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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[2] The author is indebted to Betty Rosenbohm for her excellent technical assistance.

TABLE 1

LETHAL MUTATION RATE AFTER SERIAL ADMINISTRATION OF 20-METHYLCHOLANTHRENE IN A VAGINAL DOUCHE

| Generation | Females injected | Females tested | Chromosomes tested | Lethal mutations | | | | | | Visible | | Nondisjunction | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | + chromosome | | B chromosome | | Total | | Total | | | |
| | | | | No. | % | No. | % | No. | % | No. | % | No. | % |
| 1 | 36 | 2 | 86 | | | | | | | | | | |
| 2 | 54 | 3 | 54 | | | | | | | | | | |
| 3 | 56 | 35 | 1,224 | | | 1 | .163 | 1 | .082 | 1 | .163 | 1 | .163 |
| 4 | 39 | 28 | 1,116 | | | | | | | | | | |
| 5 | 27 | 18 | 676 | | | | | | | | | | |
| 6 | 34 | 10 | 224 | | | | | | | | | | |
| 7 | 50 | 19 | 398 | | | 1 | .503 | 1 | .251 | | | | |
| 8 | 34 | 9 | 206 | | | | | | | | | | |
| 9 | 35 | 2 | 22 | | | | | | | | | | |
| 10 | 30 | 16 | 500 | | | | | | | | | | |
| 11 | 34 | 7 | 154 | 1 | 1.299 | .. | ... | 1 | 0.649 | .. | ... | .. | ... |
| Total | 429 | 149 | 4,660 | 1 | .043 | 2 | .086 | 3 | .064 | 1 | .021 | 1 | .021 |

no eggs (59.2% of the total), doubtless as a result of the trauma. (The number of males dying after administration of aerosol appears in Table 2.)

The lethal mutation rate following serial administration of 20-methylcholanthrene in a vaginal douche was found to be .064%. The rates for each generation in the series appear in Table 1. One mutation on the + chromosome appeared in the 11th generation, and one occurred on the $sc^s$ $w^a$ B chromosome in the 3rd and one in the 6th generation. These three lethal mutations occurred among 4,660 chromosomes tested. In the 4th generation a *yellow* male was found when tests were made for lethals. This gene was present on the wild-type chromosome. An instance of nondisjunction of the X-chromosome was found and confirmed in subsequent generations.

When the carcinogen was administered as an aerosol (Table 2), 5 lethal mutations were found on the + chromosome and 5 on the $sc^s$ $w^a$ B chromosome among 10,108 chromosomes tested, a mutation rate of .099%. It should be noted that only the + chromosome was treated by this method. Lethals occurred in the groups treated 15, 72, and 216 hours, but not in those when the treatment period was 24, 48, and 96 hours. Two of the lethals on the + chromosome occurred among 12 offspring of a single male. Also in the progeny from each of two other matings there were two lethals on the $sc^s$ $w^a$ B chromosome. More offspring had been tested, however, 85 in one instance and 26 in the other.

Demerec (*6, 7*) has reported a number of carcinogenic compounds to be mutagenic and has included 20-methylcholanthrene among them. Sacharow (*13*) obtained 18 lethals in 2,921 chromosomes by treating *Drosophila* eggs with methylcholanthrene, and 87 lethals among 33,975 chromosomes tested in the control series.

Preliminary experiments with feeding carcinogens gave negative results in experiments by Auerbach, and, later more extensive studies on mutation rate following the injection of carcinogens, including methylcholanthrene, also gave negative results (*1*). Bhattacharya (*3*) found no increase in mutation rate when methylcholanthrene was fed to larvae. It is pointed out in the report that there is no significant difference between the positive results reported by Demerec and the results Bhattacharya obtained in the feeding experiments, although the latter were negative when compared to controls.

The use of mice in quantitative work on mutations has definite limitations and is the reason this investigation was done with *Drosophila*. The detection of visible mutations is somewhat subjective, and inversions are not available for use in detecting lethal or visible, recessive mutants. There is the undeniable advantage, however, that mice bear tumors very similar to those in man.

