THE EFFECT OF PHOTOREACTIVATING LIGHT ON THE MUTATION RATE INDUCED IN DROSOPHILA BY TERTIARY-BUTYL HYDROPEROXIDE¹

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Received September 11, 1957

THE effectiveness of long ultraviolet and visible light (photoreactivating light) in reducing the number of mutations produced by mutagenic ultraviolet, when the light is applied as a posttreatment, has been demonstrated in a number of microorganisms (NOVICK and SZILARD 1949; NEWCOMBE 1950; KIMBALL and GAITHER 1949; HAAS *et al.* 1950; GOODGAL 1950) and in *Drosophila* (MEYER 1951; ALTENBURG and ALTENBURG 1952). Moreover, HAAS *et al.* (1950) have found that irradiation of the culture medium with visible light, subsequent to its irradiation with mutagenic ultraviolet but prior to inoculation of the bacteria, reduced the mutation rate produced in the bacteria by the irradiated medium alone, a result which they suggest may be due to the destruction of unstable molecules, such as peroxides, by the visible light. It had been shown previously (WYSS *et al.* 1948) that culture medium irradiated with mutagenic ultraviolet and from which all the hydrogen peroxide had disappeared still retained part of its mutagenicity, most of which was due to the presence of organic peroxides which could be removed by catalase, leaving the medium nonmutagenic. Since a certain organic peroxide (tertiary-butyl hydroperoxide) has been shown to be mutagenic when applied to the polar caps (early germ track) of *Drosophila* eggs (L. ALTENBURG 1954), the effect of treatment of the polar caps with long ultraviolet and visible light (3000–4200Å) following upon tertiary-butyl hydroperoxide treatment has been investigated. The posttreatment with photoreactivating light has been found to be much less effective, if not entirely ineffective, in lowering the mutation rate induced by pretreatment with tertiary-butyl hydroperoxide alone.

METHODS

The polar cap ends of one group of dechorionated fertilized eggs of *D. melanogaster* were exposed for 20 minutes to the vapor of a 0.5 M solution of tertiary-butyl hydroperoxide in di-butyl phthalate, after the manner described in a previous paper (L. ALTENBURG 1954). At the end of this time the eggs were removed from the chamber containing the vapor and the polar caps of one half of the eggs were irradiated for an additional 20 minutes at a distance of 50 cm with the light from a 100-watt General Electric CH-4 spotlight lamp, a large percent of the output of which was radiation ranging in wave length from 3000 to 4200Å (photo-

¹ This work has been supported by a grant received for work of EDGAR ALTENBURG and associates for the National Institutes of Health, U. S. Public Health Service.

reactivating light). The other one half of the eggs were not subjected to posttreatment after being exposed to the peroxide vapor but were placed in the same chamber while the posttreatment was in progress. These eggs were shielded from the long wave length ultraviolet and visible radiation; and after the posttreatment period, both sets of eggs were removed from the chamber and allowed to develop in darkness. The polar caps of a third set of eggs were exposed to the vapor of only the di-butyl phthalate (in which the peroxide used in the treated series was dissolved) for 20 minutes, then placed in the posttreatment chamber but shielded from the irradiation. All three sets of eggs were treated in each daily experiment and the experiments were repeated on successive days until a sufficient number of eggs was obtained.

After the eggs hatched, males carrying speck (*sp*) as a marker in one member of the second pair of autosomes and nonspeck (*sp*⁺) in the homologous chromosomes were selected and tested for the presence of lethal mutations by the *sifter* technique (MULLER 1951). The standard errors of the mutation rates are here calculated in such a way as to take into account the increase in error caused by some of the mutants having had a common origin (MULLER 1952).

RESULTS

The mutation rate induced in the eggs by treatment with tertiary-butyl hydroperoxide alone for 20 minutes was 5.4 ± 1.6 percent (835 tested chromosomes from 41 treated males). If data from all previous experiments in which the pole cells were similarly exposed to the peroxide vapor are included, the mutation rate becomes 5.8 ± 1.3 percent (1,471 tested chromosomes from 89 treated males). The mutation rate induced in the eggs exposed to posttreatment by long wave length ultraviolet and visible light was 5.1 ± 1.7 percent (434 tested chromosomes from 34 males). The mutation rate recovered from eggs exposed to the vapor of di-butyl phthalate alone (the solvent used for the peroxide) was 0.3 ± 0.3 percent (358 tested chromosomes from 21 males).

DISCUSSION

The present study shows that pretreatment of *Drosophila* eggs with tertiary-butyl hydroperoxide results in a mutation rate which either cannot be lowered by subsequent irradiation with long wave length ultraviolet and visible light (3000–4200Å), or, at least, which cannot be lowered to an extent comparable to the lowering (by as much as 50 to 80 percent) of the mutation rate obtained when pretreatment consists of irradiation by mutagenic ultraviolet light (2537Å), using the same experimental techniques. This result indicates, as one possibility, that the photoreparative process acts before peroxide formation, or at least before the formation of peroxide of the type used. It is also possible that the mutagenic pathway early becomes branched, with photorepair affecting only one branch, or certain branches of it, and not the "peroxide" branch. This is indicated by the fact that a fraction of the mutation rate induced by ultraviolet light cannot be reduced by photoreparative light. There exists a possibility that there may have

been a slight lowering of about 0.3 percent in the mutation rate of the posttreated series in the present experiments (or 0.7 percent when all previous peroxide experiments are considered); but because of the large statistical error involved in the polar cap method of treatment, it would not be possible to demonstrate so small a difference.

SUMMARY

Posttreatment of germ cells of *Drosophila* at the polar cap stage with photoreactivating light does not significantly lower the mutation rate induced by previous treatment of the material with tertiary-butyl hydroperoxide.

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