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THE INFLUENCE OF LIGHT UPON THE ACTION OF ULTRAVIOLET ON *PARAMECIUM AURELIA*

R. F. Kimball and Nenita Gaither

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ABSTRACT

Kimball, R. F., 1915 - and Nenita Gaither, 1919

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

1950. The influence of light upon the action of ultraviolet on *Paramecium aurelia*.

J. Cell. and Comp. Physiol.

A number of different effects upon *Paramecium aurelia* of monochromatic ultraviolet of wave lengths 2650 Å and 2804 Å were shown to be considerably decreased if the paramecia were exposed to intense light from a lamp having its major output in the long ultraviolet (3650 Å and longer) and the short visible immediately after they were exposed to the shorter ultraviolet. These effects were retardation of cell division, death before autogamy, reduced vigor after autogamy, and a change in the microscopically visible structure of the macronucleus. The reduced vigor after autogamy is probably due in large part to mutations while there is reason to believe that the other effects do not involve genetic changes. It is shown that different effects on the cell may be subject to different degrees of photoreactivation. No differences were established between material exposed to 2650 and to 2804 Å ultraviolet. No effect of light could be demonstrated upon X-ray-induced vigor after autogamy.

Physiology	cell
	death
Cytology	nucleus
Genetics	
Protozoology	

THE INFLUENCE OF LIGHT UPON THE ACTION OF ULTRAVIOLET ON *PARAMECIUM AURELIA*

R. F. Kimball and Nenita Gaither

Kelner ('49a) and, almost simultaneously, Dulbecco ('49) reported a phenomenon which has been called photoreactivation. Briefly, exposure to very long ultraviolet (3650 Å or longer) or short visible light immediately following exposure to 2537 Å ultraviolet considerably decreases the effect otherwise to be expected from the latter. This has now been demonstrated for a number of different organisms and for a variety of effects of ultraviolet. Kelner ('50) may be consulted for a review.

Kelner ('49b) and Novick and Szilard ('49) have shown that photoreactivation in *Escherichia coli* can be described by what Kelner has called the dose-reduction principle. The light can be treated as reducing the effective dose of the ultraviolet to some fraction of its measured value. Dulbecco ('50) has shown the same relation for bacteriophage. Kelner('49b), and Novick and Szilard ('49) both found a reduction of the effective dose to about one-third the measured value for inactivation of *E. coli*. Novick and Szilard ('49) found a reduction to a similar fraction for mutation induction in *E. coli*. On the basis of the similarity of the fraction for inactivation and mutation in *E. coli*, Novick and Szilard ('49) suggest tentatively that inactivation and mutation in their experiments may be due to the same action of ultraviolet. Dulbecco ('50) found rather different values of the fractional reduction for different *coli* phages. Kelner ('49b) could not find clear evidence for photoreactivation of "delayed mutation" in *E. coli* though he did find it for "zero point" mutation.

It is possible to investigate several, superficially, quite different effects of ultraviolet upon the ciliate protozoan *Paramecium aurelia*. The question may be raised whether all or only part of these effects are subject to photoreactivation and whether those that are, are reactivated to the same extent. Attempts to answer these questions will be made in this paper. A preliminary report of this work was given by Kimball and Gaither ('50).

MATERIALS AND METHODS

Sonneborn's stock 90 of variety 1 of P. aurelia was used and cultured in the standard lettuce infusion medium with living Aerobacter aerogenes as food. The paramecia for any one experiment were members of a single clone which had arisen at autogamy some 4 to 6 days before irradiation. Just before irradiation, animals which were undergoing division were picked from the available cultures of well-fed animals and concentrated in a mixture of equal parts of a dilute Ringer's solution and culture fluid. Groups of about sixty animals from this mixture were put into small quartz tubes which were approximately 18 mm long and had an inside diameter of 1.3 mm and an outside diameter of 2 mm. The tubes were left open at the ends, the fluid being held in by capillarity.

The slit image from a large quartz monochromator, using a Daniels-Heidt medium pressure mercury vapor lamp as a source (Hollaender and Emmons, '39), was centered on a groove ground in a microscope slide. A camera shutter, interposed in the beam, was closed; and one of the small quartz tubes containing the animals was centered in the groove. Exposures were started and stopped by means of the shutter. Measurements were made of the intensity of the ultraviolet just before and after a series of exposures using a standardized thermopile and galvanometer. The exposures were made in a darkened room, and the material being exposed was shielded from direct radiation from the lamp by a plywood shield and covered with a thick black cloth to exclude scattered radiation as far as possible. The quartz tubes containing the animals were kept in a covered box except during the exposure period.

After exposure, each quartz tube was dropped into a somewhat larger glass tube which was closed at one end and contained a small quantity of culture fluid. Four such glass tubes were placed in a test tube of such a size that the glass

tubes fitted tightly and were equidistant from the center. This test tube was lined with heavy paper. Another set of four glass tubes was placed in an unlined test tube. The unlined tube was rotated slowly in a water bath at 25°-27° C for half an hour in the beam of the General Electric 250-watt A-H5 mercury vapor lamp. The lined tube was put in the same bath in a metal container. The H5 lamp gives off intense light in the short visible and long ultraviolet (3650 Å and longer) region.

The animals were removed from the tubes, following exposure to the H5 lamp, and one animal was placed in each depression of a number of pyrex triple-depression spot plates. These operations were carried out in a room in which the only light was a small fluorescent microscope lamp covered with several layers of yellow cellophane. The manipulations were performed as rapidly as possible after treatment and the isolated animals were placed in a dark incubator which was not opened again until the next day. No further precautions concerning light were taken.

