EFFECTS OF MITOMYCIN C ON CROSSING OVER IN *DROSOPHILA MELANOGASTER*[1]

DAVID T. SUZUKI

Department of Zoology, University of British Columbia, Vancouver 8, B.C., Canada

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RECENT evidence indicates that a large proportion of increases in crossing over induced in *Drosophila melanogaster* females is of gonial origin (WHITTINGHILL 1955; THOMPSON 1964; SUZUKI 1965). HOLLIDAY (1964) and ESPOSITO and HOLLIDAY (1964) find that treatment of Ustilago and Saccharomyces with agents that inhibit DNA replication (mitomycin C, 5-fluorodeoxyuridine) results in increased somatic recombination. They propose that delayed DNA replication results in a meiotic-like cell cycle in which greater opportunity for pairing and crossing over may occur. The experiments reported here were undertaken to determine whether mitomycin C, an antibiotic which inhibits DNA replication without preventing RNA and protein synthesis (SHIBA, TERAWAKI, TAGUCHI and KAWAMATA 1959) affects crossing over in Drosophila and if so, what cell stages are affected.

MATERIALS AND METHODS

Crossing over was measured in chromosome 3 using the following markers (followed by map distances as listed by BRIDGES and BREHME 1944): *ru* = roughoid 0.0, *h* = hairy 26.5, *st* = scarlet 44.0, p^p = pink-peach 48.0, *ss* = spineless 58.5, e^s = ebony-sooty 70.7. The region from *ru* to *h* is designated as 1, from *h* to *st* as 2, and so on. The centromere lies between *st* and p^p in region 3.

The injection and mating procedure has been outlined previously (SUZUKI 1963a, 1965). Mitomycin C (MC) dissolved first in a drop of 95% ethanol was added to a 0.7N saline solution and injected into females at concentrations of 10 and 100 μg/ml (these series will be referred to as 10 MC and 100 MC respectively). As controls, females were injected with a sterile physiological saline solution. Each injected female was mated with three males and transferred to a fresh vial at three day intervals for five consecutive broods. Crossing over in each brood was recorded for each female.

RESULTS

The total crossover data from all females in each brood of each treatment series is listed in Table 1. An increase in crossing over in region 3 can be seen in the 100 MC series beginning in Brood 3 and increasing in subsequent broods. Although the crossover values in region 3 normally do decrease with increasing age of the female (SUZUKI 1963a, 1965), the control value of 0.3 in Brood 5 (Table 1) is exceptionally low and makes the relative increase of the MC series

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TABLE 1

Crossover values in each region of chromosome 3 for each treatment in successive three-day broods

Brood number	Treatment	Crossover regions 1	2	3	4	5	Total progeny
	Saline	19.1	20.6	4.1	14.3	14.0	608
1	10 MC	24.3	20.8	4.1	9.0	14.5	1,310
	100 MC	22.3	23.7	4.4	11.8	13.4	2,180
	Saline	23.8	20.0	2.2	8.0	14.5	808
2	10 MC	24.8	16.9	2.0	5.6	11.9	2,006
	100 MC	24.2	16.6	2.3	7.4	12.1	2,640
	Saline	24.4	17.9	3.0	7.8	13.2	676
3	10 MC	21.8	16.2	3.7	7.0	10.6	1,591
	100 MC	22.9	17.6	5.0	8.5	12.6	2,745
	Saline	25.4	16.0	2.1	5.9	12.9	1,144
4	10 MC	21.8	19.0	4.6	9.3	12.7	1,669
	100 MC	18.2	19.3	7.5	10.2	12.3	3,848
	Saline	24.4	12.7	0.3	4.8	11.6	628
5	10 MC	21.1	17.8	4.6	7.0	12.5	1,795
	100 MC	20.0	19.4	5.0	8.4	12.1	2,784

appear disproportionately large. In a similar control series, SUZUKI (1965) obtained a Brood 5 value in region 3 of 1.3 (2,584 offspring counted) which may be considered as a more representative control value.

The ratios of crossover values for each region in the MC series (p_1) to the values in the controls (p_0) for the same region of each brood are plotted in Figure 1. Any ratio and its 95% confidence limits which spans the value of 1.0 indicates that the two values compared are not significantly different.

