are involved in the formation of antibodies. As has been emphasized elsewhere (*7*), antibody formation can be interpreted as protein synthesis modified by the presence of antigen molecules. Actually, mitochondria have been considered to be endowed with the property of self-duplication (*1*), which implies protein synthesis.

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The Differential Induction of Lethal Mutations by Formalin in the Two Sexes of *Drosophila*[1]

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Several studies have been made recently in an attempt to administer chemical mutagens to eggs and sperms at various stages in ontogeny, followed by tests to detect variation in the mutation rate. It was shown that, whereas sex-linked recessive lethal mutations were induced in both eggs and mature sperms of adult *D. melanogaster* after vaginal douches with the N mustard methyl bis (ß-chloroethyl) amine hydrochloride (*6, 7*), there was no increase in the number of such mutations in either type of gamete when larvae were exposed to sublethal concentrations of this substance in the food (*8*). On the other hand, vaginal douches with formaldehyde failed to increase the mutation rate in eggs and mature sperms (*7*) but, although female larvae were not studied, large numbers of lethals occurred in the sperms of male larvae grown on food containing this chemical (*3, 9, 11*). Auerbach (*3*) has reported that formaldehyde fails to induce mutations in either type of gamete, mature or immature, when this substance is administered directly. She has presented evidence suggesting that an active mutagenic agent is produced only when formaldehyde is first mixed with the food. The ability of a chemical substance to induce mutations with some techniques and not with others may have several possible explanations (*1, 4, 7*). Such factors as the solvent for, the concentration of, and the duration of treatment with the chemical substance used may account for the results obtained with different techniques.

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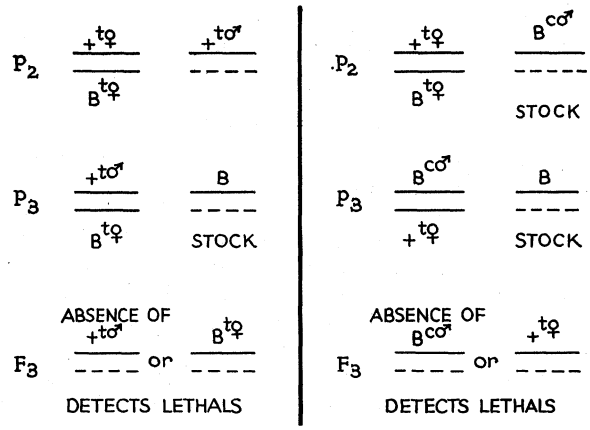

FIG. 1. Plan of matings to detect X-chromosome recessive lethals in progeny from P_1 crosses of Ore-R ♀♀ (+/+) by M-5 ♂♂ (B/Y). All ♀♀ virgin; all matings but P_1 in single pairs; solid line = X, broken line = Y chromosome; t = treated, c = untreated chromosome; ♀ or ♂ = P_2 parent from which chromosome came; up to 10 P_3 matings from each P_2 cross.

In the experiments reported here, *D. melanogaster* Oregon-R wild-type ♀♀ were crossed to Muller-5 ♂♂ and permitted to oviposit for 2 days in bottles containing 50 ml of a standard culture medium. After removal of the parents, 0.75 ml of a 8.9–17.8% solution of formaldehyde was added by pipette on top of the food, and the F_1 ♀♀ and ♂♂ were permitted to complete their development. For the P_2, half the F_1 ♀♀ were crossed to F_1 ♂♂, the other half to stock (untreated) Muller-5 ♂♂. The detailed plan of matings to detect sex-linked recessive lethal mutations arising in the gonads of P_2 parents is presented in Fig. 1. These lethals, detected in the F_3 generation, are of the following 4 types: + chromosome from treated F_1 ♀♀ ($+^t$♀); B chromosome from treated F_1 ♀♀ (B^t♀); + chromosome from treated F_1 ♂♂ ($+^t$♂); B chromosome from untreated P_2 Muller-5 ♂♂ (B^c♂). Lethals were retested for confirmation (*7*). The results are presented in Table 1.