Exposures to X rays were made with a Coolidge self-rectifying tungsten target tube operated at 250 KV and 15 ma. Animals were exposed in the dark in small lucite dishes. Shortly after exposure, the contents of each dish were distributed equally between two small glass tubes of the same kind as those used to contain the quartz tubes in the ultraviolet experiments. One tube from each dish was put into a lined and one into an unlined test tube. The further procedure was the same as for the ultraviolet-treated animals.

We are indebted to Mr. J. Moshman of the Mathematics Panel of the Oak Ridge National Laboratory for the statistical analysis of the data on division rate.

EXPERIMENTAL

1. Retardation of cell division. Giese and his co-workers (see Giese, '47

for review) have investigated in some detail the retardation of division in Paramecium by monochromatic ultraviolet. The following methods were used in the present investigation. Each depression was examined on the day after treatment, and the number of animals was recorded. One animal from each depression was transferred to fresh medium, and the number of its descendants was recorded the next day. This procedure was repeated until the line of descent either died or completed at least twelve divisions. Control lines were kept until all treated lines had recovered. The time to the sixth division was calculated from these daily counts for each line of descent using the arbitrary assumptions that the last division on a given day occurred at the end of the day and that all the divisions on that day occurred at equal intervals. It will be shown in another paper that the errors introduced by these arbitrary assumptions are probably no more than a few per cent.

A more complete discussion of the retardation of cell division in Paramecium aurelia will be given in another paper. A brief account follows. Several different components of delay can be recognized having different relations to dose. These are delay in the first two or three division intervals; a cessation period in some one division interval, usually the third or fourth; and a recovery period, usually the fifth and sixth intervals. At higher doses, the first two or three divisions are ordinarily completed within 1 or 2 days. Then the animals frequently do not divide again for some days or even weeks. This long period during which no division occurs will be called the cessation period. Some animals die during this period, but many recover. Once division starts again, the normal rate is restored within two or three divisions. The time to the sixth division furnishes a convenient measure that includes almost all the delay in division but little of the period after normal division has been restored.

Most of the work was done with two different wave lengths of monochromatic

ultraviolet, 2650 Å and 2804 Å. In one experiment, 2378 Å and 2537 Å ultraviolet were used. The results are plotted in figures 1 and 2 in which time to the sixth division is plotted against dose. The individual points are the arithmetic means for all lines given the same dose and treatment in one experiment. The number of lines per point varied somewhat from experiment to experiment from a maximum of 60 to a minimum of 5, with an average of 30. The very low numbers are due to death before the sixth division which was considerable at the higher doses of 2804 Å but was not high (maximum 14%) with any dose of 2650 Å ultraviolet.

Curves of the form $y = ax^b$ were fitted to the data where y is the difference between the controls and the experimentals in the time in days taken to reach the sixth division; x, the dose in ergs/mm^2; and a and b are constants to be determined. The means weighted by the reciprocals of their standard errors were used for the analysis. The values of the constants under different assumptions are given in table 1 together with the value of t for certain pairs of differences between the constants. The first four lines give the value calculated on the assumption that both constants could be different for different wave lengths and light treatments. The results of tests of significance are given for the differences between the values of b for the light and dark groups for the two wave lengths separately. The differences tested in this way are not significant. However, as shown in the last line of the table, the value of t for the difference between the b's calculated from the dark and from the light data for the two wave lengths combined is rather large, suggesting that with larger numbers a difference might be demonstrated. At least, it seems desirable to keep in mind the possibility that light treatment changes the form of the dose-response curve.

Tests of significance of the differences between the values of log a were made with the assumption that b, and so the form of the curve, was the same for the groups being compared. In table 1, it is seen that the difference was significant at the one per cent level for the 2804 Å group but was not significant at the five per cent level for the 2650 Å group. Nevertheless, it seems probable, though not demonstrated

Figure 1

Days to the sixth division plotted against dose of 2650 Å ultraviolet. Points for 2378 Å and 2537 Å ultraviolet are also included. Each point represents the arithmetic mean for 16 to 60 lines of descent from a single experiment. Curves of the form $y = ax^b$ were fitted to the 2650 Å data.

