QUANTUM RELATIONS IN PHOTOREACTIVATION OF COLPIDIUM*

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The degree of photoreactivation or reversal of injurious effects of ultraviolet radiations (UV) by illumination with visible light has been shown to increase with length of exposure of the irradiated organisms to visible light (Dulbecco, 1949; Kelner, 1951). After maximal photoreactivation of phage has been achieved, further illumination with white light has no further effect (Dulbecco, 1949), whereas in *Escherichia coli* and *Streptomyces griseus* additional illumination proved to be injurious in some cases (Kelner, 1951). Photoreactivation in *Colpidium colpoda* increases with exposure to monochromatic or mixed visible light to a maximal value. Additional illumination to the extent previously tried was not injurious (Giese, Brandt, Iverson, and Wells, 1952). The question arises as to how many quanta of visible light are necessary to reverse maximally the action of 1 quantum of UV. This information would be interesting for theoretical reasons. The present report records such determinations for *Colpidium colpoda*, a ciliate protozoan.

Methods and Materials

As a criterion of UV injury the retardation of the third division of the irradiated colpidia was determined in much the same way as in previous experiments with paramecia (Giese, 1939). Photoreversal was measured by recovery from such division delay. Fortunately *Colpidium colpoda* like *Paramecium caudatum* (Giese, 1939, 1945) does not show a period of cessation of division after UV treatment as does *P. aurelia* or *P. multimicronucleatum* (Giese and Reed, 1940; Kimball and Gaither, 1951) or *Tetrahymena geleii* (Christensen, unpublished). Therefore the delay in the third division after irradiation may be determined without this complication. The methods were in general similar to those described in the previous paper (Giese, Brandt, Iverson, and Wells, 1952).

As a source of radiation a quartz mercury arc was used and the radiations were passed through a natural quartz monochromator. The 2654 A line in the short UV was reflected by a right angle quartz prism upon the horizontally held quartz cell containing the protozoa. For photoreactivation the light from a high pressure Ames

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mercury arc operating at high voltage was resolved by a monochromator and either the blue (4350 A), the violet (4050 A), or the long UV (3660 A or 3350) was used. A few experiments were also performed with green light (5460 A). The light intensity for both visible and UV radiations was measured by a thermopile calibrated against United States Bureau of Standards certified lamps.

The intensity of each wave length used was always the maximal obtainable. For most experiments the intensity at λ 2654 A was about 3 to 5 ergs/mm.^{2}/sec., but when the arc was freshly cleaned, the intensity was slightly higher. The lowest intensity at the time the last experiment was performed was 1.88 ergs/mm.^{2}/sec. For λ 4350 A, the intensity varied from 136 to 239 ergs/mm.^{2}/sec.; for λ 4050 A, from 100 to 117 ergs/mm.^{2}/sec.; for λ 3660 A from 57.2 to 190 ergs/mm.^{2}/sec.; for λ 3350 A from 24.6 to 46.4 ergs/mm.^{2}/sec.

EXPERIMENTAL

1. Studies with Well Fed Individuals, Using Blue Light.—For colpidia irradiated with a dosage of 1000 ergs/mm.^{2} at λ 2654 A the third division on the average occurs some 40 hours later than in untreated controls isolated in culture tubes at the same time. When individuals treated with UV light are given a dosage of an equivalent number of quanta of blue light (609 ergs/mm.^{2}) a small degree of photoreactivation may possibly occur, but it is never striking enough to be above the margin of error (see Table I). When UV-treated colpidia are given a dosage of 10 quanta of blue light (6090 ergs/mm.^{2}) for each quantum of UV light delivered, slight reactivation may occur. A much more striking and significant photoreactivation follows exposure to 100 quanta of blue for each quantum of short UV (Table I). The latter exposure to blue light gives almost maximal reactivation producing a dose reduction of UV light to approximately one-third (*i.e.* as if the colpidia had been irradiated with only one-third the ultraviolet dosage). If 1000 quanta of visible light are delivered per quantum of UV, there may be an additional small increment of photoreactivation as seen in Fig. 1.

