THE LOWERING OF THE MUTAGENIC EFFECTIVENESS OF ULTRAVIOLET BY PHOTOREACTIVATING LIGHT IN DROSOPHILA

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ALTHOUGH there is now (since we started our present work) evidence that long ultraviolet and visible light lower the mutagenic effectiveness of ultraviolet in certain microorganisms (DULBECCO 1950; GOODGAL 1950), we have thought it desirable to carry through our investigation of this effect in Drosophila, both because considerable differences in mutagenesis, of other kinds, have been found between widely different organisms and because the deleterious effects of mutations might be greater when induced in haploid material (as in microorganisms) than in diploid and these effects might cause complications in conjunction with the physiologically deleterious effect of ultraviolet.[1]

MATERIALS AND METHODS

It will be recalled that the polar cap appears at one pole of the developing Drosophila egg shortly after fertilization and that it contains the cells of the early germ track. About 55 cells are present in the fully developed polar cap of a male egg, but only some 20 to 24 of these become incorporated in the testes when these are formed (SONNENBLICK 1941, 1951). The polar cap is separated from the outside by only the thin transparent vitelline membrane and by the chorion (shell) of the egg. The polar cap cells are therefore almost directly accessible to ultraviolet that strikes the overlying surface, particularly when the shell of the egg is removed. It is impractical to treat the adults with ultraviolet, because only a small fraction of the ultraviolet that strikes the surface of the adult fly can penetrate the thickness of tissue that separates the gonads from the outside surface.

The polar caps of two lots of Drosophila eggs were treated with ultraviolet from a 15-watt germicidal lamp. One lot was given no further treatment. The other lot was posttreated with photoreactivating light, the source of which was 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 4200 Å. The posttreatment usually lasted for 30 minutes (but only for three minutes in one case) and it followed immediately after the mutagenic ultraviolet treatment. In one series of experiments (Series 1), the shells were not removed from the

[1] We were aware that, while we were doing the present work at The Rice Institute, a parallel investigation was being carried out at Indiana University (MEYER 1951), but both groups were of the opinion that in this case, owing to the difficulties involved, it was desirable to have two separately obtained sets of results. By mutual agreement, these are being published in parallel papers.

eggs previous to treatment, neither was the light from the germicidal lamp filtered. In two other series, the shells of the eggs were removed and the light was passed through a filter (consisting of an aqueous solution of $CoSO_4$ and $NiSO_4$), so as to render it mostly monochromatic ultraviolet of wave length 2537 Å. This was done in order to eliminate all photoreactivating light from the germicidal lamp, but as it turned out, this precaution was unnecessary, as shown by the results (to be considered later) of Series 1 experiments in which the light was not filtered.

Second chromosome lethals were used as a measure of the mutation rate. These were detected by means of MULLER'S " sifter " technique (MULLER 1951), which is being fully described in another article now in preparation, but since the technique is new and complicated, it will be reviewed here. In the form used here, the technique is briefly as follows. The treated polar caps are those of eggs of genotype *cn bw sp/cn bw* (*cn* = cinnabar, *bw* = brown, *sp* = speck). When the eggs reach maturity the males (termed P_1) are selected and mated to *Cy* cn^2 sp^2/*mr bs* females (*Cy* = Curly, *mr* = morula, *bs* = blistered). The Curly offspring are of two classes (1) *cn bw sp/Cy* cn^2 sp^2 (phenotypically Curly cinnabar speck) and (2) *cn bw/Cy* cn^2 sp^2 (Curly cinnabar non-speck). About 20 F_1 brothers of each of these two classes, or some other convenient number, derived from each P_1 male are selected as P_2 and individually bred to females of a complicated stock designated by MULLER as " sifter."