TABLE 2

LETHAL MUTATION RATE (20-METHYLCHOLANTHRENE AEROSOL)

| Treatment period | Died with treatment | Males tested | Chromosomes tested | Lethals | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | + chromosome | | $sc^s$ $w^a$ B chromosome | | Total | |
| | | | | No. | % | No. | % | No. | % |
| 15 hr | 26 | 51 | 1,484 | 1 | .135 | | | 1 | .067 |
| 24 hr | 1 | 41 | 1,858 | | | | | | |
| 48 hr | 10 | 51 | 1,060 | | | | | | |
| 72 hr | | 41 | 1,786 | 3 | .336 | | | 3 | .168 |
| 96 hr | 21 | 19 | 290 | | | | | | |
| 216 hr | 71 | 74 | 3,630 | 1 | .055 | 5 | .275 | 6 | .165 |
| Total | 129 | 277 | 10,108 | 5 | .099 | 5 | .099 | 10 | .099 |

TABLE 3

LETHAL MUTATION RATE

| Treatment | Chromosomes tested | Lethals | | | | | | P |
|---|---|---|---|---|---|---|---|---|
| | | + chromosome | | $sc^{8}$ $w^{a}B$ chromosome | | Total | | |
| | | No. | % | No. | % | No. | % | |
| Control | 2,822 | 1 | .071 | 1 | .071 | 2 | .071 | |
| 20-Methylcholanthrene (vaginal douche) | 4,660 | 1 | .043 | 2 | .086 | 3 | .064 | 0.99 |
| 20-Methylcholanthrene (aerosol) | 10,108 | 5 | .099 | 5 | .099 | 10 | .099 | 0.7 |

Strong (*14*) has reported numerous variants in strains treated with 20-methylcholanthrene for a number of generations, including color changes and differences in susceptibility to tumors. He regards these as mutations induced by the chemical and thinks it is probable that methylcholanthrene may bring about malignancy by causing somatic mutations. In commenting on this work, Auerbach (*2*) has questioned the accuracy of the comparison of the experimental mutation rate (1 in 557) to the control (1 in 26,250) given by Strong, although she was impressed by the large number of variants in an animal in which visible mutations rarely occur.

Tatum (*15*) has reported the appearance of 6 biochemical mutant strains among 2,075 cultures of *Neurospora* treated with 20-methylcholanthrene endosuccinic acid. One apparently contained 2 independent gene mutations. Control studies from another of his experiments showed 1 biochemical and 6 morphological mutants. He mentioned a high incidence of morphological mutants with methylcholanthrene but did not feel that a significant difference had been established by the data at hand. He did, however, state that results with *Neurospora* suggest that this carcinogen has mutational effects. On the other hand, Latarjet (*11*) found no increase in mutation rate when methylcholanthrene was used to treat bacteria, even though enough of the chemical was present to cause the organisms to fluoresce.

The figures for mutation rate in *Drosophila* obtained in these experiments reveal that there is no significant difference (Table 3) between control rate and that for treatment by vaginal douche ($P = 0.99$) or for treatment with methylcholanthrene aerosol ($P = 0.7$). The appearance of a *yellow* male and the instance of nondisjunction are not regarded as evidence to the contrary. The experiment was not designed for determining the rate of visible mutations or the incidence of nondisjunction. Also, *yellow* is known to mutate spontaneously more frequently than many other loci in *Drosophila*.

The discrepancy between these and other negative findings and those which have indicated that 20-methylcholanthrene is mutagenic may be explained in several ways. It is possible that the carcinogen was not present in optimum concentration over a sufficient period to be effective as a mutagen. It is also possible that the route of administration or the vehicle in which it was given did not allow access to the chromosomes. Finally, mutagenic effects may be strain- or species-limited.

Factors which would operate toward giving false negative results were minimized as much as possible. The concentration of carcinogen used was sufficiently high so that further increase does not reduce the induction time of subcutaneous tumors in inbred mice, although the high concentration is not necessarily optimum. More than one route of administration of the carcinogen was utilized, and both methods have given positive results with nitrogen mustard in other work by the author. Chromosomes from the males were treated by means of the aerosol, and those from both males and females by the vaginal douche technique. In the latter the treatment was repeated and perhaps prolonged. The stock of Oregon-R used was obtained from the laboratory of Dr. Demerec, and the aerosol method he described (*7*) was duplicated as nearly as possible.

Undoubtedly chemicals can induce mutations. Some of these chemicals are also carcinogens. For example, methyl-bis(β chloroethyl)amine hydrochloride (*2*) and urethane (*16*) are capable of inducing mutations and also of increasing the incidence of mouse tumors (*9*, *12*). Others, such as formaldehyde, are mutagenic (*10*) but not carcinogenic. There is little basis at the present time, however, for the assumption that all carcinogens are mutagens. Even in the reports of Demerec (*6*), there are negative results for agents known to be carcinogenic. The results reported in this communication do not support the hypothesis that 20-methylcholanthrene is a mutagen, since the mutation rate of lethals on the X-chromosome was not increased either by administering 1% 20-methylcholanthrene in sesame oil by vaginal douche serially for 11 generations over a period of 128 days or as an aerosol during periods of from 15 to 216 hours. More work is in order to clarify the reasons for the differences in results with this carcinogen. There would seem to be a reasonable doubt that there is necessarily a connection between mutagenic and carcinogenic effects of an agent or that carcinogens are necessarily mutagens. At the present time there are even more obstacles in accepting without reservation the hypothesis that tumors are the direct result of somatic mutations.

