Technical Papers

Failure of Mercaptoethylamine to Protect against the Mutagenic Effects of Radiation I: Experiments with *Drosophila*

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The work of Bacq and Herve (*1*) has shown that β-mercaptoethylamine ($SH—CH_2—CH_2—NH_2$) affords protection against the lethal effects of radiation and produces a marked decrease in mortality when administered intraperitoneally to mice during the half-hour preceding whole body irradiation. However, Bacq and Herve did not determine whether this substance also protects against the mutagenic effects of radiation. Accordingly, upon the suggestion of Bacq, the possibility of its being an antimutagen was tested in small scale experiments using *Drosophila melanogaster* and mice as the test organisms. Consistently negative results were obtained.

In the *Drosophila* experiments 3 groups of day-old adult wild-type males were used. Flies of groups A and C had injected into their abdomens a solution of Labaz β-mercaptoethylamine diluted to a final concentration of 1/1000 in 0.75% sodium chloride. The quantity of fluid injected, 0.2–0.3 mm^3, was sufficient noticeably to distend the abdomens of the flies. Each fly, therefore, received approximately 0.25γ of mercaptoethylamine. This amount was somewhat more per unit body weight of fly than the amount injected per unit body weight of mouse in Bacq's work. Group B was injected with 0.75% sodium chloride. Groups A and B were irradiated simultaneously and received 2400 r of x-radiation (70 kv, 7 ma; filter 0.5 mm Al; H.V.L. 0.8 mm Al; 165 r/min) approximately 15 min after the injections. Group C was not irradiated. Determinations of dominant and sex-linked recessive lethals were performed on all three groups.

Dominant lethality is defined as (1 − hatchability). To measure it males of Groups A, B, and C were mated to virgin Muller-5 females, the eggs were collected, and the hatchability determined. Ten males from each group were used and pair matings were set up, each male being kept with the female for 3 days, then being presented with a fresh virgin female for a second period of 3 days. Hatchability was measured for each 3-day period. Thus, data were obtained from 2 broods, the male gametes producing the 2nd brood having been treated at an earlier stage of spermatogenesis than the gametes of the 1st brood. Table 1 summarizes these data. The results with the nonirradiated group C indicate that mercaptoethylamine, by itself, has no significant effect upon hatchability, the figures obtained being within the standard hatchability range of the stock used. Among the irradiated males there was no significant difference between the saline-injected group (B) and the amine-injected one (A). In both groups there was a sharp decrease in hatchability (increase in dominant lethals) and a falling off in fertility between the 1st and 2nd broods. This marked parallelism between groups A and B indicates that the amine was not able to modify the combination of effects, exerted by x-radiation, which are measured by hatchability determinations.

TABLE 1

HATCHABILITY OF EGGS LAID BY FEMALES MATED TO TREATED *Drosophila* MALES

Group and treatment*	Brood 1		Brood 2	
	Total eggs laid	Hatchability %	Total eggs laid	Hatchability %
Group A (I+R)*	144	37.5	138	13.3
Group B (R)	211	35.0	275	16.7
Group C (I)	227	91.6	93	93.5

* I, injected with mercaptoethylamine; R, irradiated.

For the determination of sex-linked recessive lethals mass matings were set up. Three broods were derived from each of the 3 groups of males by presenting 25 males of each group with 3 successive sets of virgin Muller-5 females, each for a 3-day period. Thereafter, at the end of each period, the standard Muller-5 test for sex-linked lethals was carried through. Thus, germ cells were sampled which were at 3 different stages of spermatogenesis at the time of irradiation. Table 2 summarizes the results obtained. The Brood 1 data clearly indicate that mercaptoethylamine had no antimutagenic action. The results from subsequent broods are of greater theoretical interest, since it is within these broods that the results of an indirect or secondary effect of x-rays upon the mutation rate might be brought to light. It might be supposed that in the maturing germ cells x-rays interfere with one metabolic process or another and so result, ultimately, in the presence of mutations.

TABLE 2

FREQUENCY OF SEX-LINKED RECESSIVE LETHALS AMONG THE OFFSPRING OF TREATED *Drosophila* MALES

Group and treatment*	Brood 1		Brood 2		Brood 3	
	a†	*b*‡	*a*†	*b*‡	*a*†	*b*‡
Group A (I+R)*	320	8.4	42	12.0	142	3.5
Group B (R)	348	8.5	137	11.7	188	1.6
Group C (I)	418	0.2	263	0	330	0

* I, injected with mercaptoethylamine; R, irradiated.
† Total chromosomes counted.
‡ Percent lethals.

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It is precisely upon such a process that an antimutagen 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

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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 uteri opened, and the numbers of live and dead embryos and of corpora lutea determined. From these data 3 ratios were calculated:

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

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

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

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

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

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

Table 1*a* 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-