THE PROPORTIONALITY BETWEEN MUTATION RATE AND ULTRAVIOLET DOSE AFTER PHOTOREACTIVATION IN DROSOPHILA[1]

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Received October 20, 1961

IT has been previously shown that when the polar cap cells of Drosophila (early germ track cells) are treated with various doses of ultraviolet light and the lethal mutation rate plotted against the dose, the resulting dose-rate curve rises rapidly at the lower doses employed. With further increase in dose it becomes less and less steep, and when the dose is large enough to give a detectable rate of from five percent to ten percent, the curve enters a plateau. Finally, it drops somewhat (MULLER *et al.* 1954). It has also been found (1) that photoreactivating light given as a posttreatment decreases the percent of lethals induced by ultraviolet in the polar cap cells and (2) that the percent decrease is less at the higher doses than at the lower (ALTENBURG and ALTENBURG 1957). Since posttreatment has a relatively smaller effect on the induced lethal rate at high doses than at low, the shape of the dose-rate curve is not the same for posttreated and nonposttreated material. The present studies attempt to determine more precisely the shape of the posttreated dose-rate curve.

METHODS

The polar caps of dechorionated Drosophila eggs were treated with the various doses of ultraviolet shown in Table 1. One series of eggs received no further treatment. A second series was posttreated with photoreactivating light immediately after each ultraviolet treatment except the lowest (posttreated series). Lethals induced in the second chromosomes were detected by means of MULLER's sifter technique.

The controls were shielded from the ultraviolet, but controls and treated were placed side by side during treatments in order to expose them equally to any ozone that might have been produced by the lamp. Some of the controls were posttreated with photoreactivating light and some were not, and it was found that there was no significant difference in the posttreated and nonposttreated control rates ($0.3 \pm 0.2\%$ and $0.6 \pm 0.1\%$, respectively). However, the survival rate of the eggs was reduced by conditions accompanying treatment with the photoreactivating light, particularly the heat of the lamp and the prolonged exposure of the eggs to dehydration. Photoreactivating light was therefore not administered to most of the controls.

[1] This investigation was supported by U. S. Public Health Service Research Grant C-2393 and C-5960 from the National Cancer Institute.

TABLE 1

Lethal mutation rates induced in Drosophila polar cap cells by ultraviolet light and ultraviolet followed by photoreactivating light

Treatment (Minutes at 180 cm)*	Ultraviolet			Ultraviolet plus photoreactivating light		
	Chromosomes	Lethals	Rate (%)†	Chromosomes	Lethals	Rate (%)†
¾	786	8	0.4±0.4	. . .	. .	. . . . .
1½	1,013	28	2.2±0.8	863	15	1.1±0.5
2½	1,057	46	3.8±1.1	682	12	1.2±0.8
3½	805	52	5.9±1.8	1,442	18	0.7±0.4
5	496	25	4.4±1.6	536	13	1.8±0.9
7½	594	27	4.0±1.4	697	17	1.8±0.9
10	773	38	4.3±1.2	1,028	32	2.5±0.8
	Controls					
(Shielded from UV)	3,128	19	0.6±0.1			

* One minute at 180 cm equals 50 ergs/mm².
† After subtraction of control rate.

In a study such as the present it would be desirable to have all the treatments completed in a relatively short time, say one afternoon, so as to insure uniform conditions of treatment for all the treated and controls. However, relatively few eggs survive the handling attendant upon the polar cap method of treatment and the toxic effect of the ultraviolet. Of those which survive only a fraction escape the sterilizing effects of the ultraviolet (especially at higher doses) and are of the right sex (male) for further breeding. In the course of an afternoon's treatment, a team of two workers in our experience cannot as a rule successfully treat more than five eggs of the right sex, from which (after further losses in the course of breeding) not more than about 80 tested chromosomes are ordinarily derived. (The number of treated chromosomes tested from each treated male is kept low in order to avoid the clustering of induced lethals referred to below.) Accordingly, in order to obtain counts of several thousand tested chromosomes and controls, as required in a study of the present sort, it is necessary to run the experiments over a period of a year or more and to administer the different doses at times often separated by undesirably long intervals. The polar cap method has a further drawback (in addition to arduous technique) in that it leads to a clustering of the data (since the polar cap cells multiply in the course of development), and this clustering increases the statistical error. With all its drawbacks, however, the polar cap method is preferable to treatment of the adult males, since only a very small fraction (less than one percent) of the ultraviolet impinging on the surface of an adult can penetrate to the depth of the gonads, as a result of which fact induced mutation rates of over two or three percent necessitate the use of a highly toxic dose of ultraviolet at the surface layers. Moreover, when the adult male is treated, the amount of ultraviolet which gets to the gonads varies with the amount of pigment in the fly's abdomen and the extent to which the abdomen is compressed (in holding the fly down for treatment). The polar cap cells, by contrast, lie directly below the thin transparent vitelline membrane of the

developing egg and are readily accessible to ultraviolet after the shell has been removed from the egg. (The living and unstained polar cap cells can clearly be seen under the microscope.)