2. Photoreactivating Starved Colpidia with Blue Light.—Since starved colpidia were found to be more sensitive to UV than well-fed ones and photoreactivation was less readily achieved per unit dose of UV, quantum experiments were also carried out with starved colpidia for comparison with the determinations on the well fed animals. The data for reactivation with blue light (4350 A) are also given in Fig. 1. The maximum photoreactivation achieved seems to be about 80 per cent. In all cases about 800 quanta are needed per quantum of UV to achieve maximal photoreactivation; at this point saturation is achieved and additional illumination does not perceptibly increase the degree of photoreactivation. Since such large dosages were necessary for photoreactivation, the effects of relatively small numbers of quanta were not tried on the starved animals.

3. Quantum Requirements for Photoreactivation at Various Wave Lengths.—Since the energy per quantum increases as the wave length decreases, the

TABLE I

Photoreversal by Illumination with Visible or Long Ultraviolet (UV) of Injury Caused to Well Fed Colpidia by Exposure to a Dosage of 1000 Ergs/Mm². of Short Wave UV(2654 A)

Date	Wave length of PRL*	Ratio of No. of quanta of PRL to quanta of UV	Dosage of PRL	Photoreactivation
	A		*ergs/mm.²*	*per cent*
4/19/52	4350 blue	1	609	8
		10	6090	8
		100	60,900	54
		1000	609,000	72
4/22/52	4350	1	609	6
		10	6090	3
		100	60,900	61
4/29/52	3660	1	726	12
		10	7260	30
		100	72,600	84
5/24/52	3660	1	726	3
		100	72,600	25
5/27/52	3660	1	726	3
		10	7260	6
		100	72,600	60
6/18/52	3660	1	726	16
		10	7260	11
		100	72,600	60
5/20/52	3350	1	793	−7
		10	7930	14
		100	79,300	24
7/ 1/52	3350	1	793	8
		10	7930	20
		100	79,300	6
7/ 4/52	3350	1	793	−6
		10	7930	27
		100	79,300	42

* PRL, photoreactivating light.

possibility exists that smaller numbers of quanta might be needed at shorter wave lengths. In the previous study it was shown that all wave lengths from 4350 A to 3350 A produced some degree of photoreactivation. Therefore quantum determinations were made at each of these wave lengths, as well as a few at 3130 and 5460 A (green).

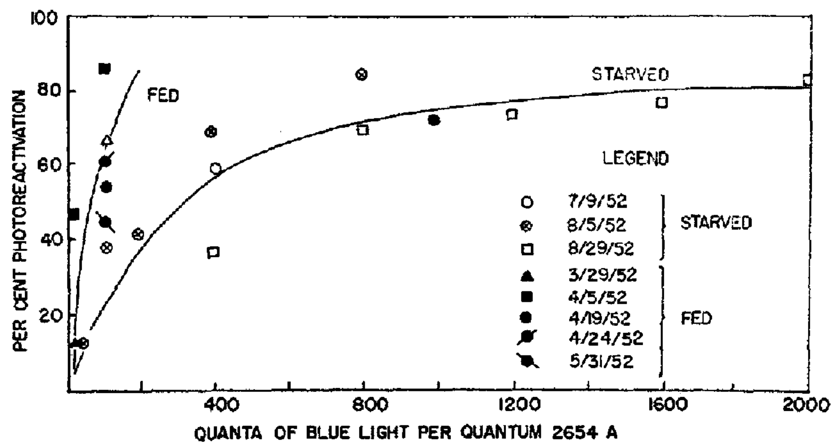

FIG. 1. Per cent photoreactivation for well fed and starved colpidia exposed to various numbers of quanta of blue light (4350 A) per quantum of injurious short UV (2654 A). In each case the dosage at 2654 A was 1000 ergs/mm.[2] for well fed and 250 ergs/mm.[2] for starved colpidia.

