SOME COMPONENTS OF X RAY-INDUCED CROSSING OVER IN FEMALES OF *DROSOPHILA MELANOGASTER*¹

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WHEN flies of the species *Drosophila melanogaster*, heterozygous for a marked chromosome, are X-irradiated and the progeny examined for crossovers, a nonrandom distribution of recombinants is observed. The proximal regions of chromosomes show the greatest response to irradiation, while in distal regions, X ray-induced exchanges are either rare (in males) or in the case of females, where the effects of X rays must be measured against the background of normal recombination, chromosomes irradiated in oögonia show less crossing over than the controls (WHITTINGHILL 1955). This peculiar pattern of response to X-irradiation has been succinctly described as a "proximal increase and compensatory distal decrease" (KIKKAWA 1934). The major structural difference between the chromosome regions is the presence of heterochromatin proximally.

In the experiments reported here, several problems relating to the pattern of X ray-induced exchanges along the X chromosome of *Drosophila* were explored:

1. The proximal region was divided into heterochromatic and euchromatic segments by means of suitable markers. Progeny testing of crossovers revealed frequent involvement of the short arm of the X in heterochromatic anisobrachial exchanges.

2. When 90% of the heterochromatin is moved distally by the *sc*⁸ inversion, a distal increase and a proximal decrease are observed instead of the pattern described above. Furthermore, X ray-induced heterochromatic exchanges occur with greater frequency than when the heterochromatin is in its normal position.

3. Experiments with the mutant *c(3)G* (which suppresses normal crossing over in the female) suggest that crossovers induced in heterochromatin are due to typical breakage and rejoining. In euchromatin, however, the distal decrease and most of the proximal increase appear to be due to radiation effects that persist through at least one cell division and alter the pattern of crossing over in oöcytes.

MATERIALS AND METHODS

Exchange was studied in two X chromosomes: one had the wild type sequence, and the other was an inversion of almost the entire X. In order to have adequate markers in both proximal and distal regions and to measure induced exchanges in oögonia as well as in oöcytes, it was necessary to run several series of crosses using brood fractionation.

For the X chromosome in normal sequence, recombination was measured distally by using

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females heterozygous for the markers $\gamma u^a spl rb$, but only recombination between the two outside markers is entered in Table 2; other regions were studied in $\gamma \cdot Dp(1;1)sc^{v1} \gamma^+ / \gamma cv v f car$ ♀♀ (the duplication $Dp(1;1)sc^{v1} \gamma^+$ is on the short right arm of the X chromosome). A description of these mutants and chromosomes can be found in LINDSLEY and GRELL (1968). The females carried either normal autosomes, second chromosome inversions $In(2L+2R) Cy$, (Cy = curly wings), or both Cy and third chromosome inversions, $In(3L+3R) CxD$ (D = bristles removed, wings held out). Females that had eclosed within the preceding 3 hr were divided into two groups. The experimental groups were exposed to 4000r of 250 kvp X rays over a period of 5–6 min, while the controls were not irradiated. All three genotypes were irradiated simultaneously, and 10–15 females were placed in a quarter-pint milk bottle containing twice as many appropriately marked males and *Drosophila* medium. The six groups of flies, three experimental and three control, were transferred daily to new bottles for 12 days. A third series used a recessive gene on chromosome 3, $c(3)G$ (GOWEN 1933), to suppress meiotic recombination. Females of the genotype $\gamma \cdot Dp(1;1)sc^{v1} \gamma^+ / \gamma cv v f car; st c(3)G/st c(3)G$ were divided into experimental (4000r) and control groups and (owing to lowered fertility) transferred only twice over a 13 day period, which resulted in one 5-day and two 4-day brood fractions.

Recombination was also studied in chromosomes homozygous for $In(1)sc^8$, a long paracentric inversion with one break at the tip (1B2) and the other in heterochromatin close to the centromere (20 B+). This inversion moves approximately 90% of the proximal heterochromatin to the tip of the X chromosome. One series of crosses used females of the genotype $In(1)sc^8, sc^8 f v cv / In(1)sc^8 sc^8 f u^a$ to measure recombination in proximal regions; no autosomal inversions were used in this series. The procedures outlined in the preceding paragraph, including crosses with heterologous inversions and a cross with homozygous $c(3)G$ present, were also used for females of the genotype $In(1)sc^8 sc^8 \gamma^{s14} / In(1)sc^8, sc^8 car f v$. These markers made it possible to study recombination in distal regions of the homozygous inversion.

The final series of crosses used markers that divided the $car-\gamma^+$ region of the normal X into heterochromatic ($su-f-\gamma^+$), and predominantly euchromatic ($car-su-f$) segments (COOPER 1959). Females of the genotype $\gamma f car su-f / \gamma f \cdot Dp sc^{v1} \gamma^+$ were exposed to 4000r of 250 kvp X rays less than 12 hrs after eclosion, mass mated to $\gamma f car su-f / Y$ males, and transferred to new bottles daily for 12 days.