RESULTS

At the lowest ultraviolet dose used in the present studies (the ¾-minute treatment) the induced rate is not significantly different from the control rate ($0.4 \pm 0.4\%$ *versus* $0.6 \pm 0.1\%$). As previously indicated, no attempt was made to get a posttreated rate at this dose. At the lowest dose for which a posttreated rate was determined (the 1½-minute treatment), the induced lethal rates for both the nonposttreated (ultraviolet only) and posttreated (ultraviolet plus photoreactivating light) are both relatively low ($2.2 \pm 0.8\%$ and $1.1 \pm 0.5\%$), and accordingly there is little difference between them (Table 1 and Figure 1). The rates for the next two doses (2½ and 3½ minutes) are well up on the steeply rising part of the dose-rate curve, and here posttreatment is causing a detectable lowering of the lethal rate, especially at the 3½-minute dose, the ultraviolet-induced rate being lowered here from $5.9 \pm 1.8\%$ to $0.7 \pm 0.4\%$ by posttreatment (Table 1). With further increase in the ultraviolet dose (5, 7½,

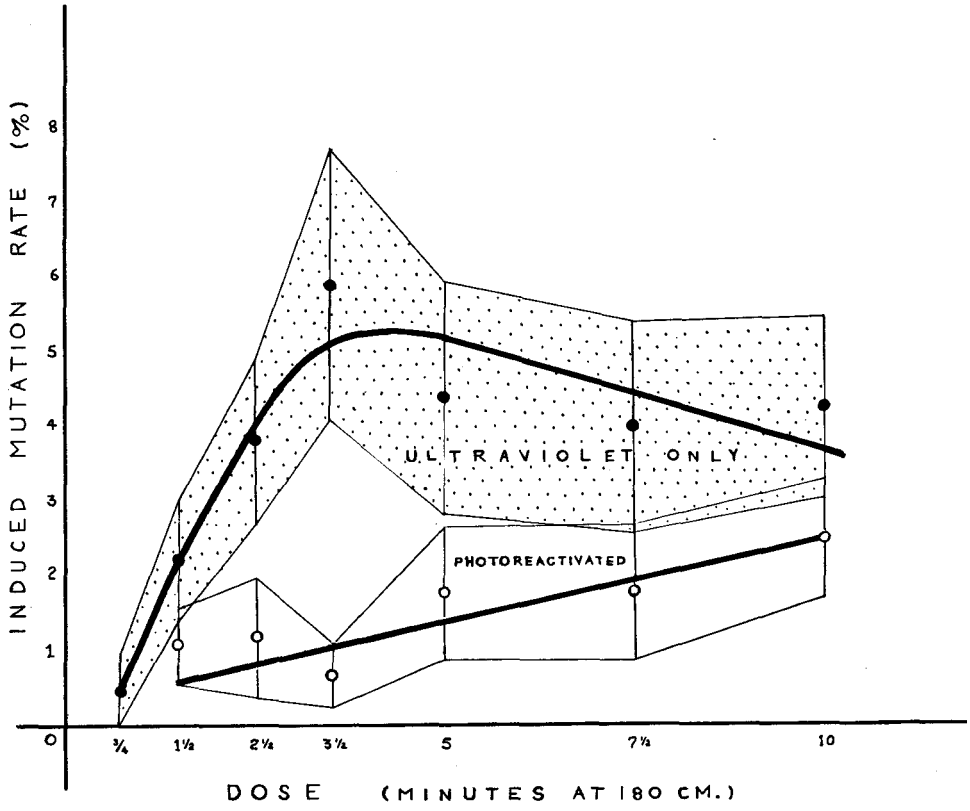

FIGURE 1.—The effect of photoreactivation on the ultraviolet dose-rate curve.

and 10 minutes), the nonposttreated series is on the falling part of the dose-rate curve (with rates of about four percent from a previous rate of about six percent at the 3½-minute treatment), but the posttreated series does not show a corresponding drop in rate. Instead it rises from a low of 0.7 ± 0.4% at the 3½-minute treatment to 2.5 ± 0.8% at the ten-minute treatment.

DISCUSSION

Largely because of the clustering of lethals previously referred to in connection with the polar cap method of treatment, the errors of the rates (graphically shown in Figure 1) are much greater than those ordinarily encountered especially when postmeiotic stages are treated. At three of the doses in the present experiments (1½, 7½, and 10 minutes), the errors of the rates of the nonposttreated and posttreated series overlap. Accordingly, the difference in these rates, considered by themselves, is not significant. However, at all doses the rates in the posttreated series are consistently lower than in the nonposttreated. Therefore, when all doses are considered, the lower rates in the posttreated series are probably significant. If only the rates are considered (not their errors also), a straight line probably represents the best fit for the points giving the relationship between the photoreactivated mutation rate and the dose of ultraviolet (Figure 1). Accordingly, the data in question indicate that the induced rates are proportional to the dose of ultraviolet in the posttreated series.