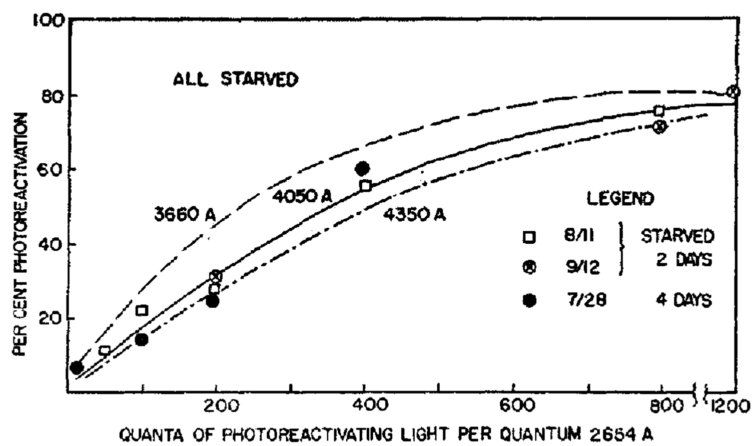

FIG. 2. Per cent photoreactivation for starved colpidia exposed to various numbers of quanta of violet (4050 A) per quantum of injurious short UV (2654 A). In each case the dosage of 2654 A was 250 ergs/mm.[2] The curves drawn through the points for wave lengths 3660 A and 4350 A were added to the graph for 4050 A. In view of the wide scatter of points (see Figs. 1 and 3) in such experiments it is questionable whether the order of the curves has real significance. The more rapid rise of the curve for 3660 A is suggestive of higher efficiency.

Experiments for the violet (4050 A) and for the long UV (3660 A) are given in Figs. 2 and 3. Approximately the same relations as those found for the blue are observed to hold here—for starved animals approximately 800 quanta are required per quantum of short UV (2654 A), whereas the number for the well fed animals is again approximately 100 at 3660 A. Therefore the conclusion may be drawn that as the wave length decreases there is little change in the general quantum relations. To bring out this point more clearly, the data for smaller numbers of quanta are given in Table 1. It is evident that although the energy of a quantum of reactivating light at λ 3660 A is greater than that of a quantum at λ 4350 A by the ratio: 4350/3660, there is no significant increase in photoreactivation with such increase in quantum size.

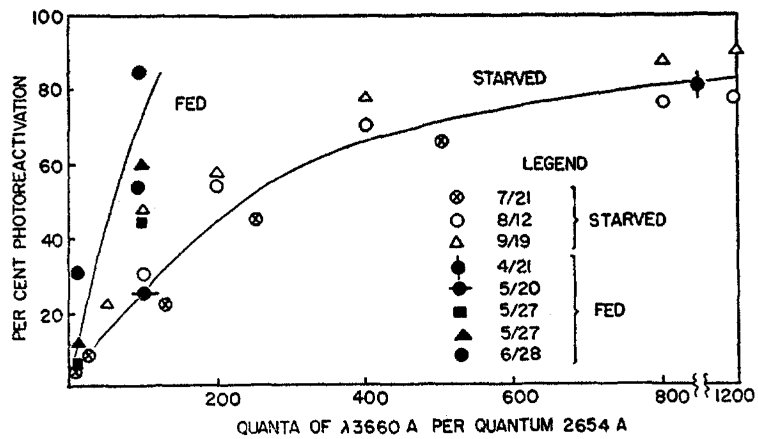

FIG. 3. Per cent photoreactivation for well fed and starved colpidia exposed to various numbers of quanta of long UV (3660 A) per quantum of injurious short UV (2654 A) previously delivered. In each case the dosage at 2654 A was 1000 ergs/mm.[2] for well fed and 250 ergs/mm.[2] for starved colpidia, with the exception of the experiment of July 21 in which case a dosage of 400 ergs/mm.[2] was used.

The largest quanta available for photoreactivation are those at λ 3350 and 3130 A. Attempts were therefore made to determine the quantum requirement for photoreactivation at these wave lengths. The data for fed colpidia are included in Table I, and for starved ones in Table II. These data reveal that light of 3350 A is injurious to starved colpidia, and rather than photoreactivating, larger dosages are additively injurious. Yet smaller dosages cause a certain degree of photoreactivation. Pretreatment with 3350 A is also additively injurious. Starved colpidia treated with short UV and subsequently exposed to series dosages of λ 3130 A showed a maximum photoreactivation of 28 per cent (Table II). A like dosage with λ 3130 A alone retarded the third division subsequent to exposure for about 1.5 hours. While the data are interesting in demonstrating that photoreactivation occurs even at this short wave length, a fact not previously observed, the quantum relationships were not determined at this wave length because larger dosages are in themselves injurious not only to starved colpidia but to fed controls.