The offspring bearing X chromosomes that had resulted from heterochromatic exchanges were progeny tested by crossing to a stock bearing no free Y chromosomes. Individual crossover males were crossed to (attached-X) $C(1)RM \gamma v bb/0$ ♀♀, and crossover females were crossed to males bearing the attached-XY chromosome ($XY^S \cdot Y^L, \gamma^2 su-u^a u^a Y^S \cdot Y^L \gamma^+ / 0$ ♂♂). The purpose of these crosses was to reveal any lethal heterochromatic deficiencies which had been viable in heterozygous females or in X/Y males; the male progeny of both of these crosses are X/0, and the presence of a recessive lethal heterochromatic deficiency would then be revealed by absence of the class of males bearing the crossover chromosome. The results of this final series of crosses will be considered first.

RESULTS AND DISCUSSION

Anisobrachial exchanges: The very low frequency of "spontaneous" recombination in X heterochromatin (ROBERTS 1965 (Table 1)) affords two advantages: (1) it ensures that virtually all of the heterochromatic recombinants recovered in the experimental series are X ray-induced, and (2) it makes it possible to progeny test recombinant females with the assurance that subsequent crossing over will not prevent recognition of the recombinant chromosomes in the next generation.

Equal numbers of males and females of each phenotype were expected, but an excess of carnation males was recovered from days 1–7 (Table 1). Progeny testing revealed that a high proportion of these males, at least in the early brood fractions, was sterile and on microscopic examination had no motile sperm. Such males

TABLE 1
X ray-induced eu- and heterochromatic exchanges recovered from various brood fractions of *D. melanogaster* females

	Days after exposure to 4000r X rays							
	1-2	3-4	5	6	7	8	9-10	11-12
Control								
Number of progeny scored	1402	1895	755	664	815	751	1259	1486
Euchromatin: percent exchange, $\overline{\text{car-su-f}}$	$5.1 \pm 1.1^*$	4.5 ± 0.9	3.7 ± 1.3	4.3 ± 1.5	3.2 ± 1.2	3.2 ± 1.2	4.2 ± 1.1	4.8 ± 1.1
Heterochromatin: percent exchange, $\overline{\text{su-f-y}}$	0.7	0	0	0	0	0	0	0
Irradiated								
Number of progeny scored	978	1957	1398	1329	1553	1618	3121	3251
Euchromatin: percent exchange, $\overline{\text{car-su-f}}$	4.8 ± 1.3	3.8 ± 0.8	3.2 ± 0.9	4.5 ± 1.1	5.6 ± 1.1	8.4 ± 1.3	10.8 ± 1.1	9.7 ± 1.0
Heterochromatin: percent exchange, $\overline{\text{su-f-y}}$	5.7 ± 1.4	3.2 ± 0.8	5.4 ± 1.3	4.6 ± 1.1	4.7 ± 1.1	2.8 ± 0.8	2.7 ± 0.6	2.7 ± 0.5
Single crossover types	26	29	26	17	22	5	7	4
	4	4	6	9	12	8	17	10
	30	33	32	26	34	13	24	14
	7	9	11	9	6	5	15	14
	9	5	13	11	16	12	23	26
	5	8	12	9	11	11	20	23
	2	5	6	5	5	4	3	6
	7	13	18	14	16	15	23	29
Double crossovers	3	3	1	1	1	1	1	4
Number and percent heterochromatic exchanges that are Y-suppressed lethals (includes above sample plus additional flies tested)								
	1/111=0.9	1/150=0.7	1/100=1.0	1/92=1.1	1/97=1.0	3/63=4.5	2/100=2.0	1/111=0.9

* Indicates 95% conf. limits.

FIGURE 1.—Possible types of exchanges involving the long and short arms of the X chromosome of *D. melanogaster*. The size of the short arm (and of the duplication) in the top diagram is exaggerated; the diagram just below the top one gives the proportions of the long and short arms observed in mitotic cells (COOPER 1959). L = long arm; S = short arm; NO = nucleolus organizer; XR = right (short) arm of X.

had received only a small fragment of the X chromosome bearing γ^+ , designated *Dp(1;f) γ^+* , from their mothers and an X instead of a Y from their fathers.

The reciprocal product of the above exchange was recovered among the γf females. Progeny testing revealed two genotypes: rod X and attached-X *C(1)RM γf* . The percentage of attached-X females recovered from the various brood fractions remained relatively constant.

