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The production of translocations in spermatogonial cells of *Drosophila* by chloroethyl methanesulphonate (CB 1506)

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It is a well-known fact that X-rays produce few translocations in spermatogonia of both *Drosophila* and mice (Alexander, 1960; Bateman & Chandley, 1959; Catsch & Radu, 1943; Hertwig, 1940; McCarthy & Nafei, unpub.; Russell, 1954; Sävhagen, 1960; Slizynska, unpub.; Traut, 1960). For mice, this statement has recently been challenged by Griffen (1958), who found a high proportion of semi-sterile sons among offspring born to irradiated males during the post-sterile period. Since, however the actual presence of a translocation was confirmed for only one case, these data are still open to different interpretations.

The shortage of translocations in spermatogonia raises a problem that cannot be resolved by reference to meiotic selection. It is true that half the premeiotically induced translocations are subject to loss at meiosis through the formation of aneuploid gametes; but against this stands the fact that the probability for a translocation to occur is about twice as high in diploid spermatogonia as in haploid spermatozoa and spermatids. On balance, then, translocation frequency per chromosome set—i.e. per tested spermatozoon—should be approximately the same in postmeiotic and premeiotic stages if conditions for the occurrence of translocations were equal. Obviously, this is not so. At least when X-rays are used as mutagen, conditions in spermatogonia are much less favourable to the induction of translocations than are conditions in spermatozoa and spermatids. Muller (1594) has discussed possible causes for this difference, the main one being that the efficient production of rearrangements from chromosome breaks is limited to the condensed stage of the chromosomes, which in spermatogonia occupies only a small fraction of the mitotic cycle.

If this is true, it might be suspected that chemicals, because of their less instantaneous action, might stand a better chance than X-rays of hitting the right stage for the production of translocations in spermatogonia. Indeed, Auerbach and Sonbati (1960) considered this as a possible explanation of their finding that the ratio of mustard gas induced translocations to lethals in *Drosophila* was not depressed during the sensitive stage, which occurs in late spermatogonia. This, however, was not the only explanation considered, and no tests were carried out on still younger germ-cells.

Mustard gas, like X-rays, has a relatively weak genetical effect on spermatogonia (Auerbach & Sonbati, 1960). Moreover, it is quickly destroyed by hydrolysis and is unlikely to have a prolonged action. A chemical that is superior to mustard gas in both these respects is chloroethyl methanesulphonate (CB 1506). This substance

acts preferentially on spermatogonia (Fahmy & Fahmy, 1956) and produces very high mutation frequencies in them. In addition, it hydrolyses slowly (Fahmy & Fahmy, 1957) and is slow also in its mutagenic action on spores of *Neurospora* (Kølmark, personal communication). An experiment was therefore carried out to test for translocations among offspring from spermatogonia that had been exposed to CB 1506. A pilot experiment by Reddi & Auerbach (1960) had given encouraging results; but these were considered inconclusive by the authors themselves because, through an unfortunate loss of cultures, it had not been possible to confirm the presumed translocations in a further generation.

MATERIALS AND METHODS

CB 1506 was kindly supplied by the Chester Beatty Institute for Cancer Research. It was made up into a 0·15% solution (approx. 10^{-2} M) in 0·4% saline, and 0·2μl. of this was injected into the abdomen of ♂♂ with the aid of an Agla micrometer syringe. The males came from a wild-type strain (*Ork*), which over the years has given spontaneous mutation frequencies for sex-linked lethals ranging from 0·1% to 0·3%. On the day following treatment, the males were mated in small mass cultures (5 males to 15 females) and this was repeated with fresh females every 3 days. No progeny was collected from the first three broods. On the 10th day and again on the 13th day the surviving males (240 out of 300 injected ones) were divided into two groups and mated individually to females of two tester stocks to produce broods *d* and *e*. The following tester stocks were used:

(*a*) *y sc*^{s1}*In49 sc*^{8}; *bw*; *st*. This is a dual-purpose stock, allowing the detection of sex-linked lethals as well as of translocations involving chromosomes Y, II and III. The F_1 females are used for the lethal test, with *y* (yellow) as the marker gene distinguishing the non-treated from the treated X. The F_1 males are crossed to *bw*; *st* (brown, scarlet) ♀♀, and their progeny are scored for apparent linkage between *bw* and *st*, indicating a II–III translocation, between *bw* and sex, indicating a Y–II translocation, and between *st* and sex, indicating a Y–III transloctaion.