The sifter stock is heterozygous for two Oenothera-like complexes which involve rearrangements of the second and third chromosomes. One of these complexes contains *Cy*, and we shall designate this as the *Cy* sifter complex. The other complex contains P^-, the Pale deficiency. The sifter stock is so constituted that in outcrosses, the only viable offspring are those that receive the *Cy* sifter complex. (Those that receive the P^- complex are inviable because of the Pale deficiency. The stock itself is viable because the *Cy* sifter complex contains P^i, the Pale insertion, which compensates for the Pale deletion. Crossovers between the two complexes are suppressed by inversions and chromosomal recombinations are made inviable by the unbalance they cause, sifter stock being a translocation heterozygote). Accordingly, when the P_2 males are bred to sifter females, the only viable offspring are those that receive the Curly chromosome from their sifter parent. Therefore they cannot also receive the Curly chromosome of the non-sifter parent (the P_2 male), since homozygous *Cy* is inviable. Thus they all receive his treated chromosome. If we indicate either treated chromosome by the symbol, *cn bw* (*sp*), then the P_2–F_2 cultures are as follows:

P_2 *cn bw* (*sp*)/*Cy* cn^2 sp^2 ♂ × sifter ♀ (with *Cy* sifter complex)
F_2 *cn bw* (*sp*)/*Cy* sifter complex ♂ and ♀.

It thus comes about that all the F_2 are of the desired constitution for further breeding; i.e., all are heterozygous for a second chromosome of treated origin as well as for a marker and crossover suppressors in the nontreated chromosome. (We shall designate the latter as the sifter *Cy* chromosome.)

A few of the F_2 are now taken from each culture and inbred (as P_3) without the selection of virgins being necessary (since all the F_2 are of the right class). The flies from different cultures are kept separate, so that there are about 20 P_3–F_3 cultures of *cn bw sp/Cy* sifter and 20 of *cn bw/Cy* sifter ♂ and ♀ for each treated polar cap (corresponding to the 20 P_2–F_2 cultures from which they were individually derived). If the P_3 do not contain a lethal in their treated chromosome (*cn bw sp* or *cn bw*), they produce offspring homozygous for this chromosome in addition to *Curly* flies heterozygous for it, thus:

P_3 *cn bw (sp)/Cy* sifter ♂ and ♀
F_3 1 *cn bw (sp)/cn bw (sp)* : 2 *cn bw (sp)/Cy* sifter.

The *cn bw* combination when homozygous (and when not modified as described below) produces white eyes, and the nonlethal cultures can therefore be scored at a glance simply by observing a few white-eyed flies through the glass container. About two-thirds of the offspring homozygous for the *cn bw* combination have cinnabar eyes instead of white, because the P_2 sifter parent carried the Pale insertion in one of its third chromosomes and this insertion contains the normal allele of *bw* (and of *sp*), thus allowing only *cn* to express itself in those F_3 that have received the insertion. The presence of *cn* flies (if straight-winged) would therefore also indicate that the culture was nonlethal. All flies that had straight wings would be homozygous for *cn bw (sp)*, so that the presence of any straight-winged flies in the culture would identify it as nonlethal. But the white eye is the most convenient trait for identifying the nonlethal cultures, since it is very conspicuous. The lethal cultures on the other hand would lack the whites and contain only the heterozygotes, *cn bw (sp)/Cy* sifter. These would have dark cinnabar eyes (cn^2 being contained in the *Cy* sifter complex) and Curly wings. A summary of the above crosses is as follows:

P_1 *cn bw/cn bw sp* ♂ × *Cy* cn^2 sp^2/*mr bs* ♀
(♂ irradiated in polar cap stage)
P_2 20 brother F_1 *cn bw/Cy* cn^2 sp^2 ♂♂ and
20 brother F_1 *cn bw sp/Cy* cn^2 sp^2 ♂♂ from each P_1 ♂
bred individually × sifter (with *Cy* sifter complex) ♀♀
P_3 *cn bw/Cy* sifter complex ♂ and ♀; *cn bw sp/Cy* sifter complex ♂ and ♀
(20 cultures) (20 cultures)
F_3 Look for *cn bw/cn bw* or *cn bw sp/cn bw sp*