Figure 1 shows the various types of exchanges that could generate the recombinants listed in Table 1 and described above. Ova bearing attached-*X*'s and *Dp(1;f)γ⁺*'s should come from $\frac{1}{2}$ *L*'*S* + $\frac{1}{2}$ *L*'*S*' + $\frac{1}{4}$ *L*'*S*' exchanges, and orthodox recombinant ova from $\frac{1}{2}$ *LL*' + $\frac{1}{2}$ *SS*'. Attached-*X*-bearing ova are recoverable only after fertilization by a *Y*-bearing sperm, and *Dp(1;f)γ⁺*-bearing ova only after fertilization by an *X*-bearing sperm. Orthodox recombinants, however, are recoverable following fertilization by either sperm type. Consequently, the efficiency of recovery of isobrachial recombinants is twice that for anisobrachial recombinants. Therefore, for the first 7 days (Table 1), 143 anisobrachial recombinants and 178 isobrachial recombinants actually represent 286 anisobrachial and 178 isobrachial crossover events, respectively. It would appear, from these data, that events giving rise to attachments are more frequent than those giving rise to orthodox heterochromatic recombinants. Attachment events of necessity involve the short arm. Therefore, it would seem that the probability per unit length of involvement in exchange is greater for the short arm than for the long.

Of 824 progeny-tested flies bearing chromosomes from heterochromatic exchanges (Table 1), only 11 (1.3%) carried *Y*-suppressed lethals (1.9% of 579 fertile progeny). There was no significant difference in the percentage of recovered deficiencies from the various brood fractions tested. Why, then, are there so few lethals among the X ray-induced heterochromatic exchanges?

If X rays produce breaks throughout *X* heterochromatin and these breaks rejoin at random, a large proportion of the X ray-induced crossovers would be expected to be lethal in the *X*/0 condition, owing to the presence of heterochromatic deficiencies. LINDSLEY, EDINGTON and VON HALLE (1960) reported that all of the heterochromatic lethals recovered at that time involved one locus, *bb*. It now seems likely that *bb* mutants are deficient for part of the nucleolus organizer region, a highly duplicated stretch of DNA coding for ribosomal RNA (RITOSSA, ATWOOD and SPIEGELMAN 1966) that is located in approximately the middle of the *X* heterochromatin (COOPER 1959) (Figure 1).

The above considerations, then, can account for the low frequency of *Y*-suppressed lethals found among heterochromatic crossovers. One possibility is that the presence of tandem duplications for ribosomal DNA minimizes the effects of "pseudocrossovers," produced by exchanges between nearby but not exactly corresponding loci (HERSKOWITZ, SCHALET and DEL VAL REUTER 1962). It is also possible that the heterochromatin of the long arm is involved in exchanges with the short arm at a distance from the centromere similar in length to the short arm and with the same high probability of exchange per unit length as the short arm. This could explain the infrequent involvement of *bb*. Such a situation might arise, for example, if the regions responsible for chromosomal movement, localized by LINDSLEY and NOVITSKI (1958) to *X* heterochromatin, comprise genes duplicated to the same extent on both sides of the centromere.

The most conspicuous feature of the curves in Figure 2 is that both the drop in recovered heterochromatic exchanges and the rise in euchromatic exchanges coincide with the transition of the cell stages sampled from oocytes to oogonia. Evidence that primitive oogonia (i.e., stem cells) (KOCH, SMITH and KING 1967)

FIGURE 2.—X ray-induced exchanges in two proximal regions of the X chromosome. $\gamma f car su-f/\gamma f \cdot Dp(sc^{v1} \gamma^+)$ females were exposed to 4000r at day 0.

Heterochromatin: Control = 0

X-irradiated { $\blacksquare$ = anisobrachial exchange
 $\square$ = isobrachial exchange

Euchromatin: $\circ$ = control

Euchromatin: $\bullet$ = X-irradiated

Bars indicate 95% conf. limits. (Data taken from Table 1.)

that will give rise to more than one oocyte) are being sampled at day 10 comes from the observation that stem cells have a characteristically low mutation rate which is not quite reached before about the 10th day after irradiation (MULLER, OSTER and ZIMMERING 1963), and also from the finding that the mutation fre-

quency was as low for eggs laid 10–15 days after acute X-irradiation as for eggs laid later than 15 days after irradiation (MEYER and MULLER 1961). Furthermore, GRELL and CHANDLEY (1965) have shown that 8–9 days elapse before eggs treated as the earliest oocytes (presumably at or near the time of crossing over) are laid.

The curves shown in Figure 2 are similar to those of HERSKOWITZ *et al.* (1962) who also studied X ray-induced recombination in proximal regions of the X chromosome. However, additional information has been provided by more extensive progeny testing of crossovers in the present experiments. For example, progeny testing has revealed that attached-X females are recovered not only from the first 5 days of brood fractionation, as reported by HERSKOWITZ *et al.* (1962), but also from early oocytes and oogonia.

