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MITOMYCIN C-INDUCED INTERCHANGE IN IMMATURE OOCYTES OF *Drosophila melanogaster*

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled MITOMYCIN C-INDUCED INTERCHANGE IN IMMATURE OOCYTES OF *Drosophila melanogaster* submitted by VIRGINIA KATRINA WALKER in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

Mitomycin C was fed to *C(1)RM, y v bb / $B^{S}Yy^{+}$; ci ey^{R} / spa^{pol} Drosophila* females so that induced chromosome aberrations in immature oocytes could be recovered. Frequencies of detachments of the compound-X chromosome, Y chromosome fragments, chromosome loss and mosaics were determined. Detailed analyses of exceptions bearing these aberrations suggests that mitomycin C has two modes of action. The drug is radiomimetic for it induces the types of aberrations recovered after X-irradiation. Mitomycin C also seems to have a delayed effect which is reflected in the relatively high recovery of gynandromorphs and mosaics.

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INTRODUCTION

The general features of meiosis in animal cells have been known since the end of the last century, but the phenomenon is still not completely understood; neither the mechanism of crossing over, the spectrum of enzymes involved, nor even aberration induction and subsequent segregation. The latter aspect should be amenable to study in *Drosophila melanogaster* oocytes which may be satisfactorily staged at a particular time in development (King *et al.*, 1956; Parker, 1968), and for which a host of genetic markers and chromosome rearrangements is readily available.

When different meiotic stages of the Drosophila oocyte are treated with X-rays, the observed aberration induction and induced recombination also varies (Parker and McCrone, 1958; Parker, 1968; Williamson, 1970, 1973, 1974; Williamson and Parker, 1974). It has been suggested that in mature oocytes (stage 14) the orientation of chromosomes on the spindle has already been established, whereas in immature oocytes (stage 7), the oldest oocytes found in a newly eclosed female, the orientation has not yet been determined and is amenable to modification by X-rays (Williamson, 1970; Parker and Busby, 1972). These stage differences were detected by genetic analysis of the recovered rearrangements in the progeny of treated females. Induced aberrations in immature oocytes involved the detachment of the attached X chromosomes to the Y and fourth chromosomes; treating mature oocytes resulted in a lower frequency in the recovery of these types of rearrangements, but with an increase in the number of rearrangements involving the major autosomes (Parker and McCrone, 1958; Williamson, 1970).

Breakpoint distribution in the Y chromosome also differs in oocytes of different stages. When X-Y interchange of Y fragment-bearing progeny of treated mature oocytes were analyzed, breakpoints in the Y chromosome were clustered distal to the fertility complexes; breakpoints in immature oocytes were more proximal (Williamson, 1969, 1970; Parker and Busby, 1972). These observations led to the conclusion that because induced translocations occur only if breakpoints in heterologous chromosomes are closely opposed, the centromeric regions of chromosomes in the immature oocytes must be close together. Indeed, it has been suggested that chromosomes in the immature oocytes are associated in a chromocentral-like arrangement, and that the X, Y and fourth chromosomes occupy positions in this configuration that increase the probability that interchange may occur between them (Williamson, 1969; Williamson and Parker, 1974). This proposal then eliminates the necessity for implied homology between those chromosomes frequently involved in interchange. As the oocyte matures, dyads may become separated at the centric regions, and by stage 14 only the telomeric regions of the chromosomes are associated (Williamson, 1970; Williamson and Parker, 1974).

Interchanges resembling those induced by X-rays may also be induced by chemicals. Mitomycin C-induced chromosome aberrations were reported in *D. melanogaster* males and females (Schewe *et al.*, 1971). Mitomycin C (Fig. 1) is an antibiotic isolated from *Streptomyces caespitosus* and has proved to be therapeutic against tumors (Usbuchi *et al.*, 1957; Early *et al.*, 1973), a carcinogen in mice (Ikegami *et al.*, 1967) and an inhibitor of DNA synthesis (Shiba *et al.*, 1959). That the drug could cross-link complementary strands of the DNA molecule was shown by the demonstration that mitomycin C-exposed DNA resisted heat denaturation

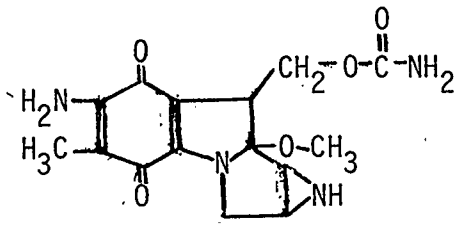

Figure 1. The chemical structure of mitomycin C

(Iyer and Szybalski, 1963). Mitomycin C may act as a monofunctional or bifunctional alkylating agent (Weissback and Lisio, 1965) preferentially reacting with guanine and covalently linking guanine residues in opposite DNA strands (Szybalski and Iyer, 1964). DNA synthesis is thus inhibited because interstrand cross-linkages prevent strand separation.

As well as inducing aberrations in the X and Y chromosomes in Drosophila, mitomycin C also induces rearrangements in Vicia, predominantly in the heterochromatic regions (Merz, 1961). In addition, mitomycin C induces a greater number of aberrations in Vicia than in Tradescantia, which has fewer heterochromatic regions in its chromosome complement (Utsumi, 1971). Breaks and rearrangements induced by the antibiotic in cultured human leucocytes most often involve chromosomes 1, 9 and 16 (Cohen and Shaw, 1964), chromosomes that contain more heterochromatic regions than other chromosomes of the human karyotype (Arrighi and Hsu, 1971). Chromatid exchanges occur most frequently between homologous chromosomes suggesting that the effects of mitomycin C treatment may be related to somatic association and crossing over of the chromosomes (Cohen and Shaw, 1964; Shaw and Cohen, 1965). It has been suggested that heterochromatic regions have a high G-C content, which

may explain these observations (Utsumi, 1971).