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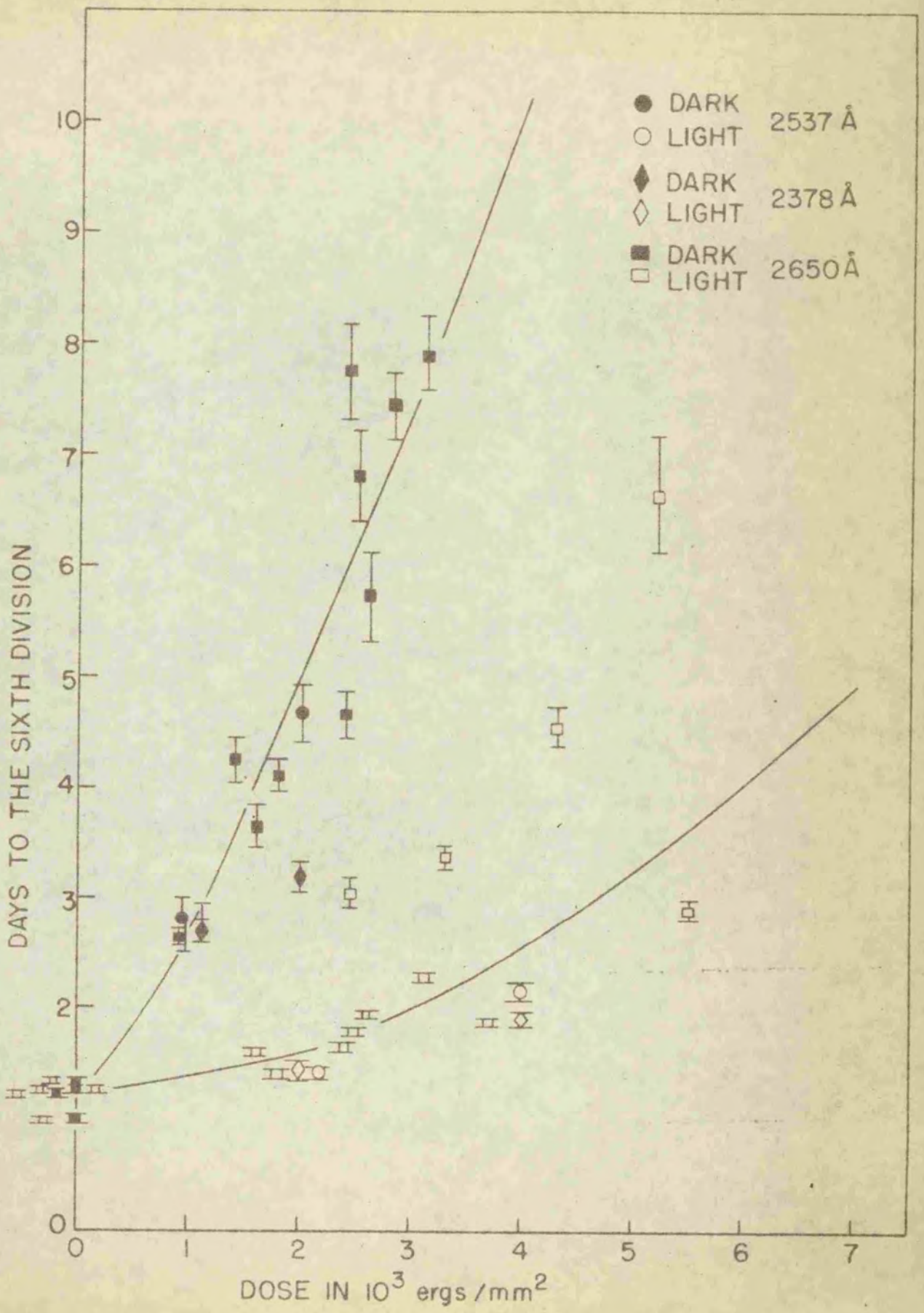

FIG. 1

Figure 2

Days to the sixth division plotted against dose of 2804 Å ultraviolet. Each point represents the arithmetic mean for 5 to 54 lines of descent from a single experiment. Curves of the form $y = ax^b$ were fitted to the data.

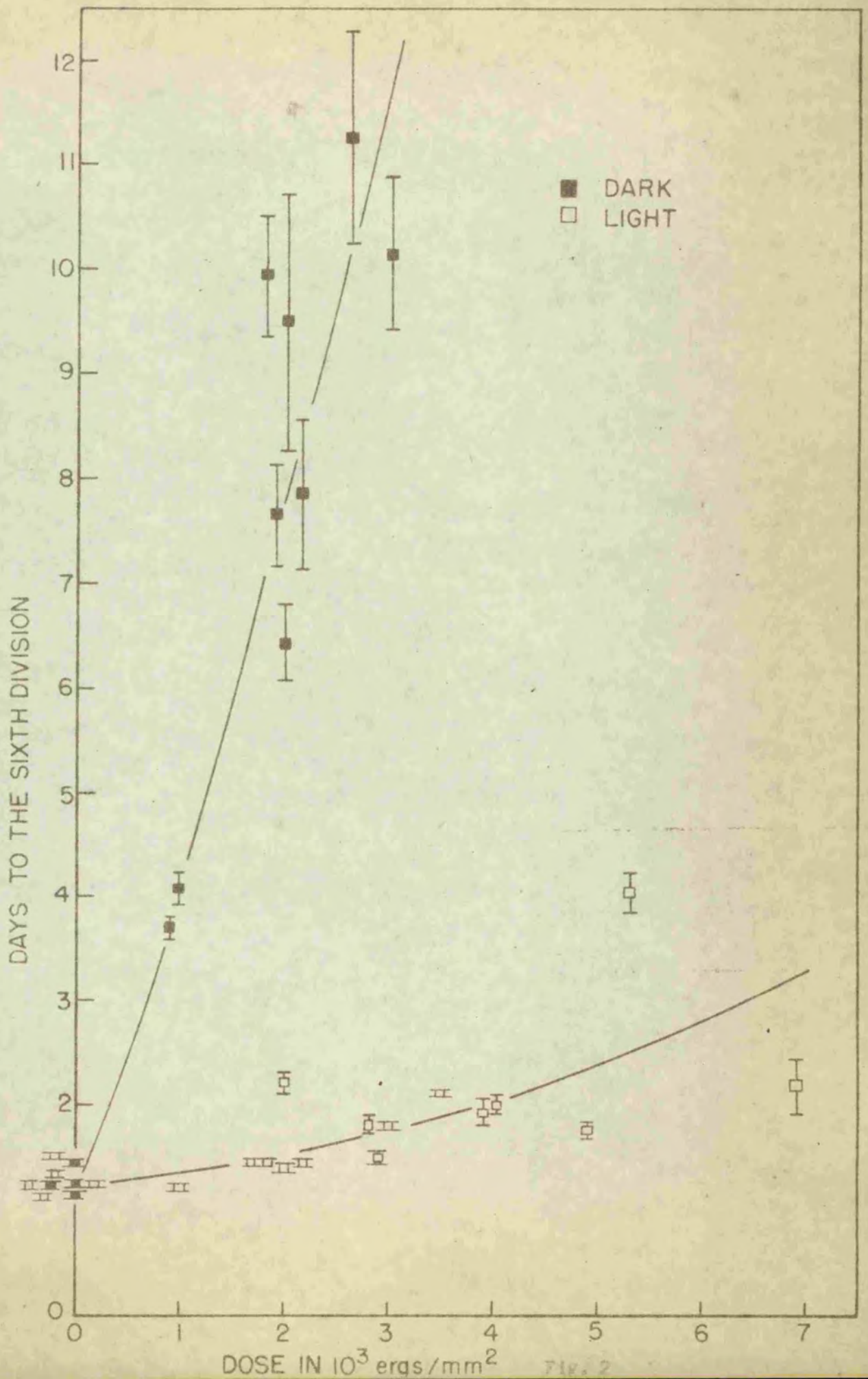
DARK
LIGHT
DAYS TO THE SIXTH DIVISION
DOSE IN 10^3 ergs/mm^2
0
1
2
3
4
5
6
7
8
9
10
11
12