4. Absorption Measurements.—By interposing a dense suspension of colpidia between the source of monochromatic light and the thermopile it is possible to determine the relative amounts of UV and blue light removed on passage through well fed as compared to starved colpidia. It is also possible to determine whether colpidia subjected to UV show an increase in absorption in the blue

TABLE II

Photoreactivation by Exposure to Light of Wave Lengths 3350 A and 3130 A after Injuring Starved Colpidia with Ultraviolet of Wave Length 2654 A

Date	Dose of 2654A	Wave length of PRL	Quanta of PRL per quantum 2654 A	Dosage PRL	Photoreactivation
	*ergs/mm.*²	*A*		*ergs/mm.*²	*per cent*
7/24/52	400	3350	2.5	793	28
			25	7930	6
			125	39,650	−3.4 injury
			250	79,300	−20 injury
8/27/52	250	3350	10	1982	6.4
			100	19,820	14
			200	39,650	−4 injury
			400	79,300	Death
9/ 4/52	250	3350	50	9912	23
			100	19,825	2
			200	39,650	−7 injury
			300	59,475	−16 injury
					Death of 60 per cent
7/24/52*	400	3350	250	79,300	−22 injury
11/ 1/52	400	3130	10	3400	0
			25	8500	5
			50	17,000	28

* In this experiment the dosage at λ3350 A was given before the dosage at λ2654 A. The injurious effect is none the less manifest.

due to the development of chromophoric material as a result of UV action. Such data are given in Table III.

A colpidium measures about 37 × 77 μ and has an area of about 1800 μ^2 as determined from a camera lucida drawing. It would take about 33,000 colpidia to fill the absorption cell used. Actually less than half this number was used in any case, as indicated in the table, therefore it is a reasonable, if approximate, assumption that the light did not pass through more than one layer of animals at any time. From the fraction of the cell area occupied by the colpidia it is possible to determine the amount of light transmitted by a single layer of colpidia over the entire cell surface (column 7, Table III). However, not all the light removed on passage through the colpidia is absorbed—a fraction is scattered. With the equipment available it was not possible to determine the scattering. However an approximation may be obtained by measuring the loss of visible light on passage through a suspension of colpidia. Since the latter are colorless in a concentrate as well as individually, it seems likely that visible light[1] is absorbed slightly if at all. Measurements of loss of light on passage through a suspension of colpidia are given in Table III for the mercury lines in the blue, yellow, and red. In all cases the loss of light is of the same order of magnitude—22 to 25 per cent. From the scatter at various visible wave lengths it should be possible to determine an empirical relationship between the wave length and degree of scattering. By extrapolation, the scatter in the UV might be obtained. However, it was not felt that the data were accurate enough for

TABLE III

Transmission of UV and Blue Light by Suspensions of Colpidia

Experiment	Light	State of colpidia	No. of colpidia	Fraction of cell occupied by colpidia	Transmission	Transmission by a layer of colpidia
1	UV	Fed	10,515	0.31	68.6	0.0
2	UV	Starved	14,547	0.43	56.4	0.0
3	Blue before UV	Fed	10,840	0.32	91.7	74.3
4	Blue before UV	Starved	12,674	0.37	94.4	85.1
5	Blue after UV*	Fed	10,515	0.31	93.0	77.6
6	Blue after UV‡	Starved	14,547	0.43	90.8	78.8
7	Yellow	Fed	11,168	0.34	92.0	76.8
8	Red	Fed	11,168	0.34	92.4	78.0

* Dosage, 1340 ergs/mm.^{2}, a dosage sufficient to just about prevent division of starved colpidia.

‡ Dosage, 3000 erg/mm.^{2}, enough to prevent division of well fed colpidia.

this purpose. While a precise determination of the difference in absorption between UV and blue is not possible, the data clearly indicate that colpidia absorb many times as much light in the UV as in the blue.