In Drosophila, too, rearrangements often involve heterochromatin. Mitomycin C injected into Drosophila females has increased crossing over on the X chromosome in the region around the *bb* locus and on the third chromosome in regions spanning the centromere (Suzuki, 1965; Suzuki *et al.*, 1967). Mitomycin C has also been reported to increase the frequency of interchanges involving the compound-X and Y chromosomes, and anisobrachial interchanges between the arms of the Y chromosome (Erasmus *et al.*, 1967; Schewe *et al.*, 1971). However, detachments of the compound-X to other chromosomes including the fourth, and interchanges involving the Y with chromosomes other than the X were not reported nor were Y breakpoint distributions in the reported aberrations analyzed. After X-irradiation detachments of the compound-X chromosome to the fourth chromosome make up approximately 15% of all detachments in compound-X females carrying a Y chromosome (Parker, 1954). Of Y chromosome fragments induced by X-rays approximately 50% were interchanges involving the fourth chromosome (Parker, 1965, 1967; Williamson, 1969).

The purpose of the experiment in this thesis was to compare in detail the types of rearrangements induced by mitomycin C with those induced by X-rays. Using the sensitive and reliable methods developed by Parker (1968) and Williamson (1974), extensive genetic analyses of the consequences of treating Drosophila meiotic cells with mitomycin C could be determined.

MATERIALS AND METHODS

A. DROSOPHILA STOCKS

All cultures, unless described elsewhere, were grown at 25 ± 5°C in quarter pint milk bottles and fed a cornmeal mixture prepared by adding 77 g cornmeal, 27 g sucrose, 53 g dextrose, 28 g yeast, 6 g agar and 8 ml of an acid mix (10 propionic acid: 1 phosphoric acid, 13 water) for mold inhibition, to 1 liter of water.

Genotypes and description (Lindsley and Grell, 1968)

1. *C(1)RM, y v bb / $XY^{L}.Y^{S}$, y w^{a}/0; ci ey^{R}*

   C(1)RM: reversed metacentric compound-X chromosome

   $XY^{L}.Y^{S}$: compound chromosome carrying all of the X and Y necessary for viability and male fertility

2. *C(1)RM, y v bb / FM6, y^{31d} sc^{8} dm B / $B^{S}Yy^{+}$; spa^{pol}*

   FM6: balancer X chromosome carrying multiple inversions

   $B^{S}Yy^{+}$: Y chromosome marked with Bar of Stone and the normal allele of *y*

3. *C(1)RM, y v bb / $XY^{L}.Y^{S}$, y w^{a} / Y; ci ey^{R} spa^{pol}*

4. *C(1)RM, y / $X{\cdot}Y^{L}$, y v f car / Y"*

   $X{\cdot}Y^{L}$: an X chromosome carrying the complex of male fertility factors on the long arm of the Y chromosome (*KL*)

   Y": Y chromosome fragment carrying fertility factors of the short arm of the Y chromosome (*KS*)

5. *C(1)DX, y f / $X{\cdot}Y^{S}$, y ac w^{a} ct^{6} f / $Y^{L}.sc^{S1}$*

   C(1)DX: reversed acrocentric compound-X chromosome

   $X{\cdot}Y^{S}$: an X chromosome carrying only *KS*

$Y^{L}.sc^{S1}$: Y chromosome fragment carrying only *KL*

6. *C(1)RA, y ac sc pn* sc^{8} / $XY^{L}.Y^{S}$, $y\ w^{a}$ / *O*

C(1)RA: reversed acrocentric compound-X chromosome synthesized by Parker and Busby (1972)

7. *y ac v:* free X chromosomes in both males and females

8. Brosseau tester stocks: a series of $y^{+}Y$ chromosomes deficient for specific male fertility factors

X chromosome markers employed

ac: achaete - missing some thoracic bristles

B: Bar - narrow eyes

B^{S}: Bar of Stone - very narrow eyes

bb: bobbed - thin bristles

car: carnation - dark red eyes

ct^{6}: cut^{6} - cut wings

dm: diminutive - small body and bristles

f: forked - bent bristles

pn: prune - brownish purple eyes

sc: scute - reduced number of scutellum bristles

sc^{8}: $scute^{8}$ - weak *sc* allele

sc^{S1}: scute of Sinitskaya - extreme *sc* allele

v: vermilion - bright red eyes

y: yellow - yellow body

y^{31d}: $yellow^{31d}$ - yellow body with black bristles

y^{+}: normal allele of *y* - grey body

Fourth chromosome markers employed:

ci: cubitus interruptus - interruption of the cubital wing vein

ey^R: eyeless-Russian - reduced eye size

spa^{pol}: sparkling poliert - glassy eyes

B. EXPERIMENTAL TREATMENT

The experimental females were obtained by crossing females of Stock 1 with males of Stock 2:

C(1)RM, y v bb / 0; ci ey^R X *FM6,* y^{31d} sc^8 *dm B /* $B^S Yy^+$; spa^{pol}

↓

C(1)RM, y v bb / $B^S Yy^+$; *ci* ey^R / spa^{pol}

Very few males were produced by this cross as the *FM6* chromosome is usually lethal in a male without a Y chromosome (Lindsley and Grell, 1968). This then facilitated the obtaining of experimental virgin females which were collected within 12 hours of eclosion. Approximately 200 females were immediately placed on tissue in a quarter pint milk bottle which had been saturated with 5 ml of 2% sucrose with mitomycin C (600 µg/ml, Sigma), or without mitomycin C (control). The flies were allowed to feed on this solution for 24 hours, at room temperature, in the dark. The females were then etherized, 15 females placed in individual, well yeasted, 8 dram vials and allowed to age for 24 hours. Thirty males from Stock 3 were placed in individual vials and allowed to recover from etherization for 24 hours. Experimental females and the stored males were then mated on fresh food in quarter-pint bottles. Twenty-four hours later males and females were transferred to new

bottles, and after an additional 24 hours they were again transferred. After remaining in the third bottle for 24 hours both males and experimental females were discarded. The above procedure ensured that a relatively homogeneous sample of immature oocytes (stage 7 and earlier) were treated with either the sucrose or mitomycin C and sucrose solution.