(*b*) attached-X; *bw*; *e*. The sons of these females inherit the treated X-chromosome and can therefore be tested for translocations involving the X as well as chromosomes II and III (*e* = ebony).

All presumed translocations were confirmed by repetition of the test on the wild-type F_2 males.

RESULTS AND DISCUSSION

The data are presented in Table 1. The high lethal frequencies show that the breeding technique was adequate to ensure that the sampled progeny traced back to treated spermatogonia. The increase in lethal frequency from brood *d* to brood *e* is on the borderline of statistical significance, suggesting an increase in sensitivity from older to younger spermatogonia. Translocation frequency, too, rises from brood *d* to brood *e*, but the actual frequencies are too small for statistical significance of the difference. An increase in mutation frequency within the spermatogonial

stage has been found also by Sonbati (1959) and by Purdom (1957), although the latter obtained it only in experiments in which germinal selection had been minimized by the use of autosomal lethals.

The result of the present experiment leaves no doubt that CB 1506 can produce chromosome rearrangements, and that it can do so in spermatogonia. In view of the tendency of chemical mutagens to produce intrachromosomal rather than interchromosomal changes (Auerbach, 1949; Slizynska, 1957; Nilan, 1961) it is quite possible that a much higher number of rearrangements might be revealed by cytological analysis. For the closely related mutagen chloroethyl cysteine Fahmy & Fahmy (1960) have come to the conclusion that it most probably is totally unable to break the chromosomes in spermatogonia because they found no rearrangement in a sample of sex-linked lethals. Since, however, the majority of large rearrangements associated with lethals are eliminated during meiosis and few occur even in irradiated spermatogonia, this conclusion does not appear well founded. So far, every adequately tested mutagen has been found to produce chromosome rearrangements as well as mutations in *Drosophila*, although the relative frequencies of these two effects may vary widely between mutagens.

Table 1.

	Sex linked lethals			Translocations between X, II and III			Translocations between Y, II and III			Total translocations	
	No. tested	No. found	%	No. tested	No. found	Type	No. tested	No. found	Type	No.	%
Brood *d* 10–12 days	546	109	19·6	824	2	Both II–III	936	2	Both II–III	4	0·22
Brood *e* 13–15 days	584	139	23·8	897	4	All II–III	874	3	One Y–III Two II–III	7	0·39

According to the data presented here, treatment of spermatogonia with CB 1506 produces very few translocations relative to lethals. This can be seen also from a similar experiment by Dr O. S. Reddi (unpub.), in which treated spermatogonia yielded 24·9% sex-linked lethals but only 0·24% translocations (1/422). Sävhagen (1960) obtained the same frequency of translocations in spermatogonia that had been exposed to the low X-ray dose of 1100 r. At first sight, this comparison seems to show that spermatogonia are even more refractory to the production of rearrangements by CB 1506 than by X-rays. This conclusion, however, remains doubtful without comparable data on CB 1506-treated spermatozoa. Many chemicals produce low frequencies of rearrangements relative to lethals, probably because of their delayed mutagenic action. There is some evidence (Auerbach, unpub.) that mutagenic delay is marked after treatment with CB 1506; this might result in a very low frequency of translocations even in postmeiotic stages. The weak action of CB 1506 on these stages makes it difficult to test this. It would seem worth while

to use chemical mutagens with less eccentric brood patterns, even though with more rapid chemical action, for a comparison between the translocation-to-lethal ratio in successive broods. Indications that the drop in this ratio is less drastic after chemical treatment than after irradiation would lend support to the hypothesis that the shortage of rearrangements in spermatogonia is due to obstacles that can be overcome more readily by chemicals than by X-rays.

SUMMARY

Adult *Drosophila* males were injected with chloroethyl methanesulphonate and late progeny, tracing back to treated spermatogonia, were examined for sex-linked lethals and for translocations involving the X, Y, second and third chromosomes. A dose yielding about 20% sex-linked lethals produced 11 translocations in about 3500 tested chromosome sets (approx. 0·3%). This result is discussed in relation to the problem of chromosome rearrangements in different germ-cell stages.

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