It can be seen (Figure 1) that there are significant decreases (region 4, Brood 1; regions 2 and 3, Brood 2) and increases (region 1, Brood 1) in the first two broods that were not observed in experiments with actinomycin D (SUZUKI 1965). In region 3, crossing over is not affected in the 10 MC series and is slightly increased in the 100 MC series in the third brood, and increases strikingly in Broods 4 and 5 (Figure 1). The p_1/p_0 values in Brood 5 were 15.33 and 16.67 for the 10 MC and 100 MC series respectively. The bottom of the 95% confidence intervals are very close to the p_1/p_0 values using the Brood 5 value of 1.3 obtained in previous experiments (SUZUKI 1965). Striking increases in crossover values in regions 2 and 4 can be seen in Broods 4 and 5 (Figure 1). The decrease in crossover values in region 1 appears to be a typical response when large increases in the proximal regions occur (SUZUKI 1962, 1963b). MC unlike actinomycin D, appears to affect crossing over primarily in Broods 4 and 5, those broods presumed to represent cells in gonial stages at the time of treatment (PLOUGH 1924).

The frequency distribution of females having different crossover values in region 3 is plotted in Figure 2 for each brood of the saline and the MC series. The

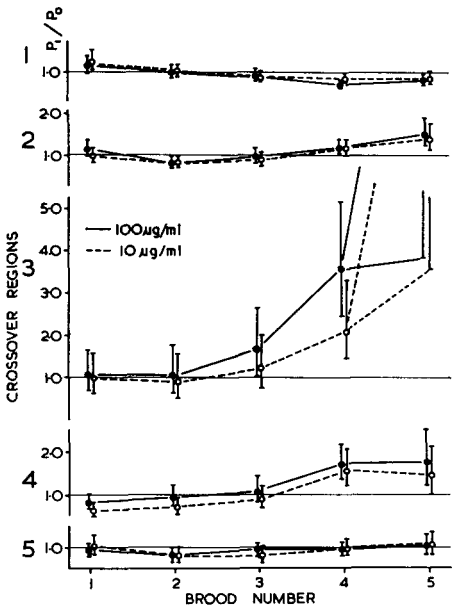

FIGURE 1.—Ratio of crossover values in each region of chromosome 3 in MC-injected females to their respective control values in each brood.

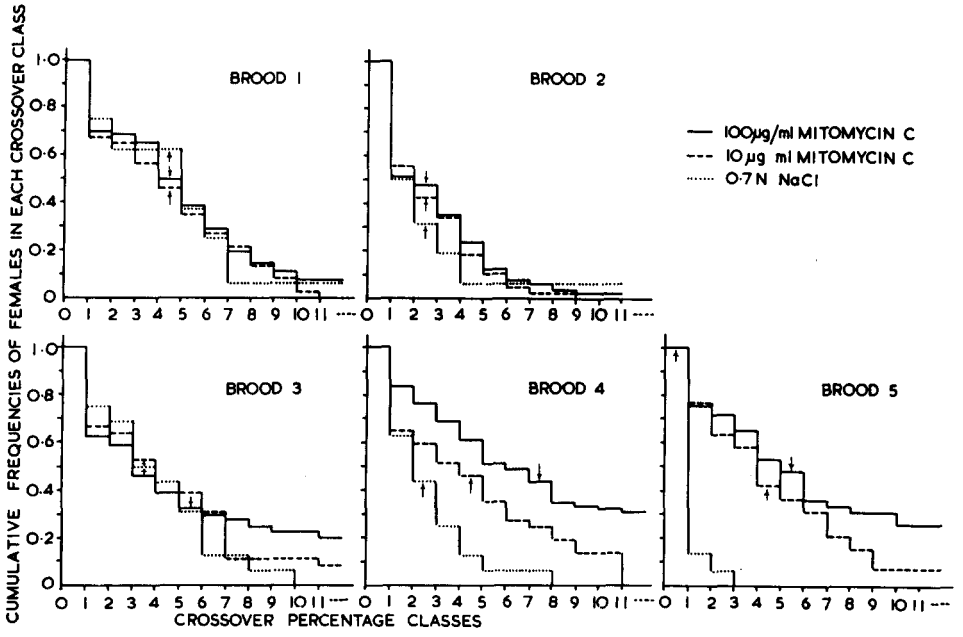

FIGURE 2.—Cumulative frequency distribution of females with values in each crossover percentage class. The numbers on the abscissa represent values in the interval between the number and the next highest integer.