The cultures were incubated at 25°C and after 10 days the progeny of the treated females were scored and recorded by phenotype at 12-hour intervals to maximize the recovery of the progeny and to ensure that all exceptional female progeny were virgin. The numbers of regular progeny were estimated by counting a sample of flies in each tray of bottles. Exceptional females (differing phenotypically from the regular female progeny) were individually mated to three males of Stock 3 on fresh food in vials at room temperature. Exceptional males were individually mated in vials to three virgin females of Stock 3. After 6 to 8 days exceptional flies and their mates were transferred to fresh vials. Progeny of the exceptional flies were scored, thus enabling a positive identification of the exception's genotype.

C. PROGENY TESTS

1. Detachments of *C(1)RM*

A breakdown of the compound-X chromosome may occur, with the result that oocytes with single X chromosomes may be recovered. Such detachments of the compound-X may be the consequence of interchanges with the Y, the fourth chromosome, the tips of the major autosomes or deletions of one of the arms of the compound-X chromosome (Parker, 1968).

(a) *C(1)RM*-Y interchange: These exceptions were detected by linkage of the X chromosome markers with a Y chromosome marker. They

were recognized as wild-type or $y\ B^S$ females and v or $y\ v\ B^S$ males. Exceptions of both sexes were also marked with *ci* ey^R or *spd*^{pol}. Balanced stocks from these progeny tests were made by selecting for detachment-bearing males and mating them to virgin females of Stock 3. These stocks were then tested for the presence of a complete Y chromosome by mating the detachment-bearing stock males to virgin females of Stock 1, which have no free Y chromosome. Mass matings of sons from this cross were then mated to virgin females in vials and the vials inspected 5 to 6 days later for the presence of larvae. If these males proved fertile, 10 males were individually mated to virgin females to ensure that the majority of them were fertile. If the individual males were fertile the original exceptional fly from which the stock was made was assumed to have all the factors necessary for male fertility and that the treated Y chromosome had simply lost one of its marker genes. If the sons were sterile, then additional detachment-bearing stock males were mated to virgin females. B^S detachment stock males were mated to Stock 4 virgins, which carry Y^S, and y^+ detachment stock males were mated to Stock 5 virgins, which carry a partial Y chromosome, Y^L. If sons of these crosses proved fertile 10 individual males were tested, and if the majority were fertile the original exceptional fly was recorded as having all of the fertility factors on Y^S or Y^L. However, if in any of these tests the males were sterile, stock males were mated to a series of Brosseau virgin females. Brosseau females have a compound-X chromosome and Y chromosome deficient for fertility factors *ks-2*, *kl-1*, *kl-3*, *kl-3* and *4*, *kl-4* and *5* or *kl-5*. The Y chromosome deficient for *ks-1* was not available. If individual tests of sons of any of these crosses proved fertile the original exception was assumed to complement

the deficient Y, thus having a partial Y chromosome which had that particular fertility factor. Sterile crosses indicated that the treated Y chromosome did not have that fertility locus. In this manner it is possible to detect where the Y breakpoint of the X-Y detachment maps on the Y chromosome.

(b) *C(1)RM*-4 interchange: A detachment of the compound-X chromosome may occur such that the X chromosome markers are linked to markers on the fourth chromosome. These exceptional progeny were recognized as *y*, *y* spa^{pol} or *y ci* ey^{R} females and as *y v*, *y v* spa^{pol} or *y v ci* ey^{R} males. This class was recognized when progeny tests of exceptional flies showed linkage of either spa^{pol} or *ci* ey^{R} with the X chromosome markers.

(c) *C(1)RM*-? interchange: Detachments of the compound-X chromosome which showed linkage neither to Y nor fourth chromosome markers were recognized as *y* spa^{pol} or *y ci* ey^{R} females and as *y v* spa^{pol} or *y v ci* ey^{R} males. Phenotypically these exceptions could have first been thought to be *C(1)RM*-4 interchanges. Progeny tests of these individuals revealed that the fourth chromosome markers were segragating independently of the X chromosome markers.

2. Y Chromosome Fragments Recovered in Males and Females

Interchanges of this type are recovered after exchanges between the two arms of the Y, or between the Y and another chromosome. Exceptional progeny representing these interchanges were detected by loss of one of the two Y markers.

(a) Y-4 interchange: These Y fragments show linkage of the Y chromosome marker with the markers on the fourth chromosome. Phenotypically they may be recognized as *y v* B^{S} or *v* females or as *y* w^{a} B^{S}

or w^a males, with or without fourth chromosome markers. Stocks from these progeny tests were made by the selection of males and females with the Y marker for several generations. Virgin females from these Y fragment stocks were collected and mated to *y ac v* males in vials. Sons of this cross then received the rearranged Y from their mothers. By individual matings of these males to virgin females, the original exceptional fly was recorded as having all the factors necessary for male fertility, or as being deficient for one or more factors. If the latter was true, y^+ stock virgin females were mated to Stock 4 males and B^S stock virgins were crossed to Stock 5 males. Stock 4 and 5 males give their sons Y chromosomes deficient for *KS* or *KL* respectively. If these males and subsequent tests of individuals proved fertile the exceptional fly was recorded as having the fertility complex which complemented the deficient Y chromosome. If, however, the sons were sterile, Y fragment stock males were crossed to a series of Brosseau females having free X chromosomes and Y chromosomes deficient for one or two fertility factors. If mass matings and subsequent tests of individuals were fertile when mated to virgin females, the original exception was assumed to complement the deficient Y, and thus the rearranged Y chromosome had that particular fertility gene. If, however, the crosses were sterile, the treated Y chromosome did not have that particular fertility factor. Thus breakpoints in Y fragments were mapped in an analogous manner to Y breakpoints in *C(1)RM-Y* interchanges.

(b) Y-? interchanges: Phenotypically these exceptional flies resembled Y-4 exceptions. Inspection of individual progeny tests revealed that in these exceptions the Y chromosome markers segregated independently from the fourth chromosome markers. Breaks in the Y-? frag-

ments were mapped in the way described for Y-4 interchanges.