Any lethal which arises in a polar cap cell is multiplied by mitotic cell division to the same extent that the cell is. Thus if a polar cap contained 10 cells, and a lethal arose in one of these, say in the *sp* chromosome of the cell in question, and further if each cell of the polar cap multiplied equally, then on the average one-tenth of the sperm cells with a *sp* chromosome (i.e., one-tenth of the *sp* genome) would contain the lethal as a result of reduplication of the one original lethal. In other words, there would be a cluster of lethals equal to one-tenth of the sperm cells with the *sp* genome. Therefore if, say, 20 of the sperm cells with the *sp* genome were tested for a lethal, then a cluster of two

(1/10 of 20) would on the average contain the lethal, on the above assumptions. (As a matter of fact, the number of germ cells per polar cap might not be 10 at the time of irradiation; and the division rate of all cells is not the same.)

In the sifter technique as here employed, it will be recalled that about 20 chromosomes of each of the second chromosome pair (*cn bw sp* and *cn bw*) are tested from each treated polar cap, and if a lethal has been induced in, say, just one cell of a polar cap and in one of the second chromosomes of that cell, then a certain fraction of the 20 P_3–F_3 cultures containing that kind of second chromosome will contain the lethal, and the rest will not. The size of the fraction (cluster) with the lethal will depend on the number of germ cells in the polar cap at the time the lethal arose, and on the extent to which the mutant cell multiplies, relative to the others, as indicated above.

When a polar cap contains just one mutant cell, it is a mosaic of mutant and normal cells, and this is reflected by the fractional transmission of the mutant chromosome to the offspring. If, on the other hand, all the cells of a polar cap contained a lethal, and at the same locus, in one of the second chromosomes, then the embryo would most likely have been heterozygous for that lethal at the time it was a fertilized egg, and we should then not consider that lethal as having been induced in the polar cap, but as preexisting (at the time of fertilization). Such lethals are not included in the totals.

Since the P_2 males derived from a given treated polar cap (of a P_1 male) are brothers, we can refer to the P_2–F_2 cultures in which they are the fathers as "brother" cultures, those with the *cn bw sp* chromosome being referred to as one series of brother cultures (the "speck" series) and those with the *cn bw* chromosome as another series (the "non-speck" series). The P_3–F_3 cultures (derived from a given treated polar cap) would be correspondingly designated. In this terminology, then, a P_3–F_3 series which is partly lethal represents a new lethal; one which is all-lethal represents a preexisting lethal.

Two or more lethals might be induced in the same polar cap (lethals of "independent origin"). When flies with two such lethals are crossed, offspring homozygous for the *cn bw* (*sp*) chromosome (i.e., whites) might survive, since the lethals would most likely be at different loci, and each would be prevented from expressing itself by its normal allele in the other chromosome. By contrast, two flies with lethals derived from the same original mutation could produce no offspring homozygous for *cn bw* (*sp*), since the lethals in this case would be alleles. Crosses were always made to test the allelism of the lethals in a given P_3–F_3 series (by crossing flies from one culture to those of all other cultures of the series).

When a mutation is induced in a polar cap cell, the nonmutant cells are multiplied on the average to the same extent (per cell) as the mutant. Therefore, the ratio of mutant to nonmutant chromosomes is the same in the adult as in the polar cap. Thus a cluster of mutant sperm cells equal, for example, to ten percent of the total sperm cells with a given second chromosome (*sp* or *non-sp*) would have resulted (on the average) from one mutant chromosome

in a total of ten germ-track cells in the polar cap and would therefore represent a ten percent mutation rate for that chromosome. Hence even though the cluster was derived from only one original mutation, the cluster must nevertheless be regarded as representing a ten percent mutation rate. This in turn means that each mutation in the cluster is scored as though it were independently induced in the sperm cells themselves, so that if for example a total of 100 sperm cells were scored (through the appropriate genetic tests) and ten found to be mutant, the mutation rate would be set down as ten percent, although all ten mutant sperm cells might belong to a single cluster derived from only one mutational occurrence.

