or ♀♀ were X-rayed and mated respectively to *al b c* sp^2 ♀♀ or ♂♂. The F_1 ♀♀ were test-crossed to *al b c* sp^2 ♂♂ for a test of crossing over within the three marked regions of chromosome 2 totalling 107.0 units.

genetic tests for inversions induced by X-rays have been made, and while the data remain scanty, they indicate the probable correctness of the foregoing reasoning. In all series the progeny was limited to the first 5 days after treatment of the parents. Table 6 presents the results. The experiments are not directly comparable. In the first two series inversions were looked for in the X chromosome; in series 3 in both chromosomes 2 and 3; but in the fourth series, for which I am indebted to MR. WILLIAM RAPPAPORT, only in chromosome 2. More important, different lengths of the chromosomes were covered in the several tests, so that only in the fourth series, where the entire chromosome 2 was checked, could all the inversions induced in that chromosome be detected. Exact comparisons between frequencies in spermatozoa and oocytes must therefore be confined to individual series, but this is not important to the conclusions, since all the tests show that inversions are about three to four times as commonly induced in spermatozoa as in mature oocytes. The ratio of inversions induced in the female germ cells to those induced in the male is therefore considerably larger and consequently nearer equality than the ratio for induced translocations.

DISCUSSION

The experiments reported above reveal that in oocytes of Drosophila there is a relatively higher frequency of inversions than of translocations, the frequency of

each type of aberration in the spermatozoa being used as the respective basis of comparison. In other words, the ratio inv. ♀♀/inv. ♂♂ greatly exceeds the ratio trans. ♀♀/trans. ♂♂. The simplest explanation of this relation is that the non-random factor responsible in spermatozoa for the excessive yield of paracentric inversions, in comparison to pericentric inversions and translocations (see CATCHESIDE 1948, p. 284), is considerably strengthened in the oocytes. This factor is supposed to be the relation between the proximity of breaks to one another and the probability that the broken ends will join. It might even be suspected from the present data that the frequency of chromosome breaks induced by ionizing radiation is no lower in oocytes than in spermatozoa; but that in the oocytes most breaks undergo restitution and thus escape detection. This hypothesis is readily testable, by comparing the frequencies in oocytes and spermatozoa of very small deficiencies. These are known to increase linearly with the radiation dose, and are thus in most cases the effects of single "hits". In such cases the two breaks produced are so very close together that the proximity factor should be virtually the same in the mature male and female germ cells. In the next paper of this series the results of such experiments will be presented.

General discussion of the relation of chromosome breakage and rearrangement to cellular conditions, and in particular to the state of the chromosomes at the time of irradiation, will be deferred until the data on the induction of small deficiencies and recessive lethals have been presented. It is sufficient at present to point out that the Drosophila data reported here agree very well with the findings of BOZEMAN and METZ (1949) on Sciara and those of A. R. WHITING (1945) on Habrobracon. In the oocytes of all three of these insects, sensitivity is apparently minimal until the meiotic prophase begins. The sensitivity then rises rapidly until it reaches a maximum in metaphase (where meiosis halts in Habrobracon and Drosophila) or anaphase (where meiosis halts in Sciara). In Drosophila, the treatment of particular stages of the meiotic prophase and metaphase in oogenesis cannot at present be identified; but there is the same general shift from insensitivity to sensitivity that probably corresponds to the progression from preprophase to metaphase.

On the other hand, even in metaphase the frequency of translocations induced in female germ cells in both Sciara and Drosophila is practically zero, although in Drosophila infrared radiation appears to magnify somewhat the probability of obtaining interchromosomal rearrangements after X-ray treatment. This finding, like the difference between the ♀/♂ inversion-translocation ratios, points to the probability that it is not so much a difference in frequency of chromosome breakage that distinguishes oocytes from spermatozoa as it is a difference in the probability of obtaining a recombination rather than a restitution of breaks.

SUMMARY

1. In series in which spermatozoa and oocytes were irradiated with identical doses of X-rays, mainly 2000 r units, only 1 translocation in 2599 oocytes was obtained, in comparison to 150 translocations in 2387 spermatozoa.

2. With supplementary near infrared radiation, no statistically significant increase in the frequency of genetically detected translocations induced by X-rays in spermatozoa was found, either when the infrared radiation was given before the X-ray

treatment or when it was given afterwards. In oocytes, 6 translocations were found in 1534 oocytes following a supplementary infrared posttreatment, and only 1 translocation in 678 oocytes following X-ray treatment alone. The difference, although not statistically significant, is suggestive.

3. Chromosomes 2 and 3 are involved in translocations equally often; the X chromosome, less than half as frequently as either major autosome; the Y chromosome, somewhat less frequently than the X; and chromosome 4, about one tenth as often as the major autosomes.

4. In spermatozoa, the frequency of translocations produced in the progeny derived from spermatozoa utilized for fertilization 8 to 11 days after treatment was found to be almost twice as great as the frequency in either the first brood (days 1–4) or the second brood (days 5–7). All the translocations found in treated oocytes were in the first brood, i.e., where induced in the most mature oocytes.

5. Inversions (detected genetically) are induced by X-rays about 3 to 4 times as frequently in spermatozoa as in oocytes. The ratio inv. ♀/inv. ♂ is 40 to 50 times as great as the ratio trans. ♀/trans. ♂. This suggests that the frequency of chromosome breakage in oocytes is little different from that in spermatozoa, and that the difference in the frequency of rearrangements results from a difference in the probability of obtaining recombinations in accordance with the proximity of the breaks.

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