(c) Y-Y interchanges: These exceptional flies resemble both Y-4 and Y-? exceptions phenotypically. However because the Y chromosome has undergone anisobrachial interchange, a particular Y marker is present in two doses rather than in a single dose. Stocks were made of individual exceptions and the Y breakpoints mapped in the way described for Y-4 interchqnge. To determine if the Y chromosome was isomarked with y^+, males of the exceptional stocks were examined for the presence of extra hairs on the second posterior wing cell (Williamson, 1968). If extra hairs were present, the Y fragment stock was derived from a non-reciprocal interchange between the arms of the Y chromosome. To determine if Y chromosome was isomarked with B^S, males of the fragment stock were mated to Stock 6 virgin females. Female progeny of this cross were examine for reduced size of the B^S eye, a consequence of two doses of the B^S allele (Williamson, 1968).

3. Nondisjunction and Chromosome Loss

Nondisjunction occurs when paired chromosomes fail to separate at metaphase. Chromosome loss, too, is responsible for the non-recoverability of chromosomes in the progeny, possibly by sister-chromatid dicentric formation (Grell, Munoz and Kirschbaum, 1966). These exceptional progeny were recognized as $y\ w^a$ males with various fourth chromosome markers, and as $v\ B^S\ spa$ or $v\ B^S\ ci\ ey^R$ females. Progeny tests were made to detect an additional fourth chromosome.

Exceptional flies which exhibited more than one phenotype (mosaics) or more than one sex (gynandromorphs) were also mated to Stock 3 males or females and their progeny, if any, examined. Mosaic flies which proved fertile were recorded as members of a particular class dependent upon the phenotype of their progeny.

RESULTS

When *C(1)RM, y v bb / $B^{S}Yy^{+}$; ci ey^{R} spa^{pol}* females are fed 2% sucrose and mitomycin C, chromosomal rearrangements may be recovered. A total of 6,195 females were treated with mitomycin C yielding approximately 90,666 progeny (15 progeny/female) while progeny from 6,845 control females numbered approximately 155,864 (23 progeny/female). Of the total progeny of mitomycin C-treated females 371 (0.41%), excluding gynandromorphs and sterile mosaics, exhibited a phenotype which differed from the regular progeny and thus may have been due to induced chromosomal rearrangements. Of these exceptions, 123 were recovered in females, and 248 were recovered in males. These results contrast with those obtained after treating genotypically identical flies with 2% sucrose: of the 155,864 total progeny 129 (0.08%) were phenotypically exceptional progeny. Sixty exceptions were recovered in females and 69 were recovered in males. The chromosomal complements of exceptions recovered as X detachments, Y fragments, recombinant fourth chromosomes, and nondisjunction and chromosome loss in progeny of mitomycin C-treated and control females are listed in Table 1.

A. DETACHMENTS OF *C(1)RM*

Each of the exceptional progeny listed as completely analyzed was scored as to the X, Y and fourth chromosome markers carried. In the case of those exceptions involving the Y chromosome a distribution of the Y breakpoints was also made. In progeny of the mitomycin C-treated females, detachments of the compound-X chromosome made up 92 of 370 total exceptional progeny. The various detachment classes and the frequency in which they were recovered are listed in Table 2. A total of

Table 1. Summary of progeny of mitomycin C-treated and control *C(1)RM, y v bb / $B^{S}Y^{+}$; ci ey^{R} spa^{pol}* females mated to attached *XY / Y; ci ey^{R} spa^{pol}* males.

	Mitomycin C-treated		Controls	
	Females	Males	Females	Males
Regular progeny (estimated)	46,993	43,302[a]	78,906	76,829
Exceptions:				
Total	123	248	60	69
Fertile	105	195	46	55
Completely analyzed	98	192	44	54
Detachment exceptions:				
Total	47	45	9	11
Fertile	42	24[b]	5	5[c]
Completely analyzed	37	23[b]	4	5[c]
Y fragment exceptions:				
Total	4	42	2	9
Fertile	3	37[d]	2	5
Completely analyzed	1	35[d]	1	4
Nondisjunction and chromosome loss:				
Total	72	160	49	47
Fertile	60	133[e]	39	43
Completely analyzed	60	133[e]	39	43
Recombinant-4	0	1	0	2

Gynandromorphs and sterile mosaics are omitted from the above data.

[a]including 9 mosaics

[b,c]including 1 mosaic

[d,e]including 4 mosaics

about 2/3 (62 of 92) of these detachments involved the Y chromosome. Linkage of X chromosome markers to fourth chromosome markers occurred in 3 of the fertile X detachment exceptions and each was recovered without a free fourth chromosome. Fertile detachments of the compound-X chromosome which showed linkage of the X markers to neither the Y chromosome nor the fourth chromosome markers numbered 19 of the total 92 detachments recovered. This class of detachments was most often recovered with a free fourth chromosome and in one exception, with a free fourth and an entire Y chromosome. Total detachments of the compound-X chromosome were recovered equally frequently in both sexes (47 in females, 45 in males).

Detachment-bearing exceptional progeny were also recovered from the control females, although they are recovered at a lower frequency (Table 2). Indeed, the frequency of detachments recovered from progeny of mitomycin C-treated females is 7.7 times greater than that observed in progeny of control females (0.001 vs 0.00013).

Detachments of the *C(1)RM* involving the Y chromosome were also analyzed using male fertility complementation tests to determine the location of the breakpoint in the broken Y. Results of these tests are summarized in Table 3. The breaks are clustered in the centromeric region and distal to the fertility complexes. For those breaks in the centromeric region it is impossible to determine if these X-Y detachment bearing progeny have capped or captured X chromosomes (i.e., if they have a centromere derived from the X or Y chromosome, respectively).

B. Y CHROMOSOME FRAGMENTS

Exceptional progeny bearing rearrangements involving the Y chromo-

Table 2. Summary of detachment bearing progeny of mitomycin C-treated and control *C(1)RM, y v bb / $B^S Yy^+$; ci ey^R / spa^{pol}* females mated to attached *XY / Y; ci ey^R spa^{pol}* males.