In one series of experiments (previously reported, ALTENBURG and ALTENBURG 1957), posttreatment caused a considerable increase in the mutation rate (from 2.9 ± 1.3% to 10.0 ± 3.0% at the ten-minute treatment). This increase occurred when the induced rate (nonposttreated) was on the falling part of the dose-rate curve (i.e., in our higher ultraviolet dosage range). However, in this particular series, a larger number of chromosomes were tested per treated male than in any of the other series, this larger number per male tending to cause larger cluster sizes and a correspondingly large statistical error. The high posttreated rate in this series (10.0 ± 3.0%) was due largely to five large clusters of lethals and was considerably out of line with the rates for the same high dosage in several other experiments. This series has therefore been omitted from the present report. If it were included in the data, then the posttreated rate at the ten-minute dose would be somewhat higher than shown in Table 1 (6.1 ± 1.5% *versus* 4.3 ± 1.2%). It would be difficult to include this higher rate in the line which fits the lower posttreated doses. However, the posttreated rate at this dose would still be rising, not falling, as in the nonposttreated.

The decreasing difference between the posttreated and nonposttreated lethal rates with increase in ultraviolet dosage shown in Table 1 might be partly explained on the assumption that ultraviolet is itself photoreactivating, and that at the higher doses, it has achieved more of its possible photoreactivation than at the lower doses. Posttreatment would therefore produce a relatively smaller decrease in the ultraviolet-induced rate at higher doses than at lower.

Moreover, in nonposttreated material, the falling off of the mutation rate with increase in ultraviolet dose is probably due in part to uneven illumination of the

polar cap cells by ultraviolet, since the more exposed layers of cells—those with the higher percent of induced lethals—would be killed by the ultraviolet to a greater extent than the less exposed as the dose was increased. Photoreactivating light given as a posttreatment would tend to counteract the toxicity of the ultraviolet and so would tend to prevent somewhat the falling off of the lethal rate which was due to uneven illumination.

In experiments on color-response mutants in *Escherichia coli*, NEWCOMBE and WHITEHEAD (1951) found that the ultraviolet posttreated dose-rate curve flattens at their high doses, unlike the apparent continued linearity in Drosophila. However, since *E. coli* is in effect a haploid organism there might have been a summation of the detrimental effects of ultraviolet on the cytoplasm and of the mutations on viability. It is therefore possible that after a certain dose of ultraviolet had been reached, the increase in the mutation rate was cancelled by a selective decrease of the mutants, with a resultant flattening of the dose-rate curve. In our experiments, a similar flattening due to the same cause would not have occurred, since we were dealing with recessive autosomal mutations in a diploid organism, and there would accordingly have been no selective viability effect of the ultraviolet on cells in which mutations had occurred.

It is true that in our nonposttreated series, there is probably selective killing off of the mutant cells at higher doses. However, as previously pointed out, this selective killing would be due to uneven illumination, not to the induced mutations (recessives in diploid material), and in the posttreated material the effect of uneven illumination would be counteracted at our higher doses (as well as our lower) by the posttreatment. Therefore the same flattening of the dose-rate curve might not occur in the posttreated series as in the nonposttreated. However, doses of ultraviolet still higher than those we employed might conceivably not be counteracted by posttreatment in sufficient amount to eliminate the effect of uneven illumination on viability, and if it were possible to achieve such high doses without killing virtually all the eggs (contrary to our experience), the dose-rate curve might flatten at these doses.

On the basis of their ultraviolet photoreactivation experiments on *E. coli*, NOVICK and SZILARD (1949) have concluded that the shape of the dose-rate curve for induced mutations (from phage sensitivity to phage resistance) after photoreactivation is similar to that for the nonposttreated, and that approximately the same dose-reduction factor (0.35) applies to the mutation rate and survival rate after photoreactivation. However, as NOVICK and SZILARD point out, because of the high statistical error of the mutation rates (±50%), their experiments on mutations were not as reliable as those on survival, in which the statistical error was smaller.

In Drosophila our results indicate that there is a difference in the nature of the photoreactivable and nonphotoreactivable parts of ultraviolet mutagenesis, as would follow from the apparent difference in their dose-rate relationships.

SUMMARY

The polar cap cells of Drosophila (early germ track cells) were posttreated with photoreactivating light after treatment with various doses of ultraviolet and the resulting lethal mutation rates compared with the rates induced by the same doses of ultraviolet in a nonposttreated series. In the nonposttreated, the dose-rate curve was found to rise rapidly at the lower doses employed, becoming less steep with increase in dose until it reaches a plateau, and finally falling off somewhat (these results confirming those earlier reported). By contrast, in the photoreactivated series, the dose-rate relationship appears best to fit a linear curve through the entire range of doses tested, the photoreactivated rates being well below those of the nonposttreated at the lower dosage ranges ($0.7 \pm 0.4\%$ *versus* $5.9 \pm 1.8\%$ at the dose which shows the greatest difference), the difference in the rates getting less with increasing ultraviolet dose and the two rates (posttreated and nonposttreated) finally becoming much closer at our highest dose ($2.5 \pm 0.8\%$ *versus* $4.3 \pm 1.2\%$). These results would indicate that there is a difference in the nature of the photoreactivable and nonphotoreactivable parts of ultraviolet mutagenesis.

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