The cells of the fully developed polar cap do not proliferate germ cells equally for very long. After they wander to the site of the future testis and become gonial cells, they undergo only very few if any equal germinal divisions. Thereafter one or a few of the cells (stem cells) in each testis do most of the germinal multiplying. Hence if one of these particular cells happened to contain an induced mutation, the estimate of the mutation rate, based on the size of the resulting cluster, would be much higher than if one of the other polar cap cells had happened to contain the mutation. This unequal division of the gonial cells therefore introduces a sampling error in the estimate of the mutation rate, for if the number of polar caps treated is small, the rate in two samples of equal size might vary considerably depending on the number of stem cells hit, even though the same percent of polar cap cells had been hit in the two samples.

It was not usually possible to derive the desired 20 P_3–F_3 cultures with each kind of treated chromosomes (*cn bw* and *cn bw sp*) from each P_1 male, partly because of sterility of the treated males and partly because of accidents. When a male happened to be unusually fertile, more than 20 cultures of each kind were sometimes derived from him in order to augment the data.

The method of estimating the sampling error herein used has been developed by MULLER. To get the standard error by this method we square the number of lethals in each cluster (an isolated lethal being considered a cluster of one), get the sum of these squares, multiply this sum by the percent of nonlethals, take the square root of this product, and divide this by the total number of tested chromosomes derived from all the treated polar caps.

In order to estimate the sampling error, it is necessary to know the number of tested chromosomes and lethals derived from each treated polar cap (of the P_1 male); but the separate presentation of the data derived from each treated male would require an excessively bulky table. In preparing table 1, therefore, the data from all males receiving a given treatment were lumped together.

RESULTS

Table 1 Series 1 gives the results of experiments in which neither the shells of the eggs were removed before treatment nor the ultraviolet light (from the germicidal lamp) filtered. It will be seen that the rate in the lot of eggs treated with mutagenic ultraviolet alone (1D) is $7.3 \pm 2.3\%$; in the posttreated lot

(1L), 1.0 ± 1.0%. The latter is very close to the control rate of .8 ± .3%. Thus the photoreactivating light has largely abolished the mutagenic effectiveness of the ultraviolet.

Table 1 Series 2 gives the results of experiments in which the shells were removed from the eggs prior to treatment and in which also the light from the germicidal lamp was rendered almost monochromatic, of wave length 2537 Å, by being passed through a filter. In this series of experiments, the eggs received a very low dose of mutagenic ultraviolet (about 835 μw-sec/cm^2), sufficient to give a gross mutation rate of only 2.7 ± .7%, which however is still

TABLE 1

Effect of photoreactivating light of ultraviolet mutagenesis in Drosophila.

D = Dark (placed in dark after mutagenic ultraviolet treatment)
L = Light (treated with long ultraviolet and visible light for 30 minutes after mutagenic ultraviolet treatment; L1, for only 3 minutes)

Series*	% hatched eggs ♂♂**	Approx. % P_1 ♂♂ sterile***	No. polar caps tested	No. chromosomes tested	No. new lethals	No. independent lethals	Mutation rate (%)	Induced rate (%)	Diff. (%)
1D	9.6	31.2	38	1,242	100	24	8.1 ± 2.3	7.3 ± 2.3	6.3 ± 2.5
1L	17.8	20.0	32	924	17	8	1.8 ± 0.9	1.0 ± 1.0	
Controls	38.0	2.6	36	1,432	11	10	.8 ± 0.3		
2D	34.4	8.1	37	1,252	34	20	2.7 ± 0.7	1.9 ± 0.8	
2L	32.0	13.2	35	1,050	17	13	1.6 ± 0.5	.8 ± 0.6	
2L1	22.2	0.0	10	408	5	5	1.2 ± 0.5	.4 ± 0.4	
Controls	41.6	4.8	20	648	5	5	.8 ± 0.3		
3D	31.7	5.3	17	528	52	28	10.0 ± 2.0	9.5 ± 2.0	5.4 ± 2.7
3L	18.8	6.3	12	326	15	9	4.6 ± 1.7	4.1 ± 1.8	
Controls	40.5	6.7	11	220	1	1	.5 ± 0.4		

