THE EFFECT OF NUTRITIONAL STATE ON PHOTOREVERSAL OF ULTRAVIOLET INJURIES IN DIDINIUM NASUTUM*

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Starvation increases the susceptibility of *Paramecium* and *Colpidium* to ultraviolet (UV) radiations (Giese and Reed, 1940; Giese *et al.*, 1954). Injury to starved colpidia is found to be less readily photoreversed than that to well fed animals, eight times as many quanta of blue light per quantum UV being required for maximal photoreversal (Giese *et al.*, 1953). Refeeding them with bacteria after UV but before blue light has little effect upon the degree of photoreversal (Giese *et al.*, 1954). Since it is difficult to tell how much food a bacterial feeder like *Colpidium* has ingested and how soon the food has been metabolized, the effect of feeding on UV injury and its photoreversal was studied with *Didinium nasutum*, a carnivorous ciliate. This species was chosen because its food intake can be easily observed under the microscope and regulated. It normally eats paramecia which can be grown under fairly controlled conditions. Therefore if the number of paramecia supplied to a didinium is controlled, the quantity and quality of food intake is known. The effect of the nutritional state upon susceptibility to UV and on photoreversal of UV injury by visible light is reported below.

Material and Methods

The didinia were irradiated with monochromatic short UV (2654 A) the intensity of which, measured by a thermopile calibrated against U. S. Bureau of Standards' standard lamps, varied from 8.67 to 4.64 ergs/sec./mm.^{2} during the course of the experiments. They were photoreversed with monochromatic blue light (4350 A), the intensity of which varied from 565 to 294 ergs/sec./mm.^{2} One fraction of a population of irradiated didinia was illuminated within a few minutes after UV treatment; another was kept as a dark control (UV). The methods have been described in some detail in previous work with *Colpidium* (Giese *et al.*, 1952). All observations were made in red light which does not photoreactivate.

Didinia were fed upon paramecia grown in test tubes on a single strain of bacteria in the manner previously described (Giese and Taylor, 1935). Freshly excysted didinia were much more constant in division rate and susceptibility to UV than

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cultures which had been kept in the laboratory for some time; therefore the following procedure was adopted to insure repeatable results. Each week cysts of *Didinium* were pipetted from old cultures, in which a small proportion of the didinia regularly encyst on the lettuce particles, into freshly bacterized 0.05 per cent lettuce and 0.00008 per cent yeast extract (Difco) medium containing a concentrated suspension of paramecia. Within 12 hours the excysted didinia were feeding on the paramecia. Selected individuals were pipetted into small test tubes (made from 5 mm. soft glass tubing) containing a suspension of paramecia concentrated by centrifugation and the cultures were kept at room temperature. Each day a single clone was selected and transferred to new medium. On the day preceding an experiment 16 didinia were pipetted from 1 tube into each of 16 small tubes containing concentrated suspensions of paramecia and the cultures were kept in the dark. On the following day 16 didinia were usually present in each of the 16 tubes and only such cultures were used in the experiments. A fresh clone was excysted weekly, except when the effect of aging on division rate and susceptibility to UV was studied.

If they were to be starved, the 16 didinia present in a small tube were pipetted into a Columbia watch glass containing fresh lettuce medium and kept at 26°C. without food for 4 hours. Longer starvation weakened the didinia to the extent that they were unable to cope successfully with paramecia, especially after irradiation. When unqualified or unfed or starved didinia are referred to, they were starved in the manner indicated above and this state is taken as the "standard state" for comparisons with other states.

By fed didinia is meant animals which have been permitted to engulf a single paramecium after being starved in the above manner. Feeding was observed through a dissecting microscope, and, as soon as one paramecium was attacked, the didinium and its prey were removed with a mouth pipette into a separate watch glass where engulfment was completed. Many such preparations could be obtained in a short time. Didinia could therefore be irradiated immediately after feeding or at any chosen interval of time thereafter.