Detachment Class	Mitomycin C-treated		Controls	
	Females	Males	Females	Males
Detachments involving Y:				
Total	38	24	7	6
Sterile (y^+Y)	1	6	1	1
(B^SY)	1	9	2	3
X-Y/4(y^+Y)	20	5	3	1
(B^SY)	15	3	1	0
X-Y/4^a/4^b(y^+Y)	1	1	0	1
(B^SY)	0	0	0	0
Detachments not involving Y:				
Total	9	21	2	5
Sterile	2	6	1	2
X-4R / 0	1	2	0	0
X-$4R^a$ / 4^b	0	0	0	1
X-? / $B^S Yy^+$ / 4	0	1	0	0
X-? / 4	6	12	1	2

Table 3. Analysis of breakpoints in detachments involving the Y chromosome by male fertility complementation tests. y^+Y detachments have all fertility loci from the breakpoint to the Y^S marker. B^SY detachments have all fertility loci from the breakpoint to the Y^L marker. (c) indicates the Y centromere.

Type of X-Y detachment	Y chromosome markers								
	B^S	*k1-5*	*k1-4*	*k1-3*	*k1-2*	*k1-1*	(c)	*ks-1*	*ks-2*	y^+
Progeny of mitomycin C-treated females:										
y^+Y	3	-	-	1	-		17	-		2
B^SY	8	-	-	-	-		5	-		2
Progeny of control females:										
y^+Y	-	-	-	-	-		4	-		1
B^SY	-	-	-	-	-		-	-		-

some but not the X are listed in Table 4. These exceptions account for 46 of the total 370 phenotypically different progeny. Some Y chromosome fragments showed linkage of Y markers with fourth chromosome markers, some were derived from interchanges between the 2 arms of the Y (anisobrachial interchange), while for others the interchange partner could not be determined. Of these classes anisobrachial exchange made up the majority of the fertile fragments (24/40). Most of these (18/24) were isomarked with y^+, the marker on Y^S, rather than with B^S, the marker on Y^L. All of the Y-Y fragments were recovered in male progeny of mitomycin C-treated females. This observation is not surprising as it indicates that isomarked Y chromosomes or Y chromosome fragments disjoin as regular Y chromosomes during meiosis. The class of Y fragments showing linkage with the fourth chromosome was represented by 4 of the total 40 fertile fragments, 3 of these were recovered without a free fourth chromosome and in one the exception was recovered with a free fourth and an additional four, the homologous fourth chromosome. Those Y fragments which had an interchange partner which could not be determined (Y-?) numbered 13 in the 40 fertile Y fragments recovered. A greater proportion of these were marked with B^s (8 B^SY to 5 y^+Y) than was the total Y fragment class (18 B^SY to 28 y^+Y). Y fragments recovered in progeny of mitomycin C-treated females were found at a frequency of 7.3 times the control frequency (0.00051 vs 0.00007).

Breakpoints in the Y chromosome fragments were analyzed using male fertility complementation tests. Results of these tests are summarized in Table 5. In all cases the analyzed exception had a Y centromere and in no case were captured Y fragments (fragments without a Y centro-

Table 4. Summary of Y-fragment bearing progeny of mitomycin C-treated and control *C(1)RM, y v bb / B^SY y^+; ci ey^R spa^{pol}* females mated to attached *XY / Y; ci ey^R spa^{pol}* males.

Y-fragment class	Mitomycin C-treated		Controls	
	Females	Males	Females	Males
Total	4	42	2	9
Sterile (y^+Y)	0	4	0	3
(B^SY)	1	1	0	1
Y-4R / 0 (y^+Y)	0	1	0	0
(B^SY)	0	2	0	0
Y-4R^a / 4^b (y^+Y)	0	0	0	0
(B^SY)	0	1	0	0
Y-? / 4 (y^+Y)	0	5	1	2
(B^SY)	3	5	1	1
Y-Y / 4 (y^+Y)	0	18	0	1
(B^SY)	0	5	0	1

Table 5. Analysis of breakpoints in Y chromosome fragments by male fertility complementation tests. Capped fragments have all fertility loci proximal to the breakpoint in the broken arm, and all loci in the other arm. (c) indicates the Y centromere. Numbers in parentheses are numbers of isomarked fragments included in the number above.

	Y chromosome markers									
Type of Y-chromosome fragment:	B^S	*k1-5*	*k1-4*	*k1-3*	*k1-2*	*k1-1*	(c)	*ks-1*	*ks-2*	y^+
Progeny of mitomycin C-treated females:										
Capped by 4R				1			1			2
Capped, not by 4R	9 (6)	1 (1)	4 (3)	1 (1)	5 (4)	4 (4)	2 -	- -		6 (4)
Progeny of control females:										
Capped, not by 4R	2 (1)				1	1	1 (1)			

mere) observed. This is probably because captured fragments would have a centromere derived from the fourth chromosome and at anaphase II meiotic drive should work against their recovery. Breakpoints were distributed along the Y chromosome, but appear to be clustered distal to the male fertility factors. In all cases the constitution of the fragment was found to be compatible with Brosseau's (1960) map sequence.

C. NONDISJUNCTION AND CHROMOSOME LOSS

Among the exceptions recovered from progeny of mitomycin C-treated females were 58 *y v* females which had 3 fourth chromosomes. The oocyte from which these females developed had not only a free fourth chromosome but also the homologous fourth chromosome. Thirty such triplo-4 females were also recovered in progeny of control females. Triplo-4 females were found 3.4 times as frequently in progeny of mitomycin C-treated females as in the progeny of control females (0.00064 vs 0.00019). These results are summarized in Table 6.

Nondisjunction, when paired chromosomes do not separate at metaphase, was recognized in 14 of 122 total exceptions recovered in females. X chromosome nondisjunction and loss cannot be differentiated in males on the basis of phenotype and thus a formula (Grell *et al.*, 1966) must be employed using female nondisjunction to determine the percent X chromosome nondisjunction in the sample:

$$\% \text{ X chromosome nondisjunction} = \frac{4 \text{ (nondisjunctional } ♀♀) \times 100}{\text{total progeny}}$$

Using this formula there is 0.06% X chromosome nondisjunction in progeny of mitomycin C-treated females. In progeny of control females X chromosome nondisjunction was 0.05%.

Table 6. Summary of nondisjunction and chromosome loss in progeny of mitomycin C-treated and control *C(1)RM, y v bb / $B^{S}Yy^{+}$; ci ey^{R} / spa^{pol}* females mated to attached *XY / Y; ci ey^{R} spa^{pol}* males.