*Series 1 (1D and 1L). Ultraviolet not filtered. Shells not removed from eggs. Distance of eggs from ultraviolet lamp, 87¼ cm. Duration of ultraviolet treatment, 19 minutes; of posttreatment, 30 minutes. Dose of ultraviolet, 37,500 μw-sec/cm^2 (only 10% of which penetrates shell).
Series 2 (2D, 2L, 2L1). Filtered ultraviolet. Eggs without shells. Distance of eggs from ultraviolet lamp, 6 cm. (filter greatly lowers intensity of ultraviolet). Duration of ultraviolet treatment, 2 minutes; of posttreatment, 30 minutes (L) and 3 minutes (L 1). Dose of ultraviolet, 836 μw-sec/cm^2.
Series 3 (3D, 3L). Ultraviolet filtered, but with deteriorating filter (dose of ultraviolet therefore uncertain). Treatment otherwise as for Series 2, with 30 minutes posttreatment.
**Percent of hatched eggs that developed into males.
***Including possibly a few males that were fertile but that failed to breed.

significantly above the control rate (.8 ± .3%), the difference between the treated and control rates being 1.9 ± .8% (t = 2.5). By contrast, the gross rate for the posttreated lot (1.6 ± 0.5%) is not significantly different from that for the controls.

One batch of eggs intended for ultraviolet treatment only, in Series 2 experiments, was inadvertently placed in the photoreactivating light and given a 3-minute posttreatment (2L 1) before the error was discovered. The experiment was nevertheless continued with these eggs, and it was found that the 3-minute posttreatment was sufficient to cause a significant lowering of the

mutation rate, as judged by the fact that the eggs in question gave an induced mutation rate of .4 ± .4%, as compared with a rate of 1.9 ± .8% for the previously mentioned ultraviolet only treatment (2D) in Series 2 experiments. (The usual posttreatment lasted for 30 minutes, it will be recalled.)

In Series 3 experiments (done before Series 2), it was discovered only late in the course of the experiments that the filter was deteriorating, and that after being used a few times it allowed much more ultraviolet to pass through it than it did at the start. (The filter was renewed before each treatment in Series 2 experiments). However, in each experiment of Series 3 (with the deteriorating filter), a comparable number of chromosomes were usually scored for the non posttreated (3D) and the posttreated lot of eggs (3L), and since the two lots would have received the same amount of mutagenic ultraviolet in each experiment, the average amount received by the two for the entire Series 3 experiments would have been about the same. Regardless of what this average dose may have been, there is a significantly lower induced rate in the posttreated (4.1 ± 1.8%) than in the ultraviolet-only treated eggs (9.5 ± 2.0%). However, the rate for the posttreated in this series (4.1 ± 1.8%) is significantly above the control rate (.5 ± .4%).

TABLE 2

Number of independent lethals per tested polar cap.

Series	D (u.v. only)				L (u.v. plus photoreactivating light)				Controls		
	No. polar caps	Independent lethals			No. polar caps	Independent lethals			No. polar caps	Ind. lethals	
		No.	No. per polar cap	No. induced		No.	No. per polar cap	No. induced		No.	No. per polar cap
1	38	24	.63	.35	32	8	.25	....	36	10	.28
2	37	20	.54	.29	35	13	.35	.10	20	5	.25
3	17	28	1.65	1.56	12	9	.75	.66	11	1	.09

The number of independent lethals induced per treated polar cap (counting each mutant cluster as one), is significantly less for the posttreated than for the non posttreated (ultraviolet only) polar caps in all three series of experiments (table 2). This method of treating the data therefore confirms the conclusion based on estimating the mutation rates from the percent of lethals per tested chromosome shown in table 1.