Because stem cells yielded attached-X chromosomes, it was anticipated that attached-X's would be recovered beyond day 16; this was confirmed by tests of a sample from day 18. The recovery of attached-X's and the free duplication throughout the germ cell stages and also the extremely high rate of recovery of the duplication from oocytes (days 1–7) make it more likely that the duplication is the product of an LS exchange rather than of a deletion of the majority of the X heterochromatin as proposed by HERSKOWITZ *et al.* (1962). The excess of *Dp(1;f)γ⁺*-bearing oocytes over attached-X's recovered through the first 7 days after irradiation can best be explained by the finding of NOVITSKI (1951) that, when two structurally dissimilar chromatids compete for inclusion in the functional egg nucleus at the second meiotic division, the smaller one tends to be preferentially recovered. In this case, the small *γ⁺*-bearing fragment is included in a functional egg nucleus more often than its large reciprocal product, the attached-X chromosome. The heteromorphic dyads, and, consequently, the difference in rate of recovery of the duplication and attached-X chromosomes, should disappear when one or more mitotic divisions intervenes between the LS exchange and meiosis. The precipitous drop in recovery of *Dp(1;f)γ⁺* chromosomes as progressively more cells, irradiated as oogonia, are recovered bears out this expectation. From day 8 on, the frequency of recovery of attached-X's and duplications is approximately equal (Table 1).

Gonial selection can be invoked to account for the excess of *γ f* crossovers over the reciprocal (phenotypically *car*) product. Since this excess is found in the products of isobrachial exchanges, it would seem that cells bearing the recombinant X marked with *γ f* are more likely to form oocytes than are cells with the reciprocal product, *γ f car su-f γ⁺*.

Induced exchanges in distal heterochromatin: It is clear, from Table 1 and Figure 2, that there is no increase in euchromatic crossovers (*car-su-f*) in X-irradiated females until days 7–8, when early oocytes and possibly the first oogonia begin to be recovered. The heterochromatic region (*su-f-γ⁺*), however, shows a pronounced increase in exchanges in X-irradiated females in all germ cell stages. Induced crossing over is compared in the noninverted X (Table 2, *car-γ⁺*), in the *sc⁸* inversion homozygote, in which 90% of the heterochromatin is moved to the tip of the X (Table 3, *γ^{31d}-car*), and in each of these chromosomes

TABLE 2

Recombination in females bearing X chromosomes in normal sequence. C = control; X = 4000r of X-irradiation at day 1; 95% conf. limits also given

Region	Autosomal inversions present	Days after irradiation																	
		1-2			3-4			5-6			7-8			9-10			11-12		
		C	X	C	X	C	X	C	X	C	X	C	X	C	X	C	X		
(Number of flies scored)																			
<u>Y-rb</u>	None	4.2±.47	4.7±.66	5.0±.45	6.0±.63	4.7±.51	4.9±.51	5.9±.58	5.1±.54	6.0±.63	5.1±.54	5.8±.68	3.1±.85						
	Cy	7.1±.56	7.4±.81	8.2±.63	6.4±.69	8.0±.70	8.9±.78	8.9±.78	9.9±.84	7.8±.78	9.0±.82	11.6±1.9	6.2±1.4						
	Cy;CxD	11.8±.98	10.7±1.2	13.9±.91	13.4±1.3	12.5±.88	15.9±1.0	13.6±.93	10.8±.94	13.6±.95	8.7±.77	16.6±1.1	8.9±.83						
(Number of flies scored)																			
<u>ev-y</u>	None	1688	1010	2251	1406	1723	1689	1636	1578	1405	1556	1172	1604						
	Cy	2076	974	1913	1239	1458	1337	1333	1262	1175	1202	1083	1152						
	Cy;CxD	1083	728	1420	612	1337	1250	1267	1086	1275	1324	1052	1194						
<u>v-f</u>	None	19.4±.95	16.8±1.0	16.5±.62	20.8±.99	19.5±.69	17.9±.58	20.6±.79	14.9±.56	22.0±.94	14.4±.63	20.5±.90	15.8±.69						
	Cy	21.0±.97	21.1±1.1	20.7±.67	21.0±.98	21.7±.72	19.3±.60	21.0±.80	20.0±.63	23.5±.98	21.2±.73	22.8±.94	20.2±.76						
	Cy;CxD	25.5±1.0	22.8±1.3	25.2±1.0	22.8±1.3	25.4±.87	22.2±.76	22.5±.91	24.0±.92	23.6±.96	24.2±.80	25.0±.88	26.2±.79						
<u>f-car</u>	None	31.7±1.6	32.9±2.2	32.1±1.4	34.0±2.6	33.4±1.4	29.0±1.5	32.5±1.3	28.4±1.3	31.8±1.4	26.3±1.2	34.8±1.3	29.3±1.7						
	Cy	7.8±.65	6.6±.72	6.2±.39	6.3±.51	6.0±.41	5.4±.33	5.5±.88	6.5±.39	7.3±.58	7.5±.47	4.5±.46	7.1±.49						
	Cy;CxD	9.5±.66	8.6±.86	8.8±.66	8.6±.66	9.5±.58	8.0±.50	10.2±.64	8.4±.61	9.1±.65	7.9±.51	8.1±.56	9.4±.51						
<u>car-y⁺</u>	None	14.1±1.2	17.6±1.8	15.0±1.1	16.5±2.1	16.2±1.1	14.2±1.2	14.3±.97	10.6±.87	15.0±1.1	12.5±.92	13.8±.92	11.8±.66						
	Cy	4.5±.50	7.0±.70	3.9±.32	5.9±.57	3.3±.31	7.4±.40	3.8±.58	7.0±.40	4.3±.44	8.4±.50	4.4±.45	8.0±.52						
	Cy;CxD	5.8±.53	7.8±.83	5.4±.52	6.5±.76	4.8±.76	9.2±.51	4.0±.42	9.7±.64	4.6±.47	10.3±.57	5.3±.45	11.4±.56						
(Number of flies scored)																			
None	None	807	639	1760	723	1509	1357	1282	1273	1193	1317	1484	1491						
	Cy	1925	1034	1845	997	2487	2901	2163	2152	1930	2800	2440	3222						
	Cy;CxD	845	447	1096	320	1089	838	1188	1146	1057	1409	1364	2195						