Fig. 2

Table 1

Delay in the sixth division: values of constants from fitting curves of the form $y = ax^b$: tests of significance of differences

Conditions		Log a	b	Difference between	t	d.f.	P
2650 A	Dark	-3.62	1.27	b's	1.22	18	0.3 - 0.4
	Light	-6.23	1.77				
2804 A	Dark	-3.18	1.21	b's	1.63	19	0.1 - 0.2
	Light	-6.35	1.73				
2650 A	Dark	-4.85	1.65	log a's	1.98	18	0.05 - 0.1
	Light	-5.84	1.65				
2804 A	Dark	-4.63	1.68	log a's	3.42	19	0.01
	Light	-6.17	1.68				
2650 A	Dark	-3.55	1.25	log a's	0.91	15	0.3 - 0.4
2804 A	Dark	-3.31	1.25				
2650 A	Light	-6.16	1.75	log a's	0.39	22	0.7
2804 A	Light	-6.40	1.75	b's	1.79	39	0.05 - 0.1

beyond question, that light reduces the effectiveness of 2650 Å ultraviolet since the value of t is somewhat large and, as can be seen from figure 1, the points for the light treated material consistently fall below corresponding points for the material kept in the dark. The few points for experiments with 2378 Å and 2537 Å suggest that light has about the same effect with these wave lengths as with longer ones but are obviously not sufficient to establish this conclusion.

No differences in response to light by material treated with different wave lengths are established by the data. This is somewhat surprising in view of the finding by Giese and co-workers (see Giese '47 for review) that, under some circumstances, 2650 Å ultraviolet is more effective in delaying cell division than is 2804 Å. However, Giese ('45) found that well-fed animals showed a slightly greater response to 2804 Å ultraviolet than to 2650 Å when the response was measured by delay of the third division. The slightly greater value of log a for the 2804 Å group kept in the dark is in line with his finding. All we can say is that our material has not served to demonstrate a difference in effectiveness of the wave lengths in either the dark or light material.

Finally, if the data are taken at their face value and the dose reduction hypothesis assumed, light reduces the effectiveness of a given dose of 2804 Å ultraviolet to about one-eighth, and of a given dose of 2650 Å ultraviolet to about one-quarter its effectiveness in the dark. However, since no difference between the wave lengths is established statistically, these two values cannot be considered significantly different from one another.

2. Death before autogamy. Two major categories of death as a result of exposure to ultraviolet must be considered in *Paramecium aurelia*, death before autogamy and death after autogamy. Autogamy results in the production of completely homozygous micro- and macronuclei derived from the micronuclei which were present before autogamy. Thus death after autogamy could well be the result of

gene mutation or chromosome aberration induced in the micronuclei by the radiation. On the other hand, mutations would be unlikely to express themselves before autogamy because the macronucleus is a multiple structure containing many complete sets of genes (see Sonneborn, '47). Thus death before autogamy is probably due to causes other than gene mutation and chromosome aberrations. The present section will be concerned only with death before autogamy, and with the material exposed to 2804 Å ultraviolet.

It also seems desirable to distinguish two categories of death before autogamy, that before the first division after irradiation and that after the first division. Almost all the latter category of death occurs in the cessation period of division delay. During this time, the animals become very small, and the impression is given that even those that do survive come very near death. In general, only some of the products of the first two or three dividions of a particular treated animal die. However, only one animal for any one line of descent was kept each day. Since the cessation period usually lasts several days, this means that the fate of only one product of the second or third division of any one treated animal was known. This should provide a reasonable measure of the probability that any product of the first two or three divisions will die during the cessation period.

The data for survival through the first division are poltted in figure 3. Different experiments yielded rather different results. To bring out the major trends, all data at approximately the same dose were combined; and the dose for the combined data was taken as the weighted average of the several doses. In the same figure is plotted the dose to the dark group necessary to produce the same percentage survival as a given dose to the light group. A linear relation is expected on the dose reduction hypothesis. It is not clear from the graph that such a relation exists. A case could be made for a sigmoid relationship. Nonetheless, the data hardly warrant a statement that the expectation is not met. Under

Figure 3

Log per cent of survival through the first division after irradiation plotted against dose of 2804 Å ultraviolet. Also plotted is the dose for the dark group which gives the same survival as a given dose for the light group as determined from the graph. The curves were fitted by eye.

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the circumstances, it can only be said that the data do not afford a critical test of the hypothesis.

If the hypothesis is correct the dose required to produce a given per cent survival in the dark group is about half that in the light group.

An examination of figure 2 shows that the light was considerably more effective in the case of retardation of division. This same fact is brought out in another way in table 2. This table compares the number of deaths before and after the first division in those experiments in which both were recorded. In all these experiments, data on retardation of cell division were also obtained. It should be noted that figure 3 includes all the experiments of table 2 and several others in which only death before the first division was studied. In the dark group, quite a few lines of descent which survived the first division died within the next few divisions while in the light group almost every animal which survived the first division produced a viable line of descent. As has been pointed out above, most of the death after the first division is associated with the cessation period of division retardation. Therefore, the difference in the relations of the two kinds of death in the dark and light groups can be looked upon as another indication of the difference in effectiveness of light in respect to death before the first division and retardation of cell division.