TABLE 3

Average size of lethal clusters, expressed as the percent of tested chromosomes of each kind (cn bw sp and cn bw) that are lethal, per lethal-yielding polar cap.

Series	D	L	Controls
1	21	18	7
2	11.5	8	5
3	17	9	9

There is somewhat of a tendency for the average size of a lethal cluster to be smaller in the posttreated than in the non posttreated polar caps, though this difference may not be significant (table 3). (The control lethals have a small average cluster size because most of these would not have arisen in the polar cap stage but later, and would therefore have been reduplicated less than lethals induced in the polar cap.) The smaller average cluster size for the posttreated agrees with the fact that the number of cells surviving per polar cap would be expected to be greater in the posttreated (because of the physiological photoreactivation) than in the non posttreated polar caps, and this would tend to make the cluster size (relative to the total surviving cells) smaller for the posttreated. However, the increase in number of surviving cells per polar cap would make the number of independent lethals per polar cap greater in the posttreated, if the mutation rate per cell was the same as in the non posttreated, so that the smaller number actually found for posttreated (mentioned in the paragraph above) now requires all the more significance.

DISCUSSION

The evidence clearly shows that photoreactivating light lowers the mutagenic effectiveness of ultraviolet in Drosophila, and that it may lower it to the extent of almost completely abolishing it. Photoreactivating light thus has an even greater inhibiting action on ultraviolet mutagenesis than on the physiologically toxic effect of ultraviolet in bacteria.

The mechanism whereby photoreactivating light lowers the mutagenic effectiveness of ultraviolet still remains to be explained. One *a priori* possibility is that it reverses the mutation process itself, either before the process is completed or after. The other is that the photoreactivating light has no effect whatever on mutation proper, but that it simply prevents this process from occurring, possibly by neutralizing some mutagenic product of ultraviolet before the product in question has time to become effective. The second of these possibilities seems the more probable, since if photoreactivating light could reverse a mutation, it would itself in effect be mutagenic, and this we know is not ordinarily the case.

SUMMARY

The polar cap of two lots of Drosophila eggs were treated with approximately the same dose of mutagenic ultraviolet (mostly of 2537 Å wave length), one lot then receiving no further treatment, the other receiving a posttreatment (usually for 30 minutes) with photoreactivating light (mostly in the range 3000–4200 Å). The lethal mutation rate induced in the second chromosome (as scored by MULLER's sifter technique), was lower in the posttreated lot than in the non posttreated, in each of three separate series of experiments. In two of the three series (Series 1 and 3) involving medium doses of mutagenic ultraviolet, the rate for the posttreated lot was significantly below that for the non posttreated; and in one series (Series 2) involving a very light dose of mutagenic ultraviolet, though the difference between the two rates

(for non posttreated and posttreated) was not highly significant (because of the low rates involved), yet the rate for the non posttreated lot was significantly above the control rate, but that for the posttreated was not. In Series 1 the rates were as follows: non posttreated 7.3 ± 2.3% (1,242 tested chromosomes); posttreated 1.0 ± 1.0% (924 chromosomes); controls .8 ± .3% (1,432 chromosomes). In Series 2 experiments the rates were: non posttreated 1.9 ± .8% (1,252 chromosomes); posttreated .8 ± .6% (1,050 chromosomes); controls .8 ± .3% (648 chromosomes). For Series 3 the figures were: non posttreated 9.5 ± 2.0% (528 chromosomes); posttreated 4.1 ± 1.8% (326 chromosomes); controls .5 ± .4% (220 chromosomes). Thus in two of the three series the induced rates for the posttreated chromosomes is very close to the control rates, even though in Series 1 the induced rate without posttreatment was fairly high (7.3 ± 2.3%). Photoreactivating light therefore has at these doses an even greater inhibiting action on the mutagenic effectiveness of ultraviolet than on the physiologically toxic effect reported for bacteria.

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