

The number of non-fluorescent cells found to be randomly labelled was 4.4 per cent based on 1,000 cells counted. Control recipient animals receiving antigen alone or antigen plus endotoxin (without cells) had no clearly identifiable antibody-producing cells by this technique. No attempt was made to identify cells as to their morphological type. Haemagglutination titration of a pool of donor sera revealed a titre of 1:500. Individual titrations of recipient serum at the time of killing failed to reveal significant haemagglutinating antibody titres.

Tritium-labelled thymidine is known to be incorporated selectively into the DNA of dividing cells⁵. The presence of label in cells producing antibody is direct evidence of DNA synthesis and donor cell division in the recipient X-irradiated mouse.

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RADIOBIOLOGY

Effect of Sulphydryl Reagents on the Frequency of X-ray-induced Autosomal Recessive Lethal Mutations in *Drosophila melanogaster*

IODOACETIC acid (or its derivative iodoacetamide) is known to enhance the lethal effect of ionizing radiation on mammals¹⁻³, mammalian cells in tissue culture⁴, and bacteria^{5,6}; but there are no reports of enhancement of damage which may unambiguously be called genetic. *N*-ethylmaleimide, on the other hand, enhances both the lethal and mutagenic effects of radiation on bacteria⁷⁻⁹, but has not been reported to affect higher organisms. Indeed, it was shown to be quite ineffective in enhancing radiation damage to red blood corpuscles in contrast to iodoacetic acid¹⁰.

It was decided to test for enhancement of genetic damage in an organism with structural chromosomes. The induction of autosomal recessive lethals in *Drosophila melanogaster* males was investigated by means of the brood analysis technique¹¹, which tests germ cells irradiated at all stages of maturation from spermatogonia to mature sperm. Irradiations with X-rays (tube voltage 250 kV, half-value layer 1.2 mm copper) were carried out under anoxia since, with bacteria, *N*-ethylmaleimide is most effective under these conditions. Flies were placed in a container through which a rapid stream of B.O.C. 'white-spot' nitrogen was passed for 20 min before and during irradiation with 800 r. Injections (approx. 0.3 ml.) (ref. 12) of 0.4 per cent saline, or 0.4 per cent saline plus 5×10^{-4} M-*N*-ethylmaleimide (NEM) or iodoacetamide (IAAm), were carried out approximately 35 min before irradiation. Immediate survival from these treatments was 100 per cent.

Mutation frequencies in flies injected with IAAm and NEM but not irradiated did not differ significantly from the expected spontaneous frequency of 0.4 per cent. The mutation frequencies in the irradiated groups are shown

in Fig. 1. So few mutants were observed in broods VI and VII that they could not be included in the statistical analysis. Cochran's test, which was applied to broods I-V, showed a significantly higher mutation frequency in the group treated with NEM compared with the saline injected group ($P \leq 0.01$). The overall frequencies of induced mutants (after subtracting 0.4 per cent spontaneous mutants) were 3.99 and 2.03 per cent respectively. If mutation induction were linear with dose and if NEM acts as a dose-modifying factor¹³ the enhancement ratio for NEM would be 1.97, that is, the same as that observed with bacteria⁷⁻⁹. It is not possible with the present data to compare the relative effect of NEM on different broods.

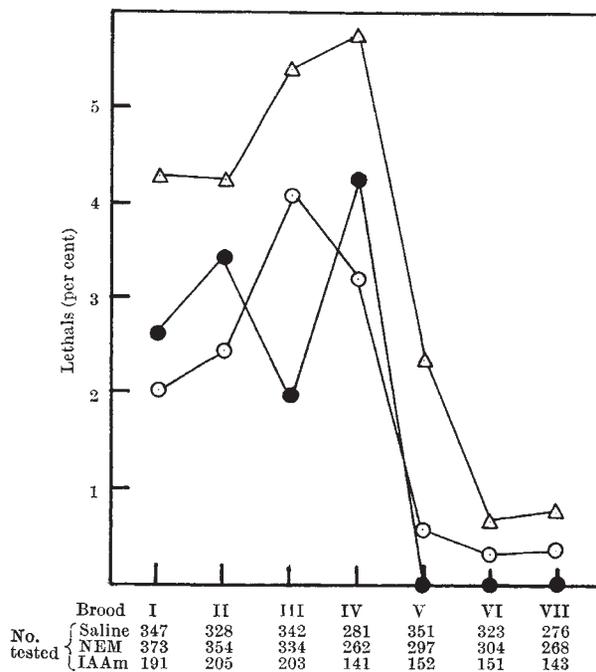


Fig. 1. The effect of injected *N*-ethylmaleimide and iodoacetamide on frequency of recessive lethal mutations after 800 rads under anoxic conditions. ○, Saline; △, NEM; ●, IAAm

Mutation frequencies in the IAAm and saline injected groups did not differ significantly from one another ($P \geq 0.1$).

The induced mutations, the radiation-induced frequency of which is increased by *N*-ethylmaleimide, are probably the expression of chromosomal damage of a much grosser kind than that in *Escherichia coli*⁹, where the lesion may well be confined to one of a few specific base pairs¹⁴. Work on enhancement with *N*-ethylmaleimide and its interrelations with the effect of oxygen is being continued.

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