TABLE 4
Recombination in the presence of homozygous c(3)G

		Days after exposure to 4000r X rays			
		Control	1-4	5-8	9-12
Normal sequence					
Total flies		6680	2149	6180	9553
Percent exchange	$v-f$	0.06	0.0	0.03	0.13
	$f-car$	0.03	0.0	0.06	0.03
	$car-\gamma^+$	0.03	$2.8 \pm .7^*$	$2.7 \pm .4$	$2.3 \pm .3$
<i>In(1)sc⁸/In(1)sc⁸</i>					
Total flies		4262	2323	5305	6833
Percent exchange	$\gamma^{31d}-car$	0.02	$3.8 \pm .8$	$4.7 \pm .6$	$5.4 \pm .5$
	$car-f$	0.0	0.0	0.04	0.09
	$f-v$	0.02	0.0	0.02	0.01

* $\pm$ 95% confidence limits.

in the presence of *c(3)G*, a recessive gene which, when homozygous, almost eliminates spontaneous meiotic recombination (Table 4). The heterochromatic exchanges given in these tables are graphed in Figure 3, where the response of heterochromatin to X-irradiation at all cell stages is evident.

FIGURE 3.—Exchanges in heterochromatin of normal and inverted X chromosomes in the presence and absence of *c(3)G* (which suppresses meiotic crossing over) following 4000r of X rays before day 1. (Data taken from Tables 2, 3, and 4.)

It should be pointed out that the *car*- γ^+ and the γ^{sid} -*car* intervals include the euchromatic *car*-*su-f* segment as well as the heterochromatin, but because there is no appreciable increase in induced euchromatic exchanges in early oocytes (days 1-4), the exchanges induced in the presence of homozygous *c(3)G* (*car*- γ^+ , days 1-4, table 4) must have occurred in the heterochromatic segment. There is a slight decrease in heterochromatic exchanges recovered from oogonia in the *su-f*- γ^+ region (Table 1; Figure 1) as well as the *car*- γ^+ region (Table 4; Figure 3). This is attributed to the decreased recovery of the small duplication owing to gonial selection, discussed above.

When 90% of the heterochromatin is moved by inversion to the tip of the X, the same X-ray exposure yields more heterochromatic exchanges in the now distal heterochromatin than occurred in the whole of the heterochromatin in the normal position. But the inverted heterochromatin does not include the regions on either side of the centromere that were so frequently involved in anisobrachial exchanges. The increased X-ray response in heterochromatin moved distally is observed in the presence (Table 3) or absence (Table 4) of spontaneous meiotic crossing over. In fact, as might be anticipated from the absence of spontaneous recombination in heterochromatin, the *c(3)G* curves in Figure 3 can be added to the control curves to give the percent heterochromatic exchanges induced by X rays in the presence of spontaneous meiotic crossing over in the normal or inverted X's. Of course, the rise in the X-irradiated normals in days 9-12 should be greater than the controls plus the X-irradiated *c(3)G* offspring because of the appearance of many crossovers in proximal euchromatin in this period. The explanation will be considered below.