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## Arylcycloalkylamines

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Sympathomimetic amines in which the basic side chain is part of a saturated ring not condensed with their aromatic portion have been the subject of recent studies (*1*). The excellent paper by Gutsche (*3*) concerning ring enlargements with diazomethane and phenyldiazomethane prompts us to record similar reactions used by us for the preparation of intermediates in the synthesis of phenyl- and diphenylcycloalkylamines in an extension of this series.

We prepared 2-phenylcycloheptanone (bp 133°–137°, 1 mm) by the slow methanol catalyzed reaction of cyclohexanone and phenyldiazomethane in a yield of 43%. The ketone yielded 2-phenylcycloheptylamine by the Leuckart reaction, and 5,5-(2′-phenylhexamethylene) hydantoin by the Bucherer method. In a similar way, 2-methylcycloheptanone (*2*) furnished 2-methylcycloheptylamine, and 5,5-(2′-methylhexamethylene) hydantoin, respectively. 4-Methylcycloheptylamine was prepared from 4-methylcycloheptanone (*5*).

Structural analogues of Methadon were obtained by subjecting 2,2-diphenylcyclohexanone (*4*) to the Mannich reaction with secondary amines. The resulting 6-dialkylaminomethyl-2,2-diphenylcyclohexanone derivatives, and the corresponding amino alcohols, are listed in Table 1. 2,2-Diphenyl-6-bromocyclohexanone, obtained from the parent ketone by bromination, has also been converted to 2,2-diphenyl-6-dialkylaminocyclohexanone derivatives.

TABLE 1

PHYSICAL PROPERTIES AND ANALYSES OF SUBSTITUTED CYCLOALKANE DERIVATIVES

| Compound | Mp, °C | Formula | Percentage composition | |
|---|---|---|---|---|
| | | | Calculated | Found |
| 2-Phenylcycloheptanone semicarbazone | 154–156 | $C_{14}H_{19}N_3O$ | N, 17.13 | 17.42 |
| 2-Phenylcycloheptylamine · HCl | 196.5–197.5 | $C_{13}H_{20}ClN$ | C, 69.16 | 68.52 |
| | | | H, 8.93 | 8.95 |
| | | | N, 6.20 | 6.45 |
| 5,5-(2′-Phenylhexamethylene) hydantoin | 204.5–207.5 | $C_{15}H_{18}N_2O_2$ | C, 69.74 | 69.57 |
| | | | H, 7.02 | 7.00 |
| | | | N, 10.85 | 10.89 |
| 2-Methylcycloheptylamine · HCl | 225.5–227.5 (dec.) | $C_8H_{18}ClN$ | C, 58.70 | 58.63 |
| | | | H, 11.08 | 10.91 |
| | | | N, 8.56 | 8.45 |
| 5,5-(2′-Methylhexamethylene)hydantoin | 216–218.5 | $C_{10}H_{16}N_2O_2$ | C, 61.20 | 61.30 |
| | | | H, 8.22 | 8.74 |
| | | | N, 14.28 | 14.78 |
| 4-Methylcycloheptylamine · HCl | 207–209 | $C_8H_{18}ClN$ | C, 58.70 | 59.24 |
| | | | H, 11.08 | 11.21 |
| | | | N, 8.56 | 8.27 |
| 2,2-Diphenyl-6-dimethylaminomethylcyclohexanone | 106–107 | $C_{21}H_{25}NO$ | C, 82.04 | 82.18 |
| | | | H, 8.20 | 8.18 |
| 2,2-Diphenyl-6-dimethylaminomethylcyclohexanol | 108.5–109.5 | $C_{21}H_{27}NO$ | C, 81.51 | 81.35 |
| | | | H, 8.80 | 8.80 |
| 2,2-Diphenyl-6-morpholinomethylcyclohexanone · HCl | 160 | $C_{23}H_{28}ClNO_2$ | C, 71.58 | 71.78 |
| | | | H, 7.31 | 7.54 |
| 2,2-Diphenyl-6-bromocyclohexanone | 117–119.5 | $C_{18}H_{17}BrO$ | C, 65.66 | 65.69 |
| | | | H, 5.21 | 5.39 |
| 2,2-Diphenyl-6-morpholinocyclohexanone | 124.5–125 | $C_{22}H_{25}NO_2$ | C, 78.77 | 78.98 |
| | | | H, 7.51 | 7.71 |
| 2,2-Diphenyl-6-piperidinocyclohexanone | 121.5–122 | $C_{23}H_{27}NO$ | C, 82.84 | 83.12 |
| | | | H, 8.16 | 7.63 |

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