Following treatment, a single didinium was added to each of 10 small test tubes containing concentrated suspensions of paramecia. The number of didinia in each tube was counted several times a day and the population was averaged and the number of divisions calculated for each count. Experiments were repeated, usually 3 to 10 times, depending on their repeatability. While controls show regular division, irradiated didinium like *Paramecium multimicronucleatum* (Giese and Reed, 1940), *Paramecium aurelia* (Giese and Reed, 1940; Kimball and Gaither, 1951), and *Tetrahymena geleii* (*pyriformis*) (Christensen, 1954), divides once or twice after exposure at about the same time as the controls, after which occurs a prolonged lag. At the end of the lag period, division is resumed. By the time the fourth division has occurred recovery is complete. Therefore the time to the fourth division was used as a measure of radiation injury, since after this time the rate of division is equal to that of the controls.

EXPERIMENTAL

1. Effects of Various Dosages of Ultraviolet Light on Didinium.—*Didinium* proved to be considerably more resistant to UV than *Colpidium*, since division in the former was not markedly delayed until a dosage of about 3000 ergs/mm.[2] had been given, whereas division was retarded by as little as 750 ergs/mm.[2] in the latter. The data for a series of experiments are given in Fig. 1 and Table I. Larger dosages, especially over 5000 ergs/mm.[2], injure didinia to the extent that they are unable to capture paramecia. They settle to the bottom of the culture dish and circle slowly. Only after a lapse of time do they attack paramecia. Part of the delay shown in the Fig. 1 may therefore be accounted for by failure to obtain food for several hours after irradiation.

TABLE I

*Effect of Feeding on Resistance to UV**

1	2	3	4	5	6	7
Dose UV	Time to 4th division control	Time to 4th division UV	Time to 4th division Fed‡ + UV	Delay to 4th division UV 3-2	Delay to 4th division Fed‡ + UV 4-2	Per cent increase resistance, = fed $\frac{5\text{-}6}{5} \times 100$
ergs/mm.[2]	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	
1500	16.7	26.9	27.2	10.2	10.5	−3.0
3000	19.0	42.3	32.2	23.3	13.2	43.3
	18.8	57.7	35.0	38.9	16.2	58.3
	18.1	45.0	34.6	26.9	16.5	38.6
4000	16.8	48.6	41.4	31.8	24.6	22.6
	16.9	72.2	40.8	55.3	23.9	56.8
	18.3	106.8	56.7	88.5	38.4	56.7
6000	16.7	173.3	147.2	156.6	130.5	16.6
	18.2	80.3	65.4	62.1	47.2	24.0

* Each experiment cited is the average of counts on progeny of 9 to 12 didinia each in a separate tube. In some cases double the number of experiments listed above were run but the data are omitted for lack of space.

‡ 15 minutes between feeding and UV.

Dosages larger than 6000 ergs/mm.[2] are likely to be lethal because the didinia are unable to obtain food and die of inanition and UV injury.

2. Effect of Nutritional State on UV Susceptibility of Didinium.—To test the effect of nutrition didinia were irradiated 15 minutes after each had engulfed a single paramecium. The delay to the fourth division of irradiated fed didinia is compared to that of unfed animals in Table I and Fig. 1. Feeding appears to increase the resistance of didinia to UV except at the smallest dosage from which recovery is rapid in either case.

The above experiment suggests that either the nutrient obtained from a paramecium is rapidly incorporated in the didinium changing its sensitivity to UV, or that protection occurs from physical screening by the bulk of material engulfed. To choose between the two possibilities the didinia were irradiated with a dose of 4000 ergs/mm.[2] immediately after engulfment of a

