Effect of Nitrogen Mustard on Tumor Incidence and Lethal Mutation Rate in Drosophila*

WALTER J. BURDETTE

(Department of Surgery, Louisiana State University School of Medicine, New Orleans, La.)

One of the most stimulating findings in recent years is that of Auerbach and co-workers (2) that the nitrogen mustards are mutagenic. It has focused attention on the possibilities of chemical induction of mutations and has prompted numerous investigations. Nitrogen mustard has also been found carcinogenic (4, 11, 12, 13) for mice, as well as palliative in certain types of cancer in man. Therefore, in any study of the relation between mutations and tumors, the action of this chemical might well be examined carefully. In this investigation methylbis(2-chloroethyl)amine hydrochloride has been administered to a tumor strain of Drosophila, and the lethal mutation rate and tumor incidence have been determined simultaneously.

METHODS

Fifty males and 50 females from siblings of the tu 36a strain with a normal sex ratio were mated in a bottle containing 50 ml. of culture medium covered with growing yeast. When 8 days old these flies were treated with 1 per cent methylbis(2-chloroethyl)amine hydrochloride in propylene glycol, administered as an aerosol. The aerosol was generated intermittently for 30 seconds every 30 minutes for periods of both 24 and 48 hours. A control culture was prepared at the same time each experimental culture was treated and followed subsequently in the same manner. One transfer to a fresh bottle was made at the end of 3 days from the beginning of treatment. Separate records were kept for these cultures. Offspring from the treated flies were examined after eclosion, and the number of tumors was tabulated. A representative number of females from this generation were mated individually to sc51 B InS wK sc* males and the lethal mutation rate determined by the Muller-5 method. A more extended discussion of the method may be found in a previous publication (6).

RESULTS

Ten per cent of the males from the group treated 24 hours bore tumors, but only 1.39 per cent of those without treatment were tumor-bearing (Table 1). The females in the treated group also had a higher percentage of tumors (5.66 per cent) than those in the control group (0.21 per cent). There were 15 tumor-bearing flies among 196 with antecedent treatment and only 7 among 904 without. All these differences in incidence are significant (P < 0.0001).

The results of 48-hour treatment on tumor incidence are given in Table 2. There were 27 males

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and 19 females with tumors after treatment, in populations of 384 and 392, respectively—an incidence of 7.03 per cent in males and 4.85 per cent in females. In comparison, the control cultures contained only 3 tumors in 362 males and 1 tumor in 407 females. The incidence of 46 tumorous flies among 776 flies following treatment is significantly higher than 4 among 769 (P < 0.0001). Although they are not truly comparable in a temporal sense, the data for original and transfer cultures are presented in Table 3.

The lethal mutation rate (Table 4) was 2.04 per cent when treatment extended over a period of 24 hours, a significantly higher rate than the 0.09 per cent among those without treatment (P < 0.0001). In Table 5 the lethal mutation rate is given for the 48-hour treatment period with nitrogen mustard. There were 29 lethals among 781 chromosomes tested, an incidence of 3.71 per cent in the treated group, and only one lethal among 983 chromosomes tested in the control group, an incidence of 0.10 per cent. This difference in mutation rate is clearly significant (P < 0.0001).

**DISCUSSION**

The incidence of tumors is definitely higher after administration of nitrogen mustard to this strain of Drosophila. This is true when individual cultures are compared to parallel controls, for males, for females, and for the entire group considered as a whole. The fact that 48-hour administration results in no higher incidence than 24-hour treatment is probably most easily explained by the rapid disappearance of the active form of the chemical. Although it is tempting to ascribe the higher incidence of tumors in the transfer cultures to a delayed effect, the absence of significant differences statistically does not justify it.

Boyland and Horning (4) treated stock mice with weekly subcutaneous injections of methylbis(2-chloroethyl)amine hydrochloride and methyltris(2-chloroethyl)amine hydrochloride. They found ten tumor-bearing mice out of fourteen surviving for more than 280 days. There were lung carcinomas and adenomas in eight, lymphosarcomas in two, one fibromyoma of the uterus, and one spindle-celled sarcoma at the site of injection. In a control group of 40 mice, killed between 14 and 18 months of age, six had adenomas of the lung and two had hepatomas. The tumors in treated mice were larger and had a more malignant appearance, and the earliest tumor was found at 284 days. Heston has shown that the incidence and average number of pulmonary adenomas in the A strain are increased by the intravenous injection of methylbis(2-chloroethyl)amine hydrochloride (12) and also by the same treatment with sulfur mustard, bis(2-chloroethyl)sulfide (13). Griffin, Brandt, and Tatum (11) report that Swiss mice and albino rats, treated intravenously, subcutaneously, and intraperitoneally with the same chemicals used by Boyland and Horning, developed tu-
strain for both 24- and 48-hour periods. This confirms the findings of Auerbach and Robson (2) and others, using tumor-resistant stocks. Nitrogen mustard is apparently a powerful mutagen in the hands of all who have used it, and conflicting reports (such as those made on the mutagenic effects of the carcinogenic hydrocarbons) are not encountered when this chemical is used.

The data presented indicate that both tumor incidence and mutation rate are increased simultaneously when nitrogen mustard is administered to a strain of tumor-bearing flies. The question of causal relationship between the increased mutation rate and increased numbers of tumors deserves some consideration. Before an affirmative answer is given too hastily, the results in other studies of similar design should be reviewed. It was found that 20-methylcholanthrene is tumorigenic but not mutagenic for this strain (9). Formaldehyde increased the mutation frequency in males but did not alter the incidence of tumors for either males or females (7). Diethylstilbestrol changed neither mutation rate nor tumor incidence (8). It is apparent that increased mutation rate following treatment is not always associated with increased numbers of tumors, and increased numbers of tumors after treatment are not always associated with higher mutation rate. The possibility that the combination of mutagenic and tumorigenic properties is fortuitous in the case of nitrogen mustard cannot therefore be eliminated at this time. The discrepancies mentioned and the lack of evidence for mutational effects of carcinogenic hydrocarbons in Drosophila (1, 3, 5, 9, 10) should be resolved before the evidence presented in this communication can be used to support the somatic mutation hypothesis of tumor etiology. The facts seem clear and are harmonious with the results of others, but the interpretation is best deferred in view of other current information. The double action of nitrogen mustard makes this group of chemicals and others with similar properties important tools in continued investigation of the causes of cancer.

CONCLUSIONS

1. Methylbis(2-chloroethyl)amine hydrochloride, administered as an aerosol to the tu 36a tumor strain of Drosophila, increased the tumor incidence significantly.
2. This chemical also caused a simultaneous increase in lethal mutation rate.
3. A causal relationship between these two properties of this chemical should not be assumed as necessarily true until certain discrepancies are resolved.

REFERENCES

7. ———. Tumor Incidence and Lethal Mutation Rate in a Tumor Strain of Drosophila Treated with Formaldehyde. Ibid. pp. 555-58.
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Walter J. Burdette