TABLE 2

Average number of offspring per fertile female in each brood of each treatment

Treatment	Brood number					Total/females
	1	2	3	4	5	
Saline	38.0	33.0	42.3	71.5	41.9	226.7
10 MC	35.4	54.2	44.2	45.1	47.2	226.1
100 MC	26.3	33.0	34.3	48.1	36.2	177.9

number of fertile females was 16 (saline), 44 (10 MC) and 98 (100 MC) in each series. Although the number of control females is small, the frequency distribution of the crossover classes is, in general, similar to those obtained in other experiments (SUZUKI 1965).

In Brood 3, there is some indication of a shift in the percentage of females having higher crossover values after injection of 100 MC. The striking effects occur in Broods 4 and 5 where the percentage of MC-treated females having crossover values greater than the highest values in the controls is significantly increased. Thus, for example, in Brood 4, almost 20% of the 10 MC and 35% of the 100 MC females have crossover values of 8.0 or more, whereas none of the control females has.

Unlike actinomycin D, MC did not significantly increase the mortality of the flies after injection. Of the surviving females, none of the controls, 13.6% and 16.7% of the 10 MC- and 100 MC-treated females respectively, were sterile for all five broods. The average number of progeny per female in each series is listed in Table 2, and it can be seen that high concentrations of MC do decrease the total. There is some indication that crossover values in region 3 of females with smaller broods are higher. For example, in Brood 5 of the 100 MC Series, the crossover value is 8.8 in those females having fewer than 20 progeny (SUZUKI, unpublished) whereas the value for the total in the series is 5.0. These data indicate that mitomycin C does have a physiological effect which is directly correlated with the degree of its genetic effect on crossing over.

DISCUSSION

Any interpretation of the genetic effects of mitomycin C treatment rests strongly on the basic assumption as to its primary physiological effect in Drosophila. Studies of MC in bacterial and *in vitro* systems indicate clearly that its primary effect is the inhibition of DNA replication without concomitant effects on RNA or protein synthesis (for a review see SZYBALSKI and IYER 1964). Obviously, the complexity of multicellular systems makes our assumptions at best, a crude approximation. However, it is felt that the experiments and intepretations reported here are useful in the setting up of testable models. MESELSOHN (1964) has demonstrated that DNA replication is not necessary for recombination in bacteriophages. TOMIZAWA and ANRAKU (1964) using KCN, and IIJIMA and HAGIWARA (1960) and YUKI (1962) using MC, showed increased recombi-

nation in phages and bacteria under conditions inhibitory to DNA synthesis. The increases in somatic crossing over in Ustilago and Saccharomyces with MC have been interpreted as the resulit of an inhibition of DNA replication (HOLLIDAY 1964). It has been suggested that the inhibition of DNA replication increases the opportunity for the chromosome pairing necessary for crossing over to occur (HOLLIDAY 1964). The results reported here are consistent with this interpretation.

If one assumes that all of the increases in crossing over after MC-treatment in Drosophila are of gonial origin, the data indicate that these induced exchanges are primarily in the region around the centromere. This has also been demonstrated in the study of somatic twin spots occurring by spontaneous exchange (STERN 1936) and those induced by the presence of Minutes (KAPLAN 1953). These localized effects could indicate that the pairing of mitotic chromosomes necessary for exchange is confined to the region around the centromere.

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SUMMARY

Crossing over in chromosome 3 was measured in females injected with 0.7 N NaCl or mitomycin C (MC) at concentrations of 10 and 100 μg/ml. The females were mated singly and allowed to lay eggs for five consecutive three-day broods. Crossing over was increased slightly in the region spanning the centromere in Brood 3 of the 100 μg/ml MC series and greatly increased in this region in Broods 4 and 5 by both concentrations of MC. Crossing over in the two regions adjacent to the centromere area was also increased in Broods 4 and 5 after MC treatment. The effects of MC appear to be primarily on cells in gonial stages.

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