	Mitomycin C-treated		Controls	
	Females	Males	Females	Males
C(1)RM / 4^{a} / 4^{b}				
Total	58	0	30	0
Fertile	47	0	23	0
C(1)RM / 4 / $B^{S}Yy^{+}$				
Total	14	0	19	0
Fertile	13	0	16	0
Nullo-X / 4				
Total	0	144	0	43
Fertile	0	120	0	39
Nullo-X/ 4^{a} / 4^{b}				
Total	0	16	0	4
Fertile	0	13	0	4

Nondisjunction is assumed to be recovered in both males and females with equal frequency, thus the difference between the number of exceptional males exhibiting a nondisjunctional/chromosomal loss phenotype ($y\ w^a$) and the number of nondisjunctional females recovered, is the amount of X chromosome loss in the sample. The per cent X chromosome loss may be calculated by the formula (Grell *et al.*, 1966):

$$\% \text{ X chromosome loss} = \frac{2\ (y\ w^a \text{ exceptional } \male\male - \text{nondisjunctional } \female\female)\ \times 100}{\text{total progeny}}$$

Using this formula there is 0.32% X chromosome loss in progeny of mitomycin C-treated females. Similarly the per cent X loss may be calculated at 0.06 for the progeny of control females. Thus per cent X loss is about the same as per cent nondisjunction in control progeny.

Exceptional progeny of the *C(1)RM, y v bb / $B^S Yy^+$, ci ey^R / spa^{pol}* females also included flies which exhibited more than one phenotype (mosaics) or more than one sex (gynandromorphs). Of the total progenh of mitomycin C-treated females, 10 were recognized as gynandromorphs, all of which proved sterile and 33 as mosaics, 14 of which were sterile. Thus 0.05% of the exceptional progeny belonged to one of these two classes of exceptions. In the progeny of the controls, however, only 2 mosaics and 1 gynandromorph were observed. Fertile mosaics were included as members of a class of exceptional or regular phenotypic flies dependent upon the phenotype of their progeny. Fertile mosaics are included in Table 1, with the exception of 1 mosaic from a mitomycin C-treated female which also proved to be a germ line mosaic.

DISCUSSION

The induction of mutations and chromosome breaks by ionizing radiation in *Drosophila melanogaster* has been known since Muller's (1927) discovery. Not until recently however has the genetic analyses of the induced rearrangements been refined enough to study the behaviour of these aberrations during meiosis. Recent advancements have included the identification of these chromosomes involved in interchange, a hypothesis on the nuclear organization which may make the type of chromosomal restitution possible, and segregational patterns of the induced aberrations during metaphase (Parker and McCrone, 1958; Parker, 1968; Parker and Williamson, 1974; Williamson, 1969; Williamson and Parker, 1974). In most cases these analyses have employed X-rays to induce chromosomal rearrangements in oocytes. In comparison it would be very interesting to determine if other agents can induce similar aberrations and subsequent segregations. If X-rays do not produce unique events then perhaps the information revealed by this tool is a true reflection of ordinary meiotic mechanics.

Mitomycin C, an antibiotic, which is believed to act either monofunctionally or bifunctionally as an alkylating agent (Weissback and Lisio, 1965) has been reported to induce interchanges similar to those induced by X-rays (Schewe *et al.*, 1971). The analyses of these induced detachments and Y chromosome fragments was incomplete and thus this work has been repeated using the refined techniques developed by Parker (1968) and Williamson (1974). Genetic analysis of the consequences of treating immature Drosophila oocytes with mitomycin C was undertaken to determine if this agent mimics X-rays in the induction of rearrange-

ments during meiosis.

Drosophila is an ideal organism on which to conduct these types of studies as there exists a variety of constructed chromosomes with markers so that rearrangements can be easily detected. Irradiation of females carrying a compound-X and a Y chromosome frequently result in the detachment of the compound-X and usually involves the Y or fourth chromosome (Parker and McCrone, 1958; Williamson, 1970, 1974). These induced interchanges are chromatid events and the heterologues involved form a *quasi*-bivalent which then separates at the first meiotic division (Parker, 1968; Williamson, 1974). Recovery of such interchange products may be detected as progeny which are phenotypically different from regular progeny. Detachment of the compound-X chromosome also occurs spontaneously although X-rays induce a higher frequency of this aberration. Mitomycin C, at a concentration of 600 µg/ml, also induces detachments of the *C(1)RM* chromosome some 7 to 8 times more frequently than the observed frequency in control females. The frequency of the mitomycin C-induced detachments represents 0.10% of the total progeny. Schewe *et al.* using 125 µg/ml induced X-Y detachments at a frequency representing 0.04% of the total progeny of the first 3-day brood. This represents 2 times the number obtained from control females. Dose response experiments with immature oocytes conducted by Parker and Hammond (1958) indicate that the frequency of detachment-bearing progeny induced with a 600 µg/ml concentration of mitomycin C corresponds to a dose of 250 - 500 R of X-rays.

As with X-rays, detachments induced by mitomycin C sometimes involved an interchange partner which could not be determined. It has been suggested that these undetermined interchange partners could be

the left arm of the fourth chromosome, the tips of the major autosomes or a deletion of one arm of the compound-X (Parker, 1968). Detachment-bearing progeny of mitomycin C-treated females more often involved an undetermined interchange partner (21% of all detachments) than did irradiated females (12% calculated from Williamson's (1974) data). This seems to be the result of a lower recovery of X-*4R* detachments recovered after mitomycin C treatment. It has been suggested that the high frequency of X-*4R* detachments recovered in progeny of X-ray treated females is a result of non-random association between the X and the fourth chromosome, perhaps as a result of a chromocentral-like arrangement (Williamson, 1969, 1970; Williamson and Parker, 1974). Some X-*4R* progeny were obtained after mitomycin C treatment and thus the chromosomes must have been close together; the difference may be that mitomycin C does not induce breaks in *4R* any more effectively than in the other autosomes, or that the agent is capable of deleting one of the attached X's. Deletions of the long arm of the X chromosome have been observed in rat kangaroo cells cultured in mitomycin C (Brinkley and Shaw, 1970). It is also possible that the action of mitomycin C is delayed so that rearrangements induced by it occur after the chromocentral-like associations of chromosomes. That this is frequently the case is suggested by the relatively high frequency of mosaics recovered after mitomycin C treatment compared with treatment with X-rays.