3. Reduced vigor after autogamy. After complete recovery from the retardation of cell division, the progeny of a number of paramecia which had been exposed to 2650 Å ultraviolet were allowed to go through the sexual process of autogamy. From each original treated animal, twenty-five autogamous animals were isolated, each into a separate depression. They were allowed to multiply for 4 days and examined for survival and amount of growth. All autogamous animals which failed to survive 4 days of produced less than a maximum population which had exhausted the available supply of food were classified as of reduced vigor. Evidence has been presented (Kimball,

Table 2

Death before and after the first division among animals exposed to 2804 Å ultraviolet

Dose in 10^3 ergs/mm^2	H5 Lamp	Number dying: Before first division	Number dying: After first division	Number Surviving	Total
1	-	0	2	82	84
2	-	5	45	121	171
3	-	42	38	28	108
1	+	1	0	51	52
2	+	15	0	137	152
3	+	18	0	59	77
4	+	27	0	63	90
5	+	24	2	31	57
6	+	27	0	2	29
7	+	48	0	6	54

- not exposed to H5 lamp

+ exposed to H5 lamp

'49a and b) that similar reduced vigor which appears after autogamy in the progeny of beta- or X-irradiated animals is due primarily to gene mutation. The necessary genetic evidence has not been obtained for the ultraviolet-treated material, however, it seems probable that much of the reduced vigor is due to gene mutation. In any case, it is clear that some kind of inherited change is involved since the effect does not appear until many divisions after treatment and until complete recovery from the immediate, and presumably nongenetic, retardation of cell division.

The results are plotted in figure 4 as log percentage of normal autogamous clones against dose of ultraviolet. Each point represents the combined data from several different experiments and involves between 20 and 100 treated animals with 25 autogamous progeny from each. Straight lines have been fitted to the data by eye since more exact fitting procedures did not seem warranted in view of the considerable scatter of the different experiments. The slope of the line for the light group is about one-fifth that for the dark group which is in fairly good agreement with the magnitude of the effect upon division rate in the 2650 Å group.

4. Change in the structure of the macronucleus. Living paramecia, examined with a Zeiss phase-contrast microscope, show a large number of small dark granules scattered throughout the macronucleus (fig. 5). A preliminary report of the changes in these granules brought about by ultraviolet has been given by Kimball ('49c). It has been possible to demonstrate an effect of light upon these changes.

The animals were exposed to ultraviolet and light as described above. Shortly after irradiation they were removed from the tubes and placed en masse in a quantity of culture medium. From time to time, samples were removed and examined as soon as possible under the phase microscope. All manipulations up to and including the removal of the sample were carried out in dim yellow light, but further manipulations and the examination of the living animals under the phase microscope were

Figure 4

Log per cent of normal autogamous clones plotted against dose of 2650 Å ultraviolet. The curves were fitted by eye.

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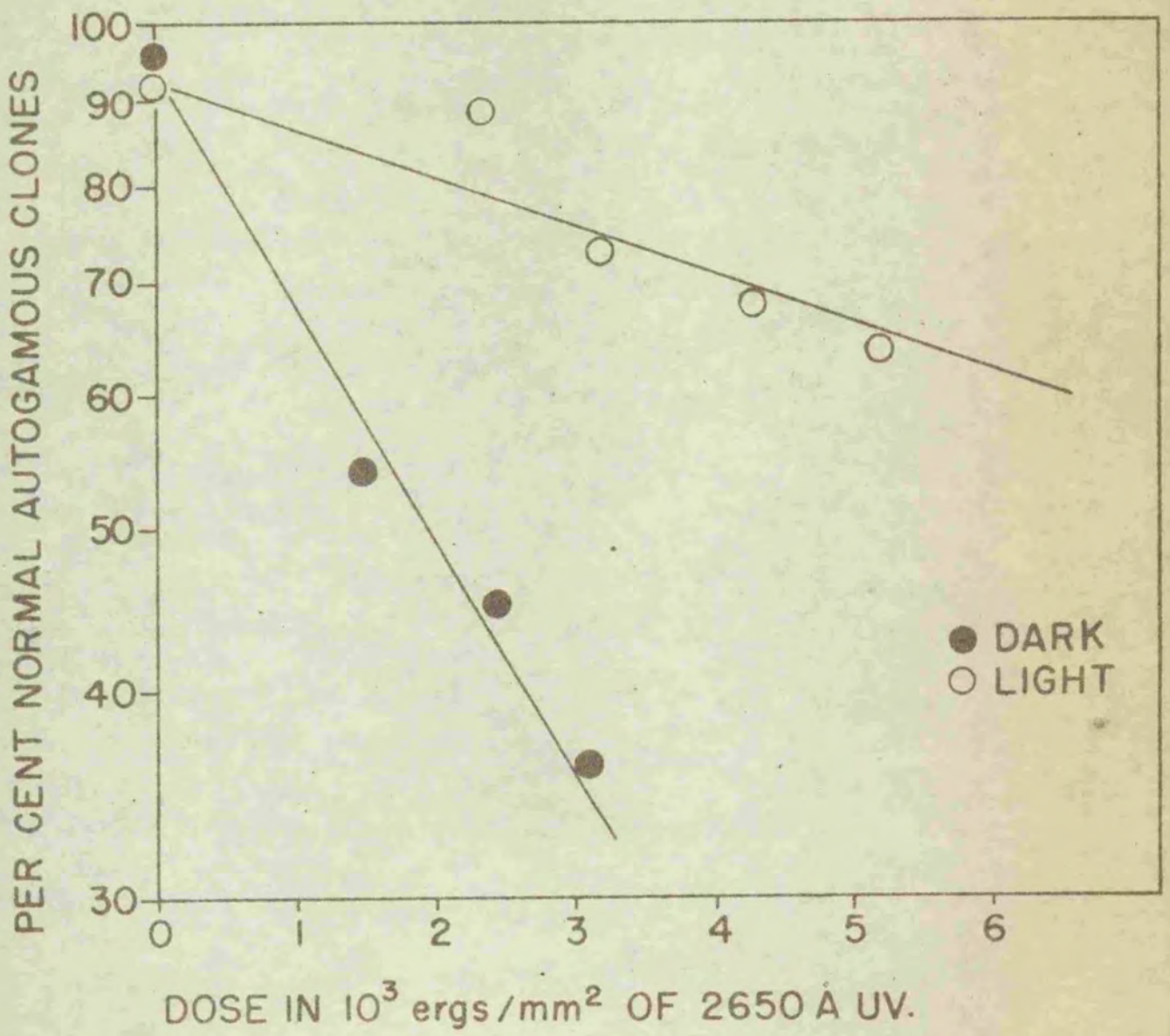

