

It is precisely upon such a process that an anti-mutagen might be expected to act. However, no reduction of the mutagenic activity of x-radiation was revealed by the methods used. The number of chromosomes tested was, however, small in Brood 2. This was due to the marked decrease in fertility which characteristically occurs in later broods following irradiation of adult males (2). Nevertheless, despite the extreme smallness of the sample, the similarity of groups A and B, both in the frequency of sex-linked recessive lethals and in the falling off of fertility, indicates that if mercaptoethylamine has any influence upon the genetic effects of x-radiation in *D. melanogaster*, it cannot be measured by the techniques employed. It did not, therefore, seem worth while to continue the work on a larger scale.

The data derived from group C and listed in Table 2 show that, by itself, this amine is not a mutagen. The spontaneous sex-linked recessive mutation rate for this stock is 0.3%.

An attempt was also made to determine whether mercaptoethylamine protects *Drosophila* against the lethal somatic effects of radiation. Two groups of flies, mercaptoethylamine-injected and controls, were each given 82,000 r in 41 min, but at this dose both groups survived. It was not possible to carry this investigation further.

References

1. BACQ, Z. M., and HERVE, A. *Bull. acad. roy. méd. Belg.*, **17**, 13 (1952).
2. LÜNING, K. G. *Hereditas*, **38**, 91 (1952).

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Failure of Mercaptoethylamine to Protect against the Mutagenic Effects of Radiation II: Experiments with Mice

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The negative results obtained using *Drosophila melanogaster* as the experimental animal did not, by themselves, rule out the possibility that β -mercaptoethylamine might possess antimutagenic properties. Two possible explanations of the results obtained are its inability effectively to reach the germ cells, and its very rapid destruction by the tissue fluids of the insect. Consequently, it was decided to carry out a test for antimutagenicity in mice, where the protective action against the lethal somatic effects of x-radiation has been so clearly demonstrated by Bacq and Herve.

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To this end male mice were irradiated with 500 r of x-radiation. After such a dose there is first a period of reduced fertility and then a period of complete sterility followed by a return to normal fertility. Snell (1) has shown that the reduced fertility immediately after irradiation is largely due to dominant lethals which cause the death of embryos soon after implantation. The proportion of implanted embryos which were inviable was therefore taken as a measure of dominant lethality in the present experiment. The effects of mercaptoethylamine on both the induction of dominant lethals and the onset of complete sterility were examined.

Two groups of 6 CBA inbred mice were used. Both groups of males received 500 r of x-radiation (70 kv, 7 ma; filter 0.5 mm Al; H.V.L. 0.8 mm Al; 165 r/min) while under Nembutal anesthesia (10% Nembutal in Ringer's solution, 0.1 ml per 10 g body weight, injected intraperitoneally). One group received in addition an intraperitoneal injection of 4 mg mercaptoethylamine in Ringer's solution, given after the onset of anesthesia but before irradiation. The control group received no such injection. The time interval between the mercaptoethylamine injection and irradiation was 4-7 min. The mice were irradiated individually and only the hindquarters and scrotum were exposed.

Directly after treatment each male of the control and experimental groups was placed in a cage with 5 normal females and remained there for 10 days. Thereafter, 3 more batches, each of 5 females, were presented to each male for 3 further 10-day periods. The presence of a vaginal plug was taken as an indication that mating had occurred. In addition, some pregnancies were detected by the "placental sign" (2) although no vaginal plug had been seen. The females were killed after 12-14 days gestation, the uterus opened, and the numbers of live and dead embryos and of corpora lutea determined. From these data 3 ratios were calculated:

$$\frac{\text{Number of live embryos}}{\text{Total number of embryos implanted}} \quad (1)$$

This ratio is equivalent to the hatchability measurements in the *Drosophila* experiments (Table 1a).

$$\frac{\text{Total number of embryos}}{\text{Number of corpora lutea}} \quad (2)$$

This ratio indicates the proportion of successfully implanted ova out of the total number shed (Table 1b).

$$\frac{\text{Number of pregnancies}}{\text{Number of vaginal plugs observed}} \quad (3)$$

This ratio serves as an indication of the fertility of the male (Table 1c). (Pregnancies which were detected without the finding of a vaginal plug were not included in this tabulation.)