DISCUSSION

While the amount of ultraviolet light absorbed by a colpidium has been estimated, the fraction of the absorbed light which is effective in retarding division of the cell is not known. There is evidence that the division retardation produced by UV is not genic (Kimball and Gaither, 1951) and that it is probably a nuclear effect (Giese, 1939; Blum, Robinson and Loos, 1951). Of the light

[1] As evidence of some absorption in the blue end of the spectrum may be cited the destructive action of large dosages of blue light especially after starvation of the colpidia. On the basis of the Grotthus-Draper law action of light is possible only if absorption occurs. The small degree of photodesensitization (protection from UV by preillumination with blue) is similar evidence of absorption of blue light by colpidia.

absorbed by a colpidium, the fraction which is absorbed by the nucleus might be estimated from photographs taken with UV light (Swann and del Rosario, 1932). This was not done, because the premise that the nucleus is the only seat of action of the UV is not fully justified. Furthermore since quanta may be absorbed in the nucleus by materials other than those directly concerned with division retardation, we cannot even assume that all the light absorbed in the nucleus is effective in retarding division, and therefore such a calculation would have little meaning.

Absorption measurements have some use in determination of the approximate number of molecules in a cell which has been affected by the time division retardation is evident. If it is assumed, for purposes of argument, that a protozoan cell or a marine egg is composed entirely of protein molecules of the average molecular weight of albumin, the cell division is affected by UV when approximately 1 in 100 molecules has absorbed 1 quantum of UV (Giese, 1939). Therefore only a small fraction of the molecules will have been affected by UV light when cell division is retarded.

To be effective in photoreactivation not only must the blue light be absorbed but it must be absorbed either (1) in the proximity of or by the UV-altered molecules or (2) by widely scattered molecules which form a pool of diffusible materials effective in photoreactivation. The data on absorption of blue light by colpidia do not enable us to choose between the two possibilities.

For maximal reactivation it is necessary to expose fed colpidia to 100 quanta of incident blue light per quantum of incident UV. For maximal photoreactivation of starved animals, 800 quanta of blue are necessary per quantum of UV. This could be interpreted as a reduction by ⅛ of absorption of blue light by the starved animals following starvation. While the transmission data in Table III appear to be contrary to such an interpretation, the uncertainty of the amount of absorption *vs.* scattering makes a decision on this matter quite arbitrary. It is possible that the less favorable metabolic state of the starved animals is a factor in their lesser capacity for photoreactivation.

A study of the recovery of capacity for normal photoreactivation following addition of different types of nutrients to starved cells should prove of interest. Experiments were attempted with colpidia but 12 hours or more are required for appreciable changes in photoreaction ability after feeding. The study could be done more effectively with an organism like yeast which responds more quickly than colpidium to nutrients added to the medium. Such studies are in progress.

SUMMARY

1. The amount of visible or long ultraviolet light (UV) required to photoreactivate *Colpidium colpoda* injured with known dosages of short UV (2654 A) was determined.

2. The effect of the short UV was tested by the delay in division of exposed animals compared to controls. Photoreactivation was tested by the effect of postillumination on the delay of division of treated colpidia compared to controls.

3. Colpidia were used in two physiological states: well fed and starved in balanced medium for 48 hours. The latter are much more sensitive to short UV although less susceptible of photoreactivation.

4. Photoreactivation occurred over the entire span from 3350 A to 4350 A for the well fed colpidia, from 3130 A to 5490 (green) for starved colpidia.

5. The photoreactivating effect of a single quantum of blue (4350 A) or long UV (3660 A) delivered per quantum of 2654 A used to injure colpidia was too slight to be considered significant. The effect of 10 quanta was usually more pronounced, but only after 100 quanta had been delivered was the photoreactivation nearly maximal for well fed colpidia.

6. The quantum requirement for maximal photoreactivation of the starved animals was greater at all wave lengths tried: 3660, 4050, 4350, and 5460 A being of the order of 800 incident quanta per incident quantum of 2654 A.

7. The transmission of UV(2654 A), blue, yellow, and red light by a suspension of colpidia was determined.

8. Large dosages of blue, violet, or long UV were slightly injurious to starved colpidia. In a few cases large dosages of 3660 A killed starved colpidia, especially after a non-lethal dose of short UV(2654 A).

9. Photoreactivation seems to be a balance between the slight injurious effect produced by the visible light or UV of long wave lengths and the injury produced by short wave length UV.

10. Possible reasons for the large number of quanta of photoreactivating light required per quantum of short UV are discussed.

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