FIG. 4

Plate 1

Photomicrographs of living paramecia taken with a phase-contrast microscope. The animals were compressed to about half their normal diameter but were still living as judged by cyclosis and ciliary movement which continued for many hours in these preparations.

Magnification 800 X.

Fig. 5. Normal animal showing small dark granules scattered throughout the macronucleus.

Fig. 6. Granules within the macronucleus are clumping together. One and a half hours after irradiation.

Fig. 7. Extensive fusion of the granules and vacuolization of the fused masses. Ten hours after irradiation.

Fig. 8. Fusion of almost all the granules into a single large vacuolated mass. Twenty-four hours after irradiation.

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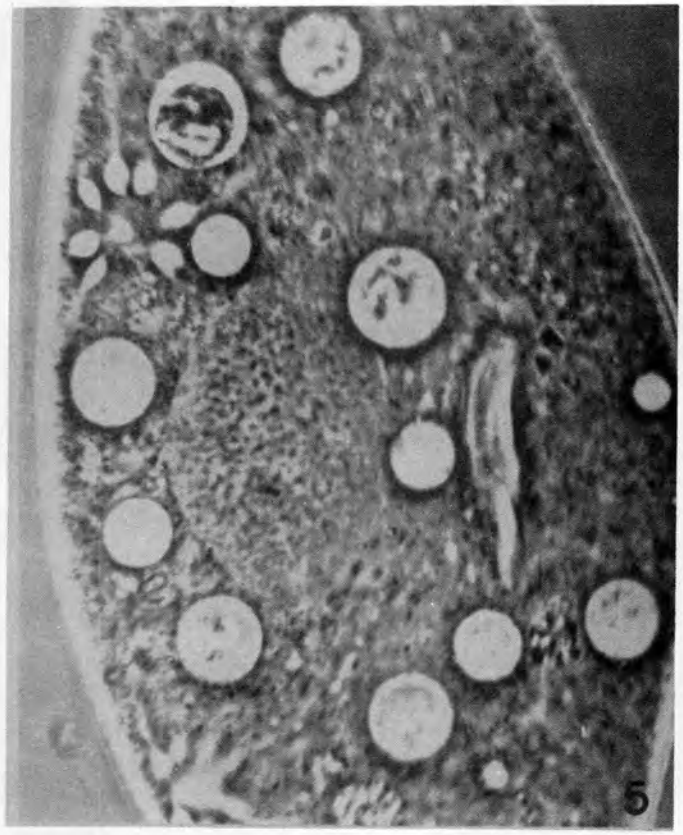