When heterochromatin is moved distally, the frequency of spontaneous meiotic recombination remains very low (BAKER 1958). The increase in recovery of X ray-induced exchanges observed here may reflect a change in chromosome coiling properties so that there are more sites (to use the concept developed by ATWOOD and WOLFF (ATWOOD 1963)) at which homologous or nearly homologous heterochromatic regions are paired. This hypothetical increase in pairing sites does not appear to be a sufficient condition for an appreciable increase in meiotic crossing over in heterochromatin (Figure 3, days 1-5, control curve + 4000r *c(3)G* equals 4000r curve). It may be a reflection of a common basis for both increases in X ray-induced exchanges in heterochromatic regions moved distally and increases in spontaneous meiotic crossovers in proximal regions moved distally by inversion (see below).

The most important conclusion from these crosses, however, is that one component of the proximal X ray-induced increase in exchange is the breakage by X rays and the immediate (contrasted with the delayed euchromatic effects discussed below) rejoining in heterochromatin in all cell stages studied. Breakage and rejoining in heterochromatin do not appear to be dependent on the occurrence of spontaneous meiotic crossing over, for X ray-induced heterochromatic exchanges are recovered with approximately equal frequency in the presence and absence of homozygous *c(3)G*.

Interchromosomal effects: Before considering the complexities of the euchro-

matic response to X rays, brief mention will be made of an attempt to determine whether inversions increase crossing over in heterologous chromosomes (interchromosomal effect, SCHULTZ and REDFIELD 1951; SUZUKI 1963) by increasing pairing between homologues. If this were the case, and if not all the pairing sites crossed over spontaneously, it might be possible to reveal the additional paired regions by inducing crossing over within these regions with X rays. Accordingly, females bearing heterozygous autosomal inversions were X-irradiated and the early oocyte brood fraction compared with unirradiated controls. We are interested in days 5–6 and 7–8 in Table 2 because these brood fractions represent cells that were early oocytes at the time of irradiation. However, at the tip of the chromosome in only one case (γ -*rb*, *Cy*; *CxD*, days 5–6) does an X-irradiated, inversion-bearing female show more crossing over than the control (inversions alone). At the base in the euchromatic *f-car* region there are no increases in the X-irradiated females over the controls. There is no evidence from these data that pairing is increased by heterologous inversions; a more plausible explanation for interchromosomal effects will be considered below.

Centromere effect: In order to relate the increase in X ray-induced exchanges observed in the inverted heterochromatin to the pattern of spontaneous crossing over in inversions, it should be recalled that when crossing over is measured between two marker genes that were initially near the centromere but have been moved distally by inversion, the same region in the distal position shows more crossing over; the converse is also true (MATHER 1939). For example, the proximal *f-car* region has 4.5% crossing over in the control at days 11–12, but the distal *car-f* region in the inversion homozygote has 7.2% crossing over (Tables 2 and 3). The same region in the presence of the *Cy* inversion shows 8.1% when proximal but 11.2% when distal. In the presence of *Cy*; *CxD* it shows 13.8% when proximal but 18.0% when distal. This relation does not hold for every day of brood fractionation; but in spite of the many factors that are known to modify crossing over, crossover values between two markers in a distal position are usually greater than between the same two markers in a proximal position. It appears that the interchromosomal effect operates within certain limitations set by the position of the region, either proximally or distally, on the chromosome. It may be, as suggested above and by other workers (MATSUURA 1940; SCHULTZ and REDFIELD 1951; WELSHONS 1955; YOST and BENNEYAN 1957) that these regional effects on crossing over are due to differences in coiling in proximal and distal regions. Owing to tighter coiling proximally, the number of pairing sites is decreased in proximal regions and increased in distal regions. This change is revealed by increased X ray-induced exchanges in heterochromatin moved distally and by increased spontaneous meiotic exchanges in euchromatin moved distally. However, if this reasoning is correct, and if the inversion of heterochromatin distally increases the number of pairing sites that can be revealed by X rays, then the reason for the absence of spontaneous recombination in heterochromatin remains obscure. It seems unlikely that crossing over fails to occur in heterochromatin because supercoiling prevents pairing of homologues. Instead, either the proximity of heterochromatic strands sufficient for an X ray-induced

exchange is insufficient for spontaneous exchange, or an additional factor, such as accretion of histones (FRENSTER, ALLFREY and MIRSKY 1963), is responsible.

Proximal increase and distal decrease: There is no doubt that X rays can induce crossovers in spermatogonia and oogonial cells (WHITTINGHILL 1955), but the crossover decreases observed in distal regions of chromosomes irradiated in oogonia have defied explanation as breakage-rejoining effects. PARKER (discussion of paper by WHITTINGHILL 1955) has pointed out that homozygosity resulting from X ray-induced gonial crossing over in proximal regions is insufficient to account for the observed distal decreases.