Most of the induced detachments (67%) recovered in progeny of mitomycin C-treated females involved the Y chromosome. This is consistent with the results obtained after X-ray treatment; approximately 72% of the detachments recovered involved the Y chromosome in irradiated compound-X females with Y chromosomes (calculated from Williamson's (1974) data). Mitomycin C-induced detachments are also recovered preferentially

in females, which is in accordance with Williamson's (1974) data.

The complete analysis of the X-detachments involving the Y chromosome also indicates that detachments induced by mitomycin C are similar to those recovered by X-rays. Using X-irradiation, Brosseau (1964) induced 115 X-Y detachments and analyzed them for the distribution of breaks in the Y chromosome by testing for the presence of individual fertility factors, with flies bearing deficiencies for these factors (Brosseau, 1960). Breakpoints were found to be clustered in the centromeric region and between the most distal fertility factors and the B^S or y^+ markers. Mitomycin C-induced X-Y detachments produce a similar distribution; of the 38 X-Y detachments analyzed only one breakpoint was found outside these three regions (Table 3).

The regions in which the Y chromosome breakpoints of X-Y detachment-bearing progeny are clustered may show some homology with the X chromosome. Both X and Y chromosomes have nucleolar organizers in the centromeric regions, the *C(1)RM* centromere is probably Y derived, and the Y chromosome markers are derived from the X. It is possible, however, that the chromosomes are associated in a chromocentral-like arrangement, not based on homologies, such that associations of Y centromeric and telomeric regions with the X chromosome are more likely to occur (Williamson, 1970; Williamson and Parker, 1974). Perhaps too it is possible that some regions of the Y chromosome are more susceptible to breaks by X-rays and mitomycin C than others. The nucleolar organizer could be such a region. In human leukocyte cultures, breaks and rearrangements of the chromosomes occur primarily in chromosomes 1 and 9 at the secondary constrictions (Shaw and Cohen, 1965). The centromere is also reported to appear damaged after mitomycin C treatment of human lympho-

cyte cultures (Brinkley and Shaw, 1970). At present there is no evidence suggesting which, if any, of these possibilities may be true.

Interchange involving the Y, but not the X may also be recovered in progeny of females carrying a compound-X and a free Y chromosome. Y chromosome fragments arise by interchange with the fourth chromosome, with a partner which cannot be determined, or by a nonreciprocal exchange between the arms of the Y chromosomes (Parker, 1968; Williamson, 1974). These induced events involve chromatids and these conjoining heterologues form *quasi*-bivalents which separate at meiosis. Progeny of mitomycin C-treated females showed loss of one of the Y markers 7 times as frequently as progeny from control females. The Y fragments less often involved the fourth chromosome as an interchange partner (8.9%) than did Y fragments recovered in progeny of females irradiated with 2000 R of X-rays (42.1%, calculated from Williamson's (1974) results). Detachments of the X chromosome which showed linkage of X chromosome markers to fourth chromosome markers in progeny of mitomycin treated females were also less frequent than in the irradiated females. In both aberration types, radiation-induced interchange involving chromosome 4 was about 5 times more frequent than mitomycin C-induced interchange. The lower recovery of Y-*4R* fragments from mitomycin treated females may indicate that the drug is a less efficient agent in breaking *4R*, or that it exerts its effects later than do X-rays, that is, after the regularly associated chromosomes have begun to move apart. The large representation of the Y-?/4 class of Y chromosome fragments (Table 4) could be a result of such a mechanism. These could represent deletions of part of the Y chromosome.

Most of the induced Y chromosome fragments recovered in mitomycin

C-treated females were anisobrachial interchanges. These nonreciprocal interchanges between the arms of the Y chromosome lead to "homozygosis" of the distal marker which may be recognized by extra hairs on the wings of male flies (y^+Yy^+) or by reduced eye size ($B^SY\ B^S$) (Williamson, 1968). Of the total progeny recovered from females treated with 600 µg/ml mitomycin C, 0.03% were anisobrachial interchanges. These results contrast with those reported for progeny of females treated with 125 µg/ml where 0.13% of the total first 3-day brood progeny represented Y-Y fragments, the only class of Y fragment reported by Schewe *et al.* (1971). This represents a vast increase over the controls (in which no Y-Y fragments were reported). These results (Schewe *et al.*, 1971) were obtained without analyzing the presumptive Y fragments, and scoring individual flies for 1 or 2 doses of the Y marker is difficult. This does not explain the discrepancy between the two results, however, and it is puzzling that although a lower concentration of the drug induces a lower frequency of X-Y interchange, there is a five-fold increase in the frequency of Y-Y interchange.

The complete analysis of the Y fragments with the male fertility complementation tests indicates that Y fragments induced by mitomycin C are similar to those recovered by X-rays (Table 5). Breakpoints in Y fragments induced by X-rays occur along both arms of the Y chromosome but are clustered near the ends of the arms, distal to the fertility factors and near the centromere (Williamson, 1969). The mitomycin C-induced Y breakpoints have a similar distribution with most of the breaks occurring between the distal fertility factors and the Y chromosome markers. These must be sites where the two arms of the Y chromosome are close enough together so that interarm exchange may occur. It is

interesting that these breakpoint locations are also the sites of breakpoints in X-Y detachments. Alternate explanations of these distributions are that these locations are more easily broken, both by mitomycin C and X-rays, or that they are very long regions of the Y chromosome. At the present time one cannot discriminate between these possibilities.