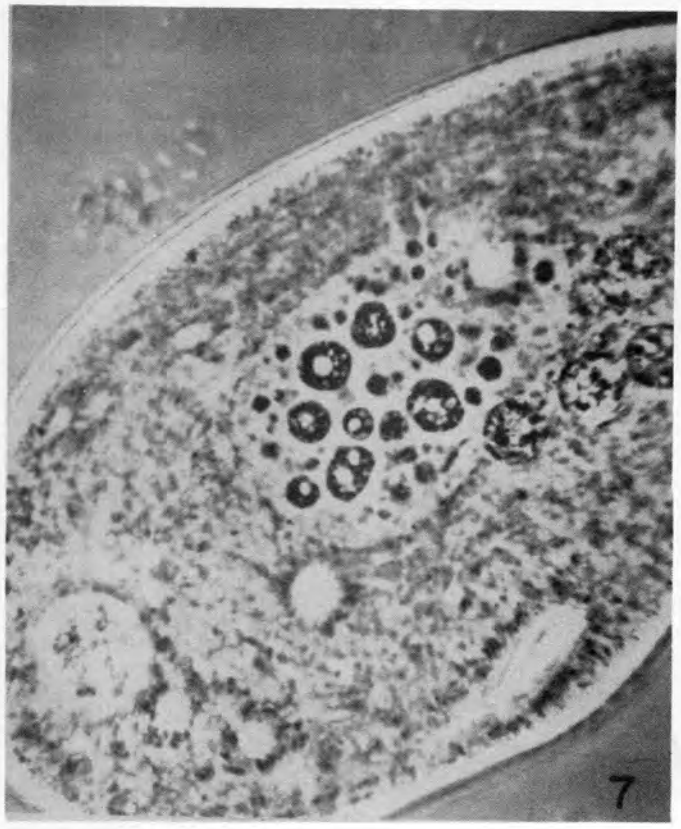

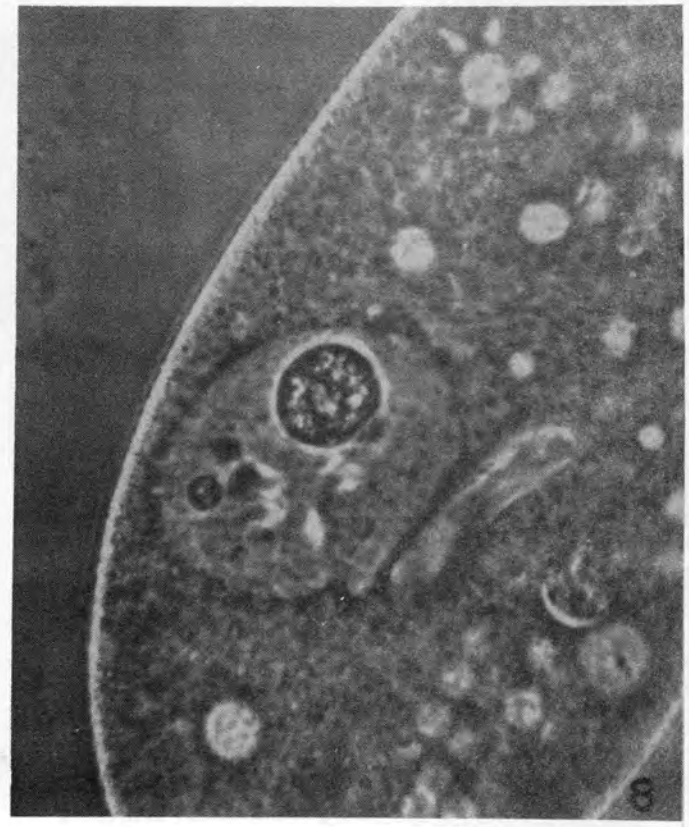

carried out in ordinary laboratory illumination. These latter operations occupied about 15 minutes.

Shortly after exposure to 2804 Å ultraviolet, the macronuclear granules start to clump together (fig. 6), and then to fuse into large masses which become highly vacuolated (fig 7). The fusion continues until nearly all the granules form a single large mass (fig. 8), a stage which is usually reached some 12 to 24 hours after irradiation. So far the phenomenon has been adequately studied only in animals exposed to doses which were sufficient to lead to death before the first division and within about 2 days after irradiation. Thus it is not clear whether the complete fusion of the granules is always followed by death.

When animals were exposed to a dose of ultraviolet sufficient to kill both the light and the dark groups, the fusion of granules proceeded at about the same rate in both groups. With a dose of ultraviolet sufficient to kill only the dark group, clumping of the granules occurred at about the same time in both groups. However in the dark group, fusion of granules proceeded in the usual way while in the light group no fusion occurred. The clumped granules apparently separated again, for samples taken some hours after treatment showed completely normal macronuclei although samples taken earlier showed some clumping in every animal. It can be concluded that exposure to the H5 lamp can prevent the change in the macronucleus from going to completion although it appears to have no effect upon the rate at which the change proceeds.

Carlson and McMaster (unpublished) have also found that a change induced by ultraviolet in the structure of the nucleus, in their case in the structure of the nucleolus of grasshopper embryo neuroblasts, is subject to photoreactivation. We are indebted to Dr. Carlson for permission to mention this rather similar case.

5. <u>Lack of effect of light upon reduced vigor due to X rays</u>. Attempts were made to demonstrate photoreactivation in X-irradiated animals using reduced vigor after autogamy as the criterion. The data are given in table 3. It is obvious

Table 3

Light and reduced vigor after autogamy in X-irradiated material

Experiment	Dose in roentgens	Number of treated animals†	Per cent of autogamous animals of reduced vigor	
			Dark	Light
1	none	20	0.2	1.0
	2400	20	38.4	33.4
	4800	20	53.6	43.4
	9700	20	85.2	76.4
2*	none	20	0.8	2.2
	1	20	4.2	9.2
	2	20	59.0	48.6
	3	20	91.4	85.0
3	none	20	4.2	4.4
	6000	60	68.0	66.6

* Doses were not exactly known because of an error in timing. Arbitrary numbers were assigned in order of increasing dose.

† The number of treated animals was the same for the dark and light groups. From each treated animal, 25 autogamous animals were obtained. The percentages are for the whole group of autogamous animals (500 in most cases).

that light has had little, if any effect. However, in the majority of cases, the per cent of reduced vigor is slightly less in the progeny of the light-treated group. To test whether any significance can be attached to this apparent trend, an analysis of variance was carried out after converting percentages to angles using the table of this transformation given by Snedecor ('46). The control groups were omitted from the analysis since there was no reason to believe that animals which had not been exposed to X rays would react to light in the same way as those that had. The group of sixty animals exposed to 6000 r in experiment 3 was divided at random into three groups of twenty each. These three groups and the three groups from each of the other two experiments were treated as a series of nine different doses for purposes of the analysis. The results are shown in table 4. The variance attributable to the differences between the light and dark groups was not found to differ significantly from the error variance when the t test was applied. Therefore, the small difference between the two groups can be attributed to chance, and it can be concluded that the present data are consistent with the view that light has either no influence at all upon the action of X rays or an influence so slight as to be undetected by the present experiments.

DISCUSSION

Photoreactivation has been found for a number of different organisms and for a variety of effects of ultraviolet. The present investigation shows that it occurs for a number of different effects in Paramecium, in fact all that have been investigated. Therefore, it is tempting to think that there is one primary effect of ultraviolet which is subject to photoreactivation and that from this primary effect follows such varied consequences as retardation of cell division, mutation, death, etc. Such a hypothesis has been tentatively suggested by

Table 4

Analysis of the variance of the reduced vigor data for X-irradiated material

Component of variance attributable to:	Degrees of Freedom	Mean Square	Variance ratio or t	Probability
Light-dark difference	1	877	1.6	cir. 0.1
Dose differences	8	12600	37.0	< 0.001
Remainder (error)	350	341	—	—

Novick and Szilard ('49) on the basis of their finding that photoreactivation for both inactivation and mutation of E. coli could be described as reduction of the effective dose to approximately one-third its measured value. Before considering the bearing of our data upon this conclusion, the question must be raised as to how far the apparently different phenomena which we have studied can be shown to be connected on grounds other than the facts that they are all produced by ultraviolet and that they are subject to photoreactivation.