The third chromosome mutant $c(3)G$, when homozygous, virtually eliminates meiotic recombination on all chromosomes (GOWEN 1933). One might question the validity of extrapolating from the radiation response of oocytes homozygous for $c(3)G$ to $c(3)G^+$ oocytes, since it has been shown that synaptonemal complexes are present in $c(3)G^+$ oocytes but absent in $c(3)G$ homozygotes (SMITH and KING 1968). For this reason, it is not surprising that there are no X ray-induced crossovers in euchromatin in oocytes (Table 4, days 1-4), but the similarity of the heterochromatic response in $c(3)G$ and $c(3)G^+$ cells, discussed above, is remarkable. It is possible that the heterochromatic regions of homologues are paired in both $c(3)G$ and $c(3)G^+$ cells without forming synaptonemal complexes, and that the supposed high breakability of heterochromatin (HANNAH 1951) is really a reflection of a higher probability of exchange in heterochromatin, owing to persistent pairing in a wide variety of cells.

The hypothesis that a chromocenter may be present in oocytes, as in other cells, is not new (NOVITSKI 1964). In fact, a chromocenter has been reported in oocytes of the fly, *Rhynchosciara angelae* (R. BASILE, personal communication). However, radiation data inconsistent with the hypothesis of a chromocenter in oocytes can be cited. Few translocations are recovered from X-irradiated oocytes (KANELLIS and RADU 1943; THOMAS and ROBERTS 1966), an observation at variance with the expectation of recovery of appreciable numbers of translocations with heterochromatic breaks, if these regions of nonhomologous chromosomes are paired in a chromocenter. It is possible that only homologues are paired at the time of crossing over. In fact, PARKER (1963) has shown that detachment of attached-X chromosomes, the most frequently recovered chromosomal rearrangement from X-irradiated oocytes, involves mostly the partially homologous Y and fourth chromosome in immature oocytes. Moreover, the regions of the Y chromosome that are homologous to the X are preferentially involved in detachments in immature oocytes (BROSSEAU 1964). Thus there is considerable evidence that heterochromatic pairing in immature oocytes, like euchromatic pairing, is governed by homology. If, instead, nonhomologous chromosomes are paired in chromocentral regions at the time of crossing over, it is possible that translocations with heterochromatic breakpoints are regularly induced by X rays but are usually not recovered. This could come about through regular segregation of the elements of a newly-induced translocation at the first meiotic division (PARKER 1969).

It does not seem unreasonable, however, to assume that pairing in oogonial cells in $c(3)G$ homozygotes is like that of $c(3)G^+$ females, because LE CLERC

(1946) has shown that $c(3)G$ has no effect on mitotic crossing over. Therefore, using $c(3)G$ homozygotes with marked X chromosomes, we can obtain an estimate of the number of euchromatic crossovers induced by X rays in oögonia, since the obscuring effect of subsequent meiotic crossing over is eliminated. As shown in Table 4, the percentage of crossovers induced in euchromatin ($v-f$; $f-car$) in oögonia (days 9–12) never rises above the control value of 0.1%. In other words, the percentage of X ray-induced euchromatic crossovers in oögonia is negligible. The proximal euchromatic interval $f-car$ (Table 2, $f-car$, days 11–12, control = 4.5%, while X-irradiated = 7.1%) shows $2\frac{1}{2}\%$ more crossing over when cells that were irradiated as oögonia are recovered than in the unirradiated controls. The difference between the experimental and control groups must represent radiation-induced crossovers, but the $c(3)G$ cross shows that the euchromatic component of the proximal increase (also the $car-su-f$ region—Table 1; Figure 2) is not due to breakage and exchanges completed in oögonia, as is the case for heterochromatic regions. $c(3)G$ should not affect the yield of induced crossovers unless these exchanges are dependent on subsequent meiotic crossing over. The proximal euchromatic increase, as well as the distal euchromatic decrease, apparently result from radiation effects on oögonia that persist through one or more cell divisions and alter the behavior of chromosomes undergoing meiotic crossing over in early oocytes, as reported previously (ROBERTS 1964). Similar conclusions were subsequently drawn from dose-effect studies (BATEMAN and CHANDLEY 1965).

Figure 4 shows the proximal increase and distal decrease (normal sequence chromosome) observed when cells irradiated as oögonia go through meiosis. The

FIGURE 4.—A comparison of recombination along normal and inverted X chromosomes 11–12 days after exposure of females to 4000r of X rays. (Data taken from Tables 2 and 3.)

euchromatic increase in the normal-sequence *X* is confined to the euchromatin immediately adjacent to the centromeric heterochromatin (*f-car* and, from Table 1, *car-su-f*). But when the bulk of this heterochromatin is inverted to the tip, the adjacent euchromatic *car-f* region fails to show the expected increase. The *sc^s* inversion homozygote shows a distal increase with a decrease of about 25% over the remainder of the chromosome (excluding that 10% of the heterochromatin that remains adjacent to the centromere—which, if marked, would undoubtedly show an increase (see above discussion)). The euchromatin of the inversion shows a rather uniform decrease throughout its length: Crossing over is not halved in the *car-f* region near the tip of the inversion as it is at the tip of the normal *X* (*y-rb*); nor is there an increase in the *cv-w^a* region, which has now been placed near the remaining 10% of the proximal heterochromatin.