Numerical aberrations as well as chromosome aberrations are recovered in progeny of X-ray (Mavor, 1924) and mitomycin C-treated females (Hayashi and Suzuki, 1968). Monosomy and trisomy need not arise from the same events; indeed, the consistent observation that cases of monosomy outnumber cases of trisomy (Parker and Busby, 1973) suggests that different mechanisms may be responsible. *Triplo-4* individuals recovered from *C(1)RM / diplo-4* oocytes were found 3.6 times more frequently than trisomy recovered from *nullo-X / diplo-4* oocytes in mitomycin-fed flies (Table 6). After X-ray treatment 97% of all recovered triplo-4 exceptions were found in males (Parker and Williamson, 1970). That mitomycin C induces fewer *nullo-X / diplo-4* oocytes may be related to the relative low recovery of Y-4 chromosome fragments. It has been suggested that when Y-4 interchange occurs in immature oocytes a *quasi-* bivalent is formed which subsequently separates at division I (Parker, 1969; Parker and Williamson, 1974). This conjoining of two heterologues effectively removes both these elements from the distributive pairing pool (Grell, 1962), leaving the homologous four and the *C(1)RM* chromosomes to distributively pair and segregate from each other. As a result of these events and of subsequent anaphase II non-random segregation (recovery of the shorter element of the heteromorphic dyad is favoured at the second division in Drosophila oocytes; Novitski,

1951) Y-4 exceptions and *nullo-X, diplo-4* eggs are often recovered. In an analogous manner, X-4 interchanges also produce *nullo-X, diplo-4* eggs (Parker, 1970; Parker and Williamson, 1970, 1974). Interchange involving chromosome four would also be expected to affect the nondisjunction of the X chromosomes. Both Y-4 and X-4 interchanges may result in X, Y, *nullo-4* gametes. Because mitomycin C induces few Y-4 and X-4 exceptions, it would be expected that there would be few *nullo-X, diplo-4* oocytes generated, as well as few X-Y, *nullo-4* oocytes (recovered as XXY nondisjunctional females).

Because nondisjunction recovered after mitomycin C treatment was not more frequent than that found in the controls (.06% vs .05%), it is unlikely that the drug can affect spindle formation or the centromeric attachment to the spindle. Nondisjunction in progeny of irradiated flies is believed to be a result of interchange and not a result of an effect on the centromeric properties or chromosomal "stickiness" (Parker and Busby, 1972; Parker and Williamson, 1974).

Chromosome loss in progeny of females fed 600 µg/ml mitomycin C was 5.3 times that of the controls (Table 6) while chromosome loss after treatment with 125 µg/ml was 2.6 times that of the control in the first 3-day brood (Schawe *et al.*, 1971). Mitomycin C, at 600 µg/ml, thus increases chromosome loss slightly less than chromosomal interchange. Ionizing radiation also increases chromosome loss (Muller, 1940). It is possible that chromosome loss is a result of *C(1)RM*-4 interchange. Because not many of these aberrations were recovered in progeny of mitomycin C-treated females it would seem likely that this is not the only way losses may be induced. Losses of the X chromosome may be recovered after the induction of chromatid breaks, which may then join,

leading to the formation of dicentric bridges and acentric fragments. The dicentrics could then enter breakage-fusion bridge cycles and be eventually lost (Grell *et al.*, 1966). Mitomycin C may induce sister-chromatid fusions as dicentric bridges and acentric fragments have been observed in human leukocyte cultures treated with mitomycin C (Shaw and Cohen, 1965).

Genetic mosaics were observed 25 times more frequently in progeny of mitomycin treated females than in the controls. These types of exceptional flies are recovered at relatively lower frequencies in progeny of irradiated flies. Mosaics are characterized by two genetic constitutions, and in gynandromorphs these constitutions may be sex-related so that characteristics of both sexes are found in one individual. It is believed that gynandromorphs originate from fertilized eggs in which, at the first division, one of the two X chromosomes lags behind and is lost (Morgan and Bridges, 1919), or both X chromosomes go to the same pole (Stern, 1960). These events occur during the first mitotic division, long after the time of mitomycin treatment of the immature oocyte. Mitomycin C then must be capable of inducing aberrations or chromosomal instabilities over a very long period of time. Of the 10 gynandromorphs recovered from progeny of mitomycin C-treated flies, 8 were *yellow* and *vermilion*. This would suggest that elimination of one of the X's of the compound-X chromosome had taken place. Because of the evidence that mitomycin C can cause large deletions, breaks and gaps in chromosomes of both animal cells (Cohen and Shaw, 1964; Shaw and Cohen, 1965) and plant cells (Merz, 1961), it is suggested that the gynandromorphs result from partial elimination of the compound-X, perhaps resulting from breaks in the heterochromatic region bracketing the centromere, as it

is in the heterochromatic regions that mitomycin C preferentially induces breaks in human (Cohen and Shaw, 1964) and plant (Utsumi, 1971) chromosomes.

Brøgger and Johansen's (1972) model for the production of chromosome damage by mitomycin C suggests that after cross-linking the DNA (Iyer and Szybalski, 1963), mitomycin C residues are detached from one strand by a repair enzyme. Excision of the residue and of the bases bonded to it with subsequent repair replication would result in an undamaged chromosome. Exchanges result if another break occurs nearby, and losses of chromosomal segments are a result of the excision of the bases on the opposite strand of the DNA. Perhaps it is a "misrepair" mechanism acting in the egg which is responsible for the gynandromorphs observed.

The other mosaics observed may be a result of somatic crossing-over. This mechanism has been suggested to explain aberrations observed after mitomycin C treatment of human cell cultures (Cohen and Shaw, 1964; Shaw and Cohen, 1965). Somatic crossing-over, however, requires that homology exist between the chromosomes. This may not be true for the heterologues involved in the production of mosaics observed after mitomycin treatment of Drosophila. Perhaps the partial elimination of the marked chromosomes could account for most of the mosaics recovered.

Mitomycin C then seems to have two modes of action. The drug is radiomimetic for it induces the types of aberrations recovered after X-irradiation. All detachment and Y chromosome fragment classes of exceptions reported in the progeny of irradiated flies are recovered, the Y breakpoint distribution in both X-Y and Y fragments are similar to those analyzed in progeny of irradiated females, and with both agents nondisjunction does not seem to be a result of spindle or centromere

damage. In addition to these characteristics, mitomycin C seems to have a delayed effect which manifests itself in the production of gynandromorphs and mosaics. This delayed effect is perhaps the reason for an increase in the relative number of X-? and Y-? exceptional progeny from females fed mitomycin C compared to females treated with radiation; it may also explain the increase in chromosome loss recovered in *diplo-4* flies.

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