Table 1a records the embryo viability within the four 10-day mating periods for the controls and mercaptoethylamine-treated males. The differences between the 2 groups, for any one period, are not statistically significant. Thus, the amine had no de-

TABLE 1
OBSERVATIONS ON THE OFFSPRING Sired BY IRRADIATED MALE MICE

Treatment of male	1		2		3		4		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
<i>a. Ratio of Live to Total Embryos*</i>										
Control (R)†	41/67	61.2	37/80	46.3	27/47	57.4	1/1	—	106/195	54.4
Mercaptoethylamine (I + R)	62/105	59.0	35/85	41.2	15/18	83.3	1/2	—	113/210	53.8
<i>b. Ratio of Total Embryos to Corpora Lutea</i>										
Control (R)	67/91	73.6	84/103	81.6	47/69	68.1	1/9	—	199/272	73.2
Mercaptoethylamine (I + R)	97/126	77.0	81/101	80.2	18/38	47.4	2/7	—	198/272	72.8
<i>c. Ratio of Pregnancies to Vaginal Plugs Seen</i>										
Control (R)	6/11	—	6/9	—	7/13	—	0/14	—		
Mercaptoethylamine (I + R)	13/14	—	10/14	—	4/14	—	1/9	—		

* Small discrepancies in total numbers of embryos between *a* and *b* were due to the exclusion of 1 or 2 litters in which the number of corpora lutea or viability of particular embryos were in doubt.

† I, injected with mercaptoethylamine; R, irradiated.

monstrable influence upon the induction of dominant lethals by the radiation.

Tables 1*a* and 1*b* show further that between the 2nd and 3rd periods there was a marked falling off in the total number of embryos in both the control and the amine-injected groups. The 4th period was one of almost complete sterility. Table 1*c* shows that the proportion of fertile matings fell off similarly. These findings agree with Hertwig's (3) histological study which showed that spermatogonia were almost entirely destroyed by a comparable dose of radiation, resulting in a complete lack of mature sperm after an interval of a few weeks. In none of these tables is there any significant difference in fertility between the control and treated males. This clearly shows that male germ cells were not afforded any protection by mercaptoethylamine against radiation death.

After the end of the 4th mating period several males from the treated and control groups were killed, their testes fixed and sectioned, and subsequently compared, histologically, with those of nontreated males. The testes from the 2 x-rayed groups were similar but differed from those that had received no radiation chiefly in the relative numbers of mature spermatozoa and spermatids. In the latter case there were many mature sperm cells and spermatids in various stages of spermiogenesis, whereas in the former case there was only a small number of mature or maturing sperm. There were no obvious differences in the relative numbers of spermatogonia and spermatocytes in the seminiferous tubules of the 3 groups of testes. This picture indicates that the period of sterility was approaching its end. However, the histological picture strengthens the genetic results in indicating that the mercaptoethylamine had no effect in protecting the germ cells against radiation.

The combined *Drosophila* and mouse studies clearly

indicate that mercaptoethylamine has no influence upon the genetic effects of radiation as measured in these experiments; nor does it protect the male germ cells against radiation death. These findings are thus in line with the more recent work of Bacq and his colleagues (4) suggesting that mercaptoethylamine exerts its protective action through the liver and that the primary effects of radiation on other organs are not prevented.

References

1. SNELL, G. D. *J. Exptl. Zool.*, **65**, 421 (1933).
2. ———, *Biology of the Laboratory Mouse*, p. 40. Philadelphia: Blakiston, 1941.
3. HERTWIG, P. *Arch. Exptl. Zellforsch. Gewebezücht.*, **22**, 68 (1938).
4. BACQ, Z. M., *et al. Science*, **117**, 633 (1953).

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Preparation of High-Purity Lithium Metal by Vacuum Distillation

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In connection with the stable isotope program of this laboratory, measurements of physical properties of isotopically pure, or nearly pure, isotopes and their compounds are being carried out to determine possible significant differences in their values. For example, as a result of mass difference, there may be some variations in melting points and boiling points of the isotopes. For dependable values, however, it is also necessary to have high chemical purity.

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