Two of the phenomena, retardation of cell division, and death before autogamy but after the first division, have already been shown to be closely related to one another; and it is probable that the changes culminating in the long cessation of division, which accounts for much of the total delay in the sixth division, also lead to death after the first division. The effect upon macronuclear structure has been investigated up to now only at doses causing eventual death of nearly all animals before the first division. It is possible that the alteration in macronuclear structure is simply one expression of the changes which lead to death. However, the possibility remains that, at some lower dose, this alteration is reversible. In that case, it would have no direct connection with death.

It seems quite clear that reduced vigor after autogamy is not closely connected with any of the other changes. It is probable that at least a part, perhaps nearly all, of the reduced vigor is due to gene mutation. On the other hand, it is unlikely that retardation of cell division, death before autogamy, or change in macronuclear structure can be attributed to genetic changes. As mentioned above, the macronucleus has been demonstrated to contain many sets of genes (see Sonneborn, '47 for review). It would be highly improbable in such a situation that any gene mutations of chromosomal aberrations which did occur would lead to immediately detectable effects. Moreover, retardation of cell division is quite temporary, disappearing after about six divisions. Further evidence of a lack of

connection is given by the fact that there is no correlation between the time to the sixth division and the per cent of reduced vigor after autogamy in groups of lines given the same dose of ultraviolet.

According to Kelner ('49) Novick and Szilard ('49), and Dulbecco ('50), the action of light can be described as a reduction of the effectiveness of a given dose of ultraviolet without alteration of the form of the dose-response curve. All our data are in fair agreement with this generalization although only rather drastic departures from it would have been clearly demonstrated. However, the data for retardation of cell division show a suspiciously large difference between the exponents for the dark and light groups suggesting that the form of the curve may not have been the same in the two cases. Two possible causes of such a discrepancy suggest themselves. The relation $y = ax^b$ is certainly no more than approximation of the form of the response curve. Therefore, the value of b might be different when calculated from data with different proportions of long and short delays, as was the case for the dark and light groups. Secondly, there is reason to believe that retardation in cell division is due to several different processes with different relations to dose. If these processes were unequally affected by light, a change in the form of the curve could occur.

This brings us to the question whether different effects of ultraviolet upon a cell are subject to photoreactivation to the same extent. Novick and Szilard ('49) found that the amounts of photoreactivation for inactivation and mutation in E. coli were approximately the same. Kelner ('49b and '50) has reported the dose-response curve only for inactivation of E. coli. However, he could find no clear evidence in this organism for photoreactivation of "delayed" mutations although he did find it for "zero point" mutations. Kelner ('49b) points out that the evidence on photoreactivation of delayed mutations is not critical since only one dose of ultraviolet was used and the dose-response curve is known to rise

to a maximum and then decline again. Under the circumstances, it is possible that photoreactivation occurred but was not detected in this one experiment. However, the possibility remains that delayed mutations either are not subject to photoreactivation or are subject to a considerably lesser extent than are "zero point" mutations and inactivation. In Paramecium, the evidence appears quite clear that the amount of death before the first divisions much less affected by light than is the delay to the sixth division or its correlated effect, death after the first division.

Both Novick and Szilard ('49), and Dulbecco ('50) have assumed that there are two components in the ultraviolet effect, one which is subject to photoreactivation and one which is not. It is obvious from the above that these two components cannot be equally involved in all effects of the ultraviolet. It seems quite possible from the fact that such a variety of different effects of ultraviolet are subject to photoreactivation that there is some primary action of ultraviolet which is subject to photoreactivation and has widespread effects upon the cell. However, it seems equally possible that the component of ultraviolet action which is not subject to photoreactivation is a multiple one differing in amount and kind for different effects of the radiation. If this is so, it is a little surprising to find no difference in the degree to which retardation of cell division by different wave lengths is subject to photoreactivation, since it would not seem at all improbable that the different primary effects of ultraviolet would have different action spectra. All that can be said is that such differences have not appeared in statistically significant fashion in our data.

The failure to obtain photoreactivation of mutations induced by X rays is in fairly good agreement with Dulbecco's ('50) findings. He found a very small amount of photoreactivation after X irradiation of phage. Our material is less satisfactory for exact quantitative study than his, so our failure to establish an effect is in

reasonable agreement with his finding that the effect is small. It indicates strongly that the process of mutation induction by these two agents is not the same despite the apparent similarity of the mutations produced. Ultraviolet must either induce mutations indirectly through some process which can act some time after the irradiation has ceased, or part of the mutations must remain capable of reversion by light for some time after irradiation.

SUMMARY

1. It has been demonstrated that the exposure of Paramecium aurelia to high-intensity light immediately after exposure to ultraviolet reduces the effect otherwise to be expected from the ultraviolet.

2. This has been demonstrated for the following effects of ultraviolet: retardation of cell division, death before autogamy, reduced vigor after autogamy, and fusion of macronuclear granules.

3. No influence of light upon X-ray induced reduced vigor after autogamy could be demonstrated.

4. The hypothesis that light reduces the effectiveness of a given dose of ultraviolet without changing the form of the dose-response curve can be fairly well applied to the data.

5. Light is less effective in reducing the amount of death before the first division after irradiation than in reducing retardation of cell division. Thus, different effects of ultraviolet upon the cell may be subject to different degrees of photoreactivation.

6. No difference could be demonstrated in photoreactivation of retardation of cell division brought about by 2650 Å and by 2804 Å ultraviolet.

7. The relation of these findings to mechanisms of photoreactivation and action of ultraviolet are discussed.

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