Obviously, we are dealing with a system of considerable complexity in which the nature of the radiation-induced lesion(s) is in doubt. Because several days may elapse between the radiation injury and the passage of the *X*-irradiated cells through meiosis, it would seem unlikely that short-lived free radicals produced in the cytoplasm or nucleoplasm could be responsible for the distal decrease or proximal euchromatic increase (although long-lived products in the internal milieu are not excluded). Since it has been shown that bacteria with a high GC content are particularly vulnerable to killing by *X*-irradiation (KAPLAN 1964), let us assume, from this point on, that innumerable lesions in the DNA are produced by a large dose of *X* rays, such as 4000r. These lesions do not usually lead to euchromatic crossovers because of the infrequency with which an ion track crosses a point where homologous strands are exactly paired at the molecular level; a viable crossover is more likely to occur in heterochromatin, owing to more persistent pairing, or more gene duplications, or both.

What may be another clue is provided by the antagonistic action of *X* rays and the interchromosomal effect. MATHER (1936) interpreted interchromosomal effects in *Drosophila* as one example of a negative correlation of the frequencies of crossing over and chiasma formation in bivalents of the same cells due to competition between bivalents for some material, essential for crossing over, that is present in a limited quantity. Evidence for common enzyme-mediated steps in the processes of crossing over and repair of *X* ray-induced damage in *E. coli* (HOWARD-FLANDERS and THERIOT 1966) suggests that it is not unreasonable to assume that the crossover decreases observed throughout most of the euchromatin in *Drosophila* oocytes when *X*-irradiated oogonia pass through meiosis are due to the employment of a limited supply of enzyme(s), normally involved in crossing over, to repair chromosomal lesions that have persisted from oogonia to oocytes. In support of this position, it should be noted that crossing over at the tip of the *X* chromosome (*y-rb*, days 11–12, Table 2) is halved when irradiated oogonia pass through meiosis. However, in the presence of the *Cy* inversions (which greatly reduce crossing over in the second chromosome) the amount of crossing over in the *X* chromosome increases as the irradiated chromosomes pass through meiosis. Another increase in crossing over in the irradiated *X* occurs when crossing over is reduced in chromosome 3 by the *CxD* inversions. It appears

that reducing crossing over in the autosomes permits more crossing over on the X along with the repair of X ray-induced damage. The finding that autosomal inversions increase the frequency of exchanges between the attached-X and Y chromosomes (LUCCHESI 1966) can also be explained by this mechanism.

While the above considerations can account for the heterochromatic component of the proximal increase and the distal decrease, the cause of the euchromatic proximal increase remains obscure. Comparison of the euchromatic responses in Tables 2 and 3 (Figure 4), suggests that proximity of the *f-car* region to heterochromatin is not a sufficient condition for increases if the region is distal (inverted) instead of proximal. It is also apparent that movement of the formerly distal *cv-w^a* region near the remaining 10% of the proximal heterochromatin yields, instead of an increase, a pronounced decrease in this region. It may be necessary to invoke an additional effect of radiation (perhaps on coiling and consequently pairing, as proposed by YOST and BENNEYAN 1957) to account for the tendency of the hypothesized lesions in proximal euchromatin to be converted to crossovers instead of merely being repaired. (BATEMAN and CHANDLEY (1965) have suggested, on the basis of dose-response studies, that there are opposing forces operating in this region.) The crossover increases occurring in euchromatin are often slight or absent (Tables 2, 3; Figure 4, *sc^s* homozygote) compared with the crossover decreases observed throughout the rest of the chromosome. But if crossing over and repair are mediated by the same enzyme(s), a minor part of the distal decrease would be caused by the proximal euchromatic increase—compensation in the sense proposed by KIKKAWA (1936).

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SUMMARY

Evidence is presented that crossing over between homologous chromosomes is modified in two ways by X rays, depending on the position and structure of the chromosomal region: (1) In heterochromatic regions exchanges occur by breakage and rejoining in both early and late oocytes and in oogonia. There is a high frequency of exchange between the short and long arms of the X (anisobrachial exchanges). (2) In euchromatin a low level of X ray-induced exchanges occurs in all cell stages, but the conspicuous increase in proximal and decrease in distal crossovers in the normal X apparently result from radiation effects on oogonial chromosomes that persist through one or more cell divisions until manifested at the time of crossing over in early oocytes. A possible explanation for crossover decreases in terms of enzyme-mediated steps common to the processes of crossing over and chromosome repair is considered.

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