A total of only 10 lethals occurred in 4,493 X chromosomes tested from control males (B^c♂) and treated

TABLE 1

TYPE OF LETHAL

	$+^t$♀	B^t♀	$+^t$♂	B^c♂
No. lethals	3	4	57	3
No. X chromosomes tested	1,546	1,401	1,401	1,546

female larvae (+ t♀ and Bt♀). However, in the 1,401 X chromosomes tested from treated male larvae (+ t♂), there were 57 lethals arising from 47 independent origins. Although this increase in the mutation rate of treated males is comparable with the results obtained by others (*3, 9, 11*), the mutation rate of females treated in the same environment is not detectably different from that of the untreated controls. Differential frequencies of lethals in the two sexes have been reported previously for spontaneous mutation (*3*) and for mutations induced by mustard gas (*2*), x-rays (*5*), and P^{32} (*10*). Whether this sexual difference in mutation rate is due to some morphological or physiological difference between male and female *Drosophila* or whether it is due to an innate difference in the mutability of the sex cells themselves remains undetermined. Nevertheless, should this type of phenomenon prove to be of general occurrence it would have interesting implications concerning the function of sex in the statics and dynamics of evolution.

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Lethal Mutation Rate in *Drosophila* Treated with 20-Methylcholanthrene[1]

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Reports on chemical mutagenesis (*2*) have revived the old idea that mutations are responsible for cancer. A number of authors have inferred that the parallelism between mutagenic and carcinogenic properties of certain chemicals lends support to this hypothesis (*6, 14*). The data presented are results of tests for the possible mutagenic activity of the carcinogen 20-methylcholanthrene in *Drosophila melanogaster.*

In the first group of experiments, virgin, heterozygous females derived from the Oregon-R and Muller-5 stocks were treated with 1% 20-methylcholanthrene in sesame oil by the vaginal douche technique described by Herskowitz (*8*). The carcinogen used was from a solution which previously had been tested and found to be fully potent in producing sarcomas in C_3H and JK mice and their progeny (*4*). It was injected into the vagina, which was partially everted by lateral pressure on the abdominal wall. Adult females 3 days old were treated and mated individually to Muller-5 males 5 days old. The male was removed after 24 hr and the female allowed to oviposit for 3 days in each of 3 vials. One portion of the offspring was then tested for lethals and another group treated, repeating this each generation.

The Muller-5 method of testing for recessive lethal mutations on the X-chromosome was utilized. Ordinarily the Oregon-R/Muller-5 heterozygous female has both wild type (+) and sc^8 w^a B sons. However, if a lethal is present on the chromosome from the Oregon-R stock, only sc^8 w^a B males appear among the offspring. Conversely, a lethal present on the Muller-5 (sc^8 w^a B) chromosome will result in + offspring only. The advantages of the method in this investigation are that heterozygous females which appear each generation may be used subsequently for retesting for lethals when poor cultures are obtained, and serial treatment is possible. In all cases where lethals were suspected, the chromosome was retested by the same method. All lethals reported have shown no male bearing the lethal chromosome among at least 50 males of the opposite type. Every lethal was retested at least once. The vaginal douche treatment was continued serially for 11 generations over a period of 128 days, mating females heterozygous for the wild-type and Muller-5 chromosomes to Muller-5 males. Since the wild-type chromosome may be recovered in the female progeny in each generation, it is possible to continue treatment of the chromosome in subsequent generations. The wild type chromosome tested in each successive generation had therefore been treated from 1 to 11 times. The total period of treatment of the + chromosome, therefore, was longer than the time required to induce tumors with this carcinogen in certain strains of mice (*5*).

The investigation originally was designed to test the effect of serial administration of the carcinogen on mutation rate, but, when it was found that very few mutations appeared, a different method for administering the chemical was adopted. In the second series of experiments Oregon-R males were treated with 1% 20-methylcholanthrene in sesame oil in the form of an aerosol with air flowing through the nebulizer at the rate of 6 l per min for a period of 30 sec every 30 min. The lethal mutation rate was then determined by the Muller-5 method for flies treated 15, 24, 48, 72, 96, and 216 hours, respectively.

Control values for mutation rate were obtained by mating females heterozygous for the Oregon-R and Muller-5 chromosomes to Muller-5 males, rather than obtaining the rate in each stock separately. The medium used contained Cream of Wheat, molasses, agar, and yeast. The matings were made in vials and the temperature was maintained at 25° C.[2]

There was a high mortality with both forms of treatment. It was found that 52% of the females died following vaginal douche and that only 37.2% of those surviving were fertile. Most of those that were sterile laid

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