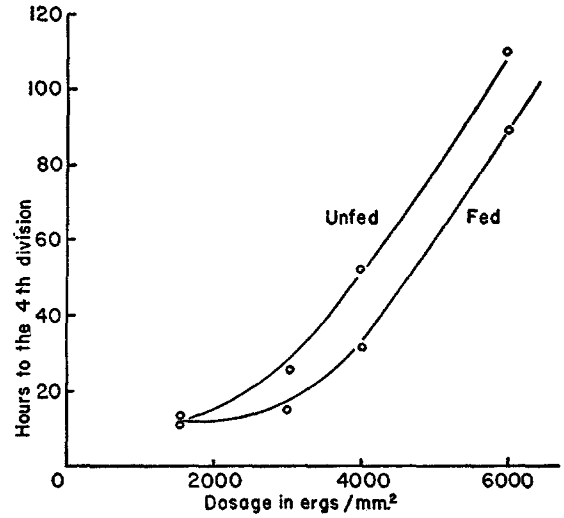

FIG. 1. Effect of feeding on ultraviolet injury to *Didinium*. Didinia each fed one paramecium 30 minutes before irradiation are compared to controls not fed. Note that feeding has most protective effect against larger dosages of UV. For details see text. Points are averages of three or more series of experiments.

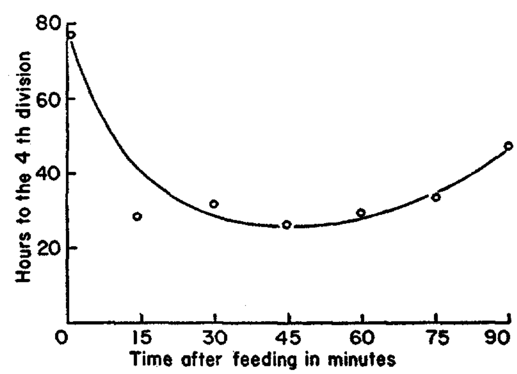

FIG. 2. Effect of time after feeding on sensitivity of *Didinium* to ultraviolet radiations (2654 A, 4000 ergs/mm.[2]). Each didinium fed one paramecium; data are averages from three or more series of experiments.

paramecium, at which time physical screening would be maximal. Didinia were also irradiated at various times after feeding and the data for the various experiments are added to Fig. 2 and Table II.

TABLE II

Effect of Time after Feeding on UV Resistance

1	2	3	4	5	6	7
Dose UV	Time to 4th division control	Time to 4th division UV	Time to 4th division fed + UV	Time between feeding + UV	Delay UV 3-2	Delay fed + UV 4-2
ergs/mm.²	*hrs.*	*hrs.*	*hrs.*	*min.*	*hrs.*	*hrs.*
3000	16.2	27.8	39.0	0	21.6	22.8
			27.0	10		10.8
			26.9	30		10.7
			27.4	45		11.2
			31.2	60		15.0
			26.8	75		10.6
			31.4	90		15.2
3000	15.4	35.0	29.8	0	19.6	14.4
			25.8	15		10.4
			26.4	30		11.0
			27.3	45		11.9
			25.3	60		9.9
			25.1	75		9.7
4000	16.8	48.6	63.3	0	31.8	46.5
			41.4	15		24.6
			34.2	30		17.4
			41.2	45		24.4
			42.7	60		25.9
			66.5	75		49.7
4000	16.9	72.2	77.7	0	55.3	60.8
			40.8	15		23.9
			46.0	30		29.1
			44.3	45		27.4
			44.3	60		27.5
			41.3	75		24.4
4000	18.3	106.8	133.2	0	88.5	114.9
			56.7	15		38.4
			68.2	30		49.9
			47.3	45		29.0
			53.6	60		35.3
			46.2	75		27.9
			66.8	90		48.5

The experiment demonstrates that even when the protoplasm of the paramecium is present inside a didinium at the time it is irradiated, division is delayed more than in the unfed animal. On the other hand, with the lapse of only 15 to 30 minutes after engulfment, the didinia became much more re-

TABLE III

Effect of Feeding on Photoreversal (PR)

1	2	3	4	5	6	7	8	9	10
Quanta blue/quantum UV	Time to 4th division control	Time to 4th division UV	Delay UV (3-2)	Time to 4th division UV + blue unfed	Time to 4th division UV + blue fed	Delay UV + blue unfed (5-2)	Delay UV + blue fed (6-2)	Per cent PR unfed $\frac{4-7}{4} \times 100$	Per cent PR fed $\frac{4-8}{4} \times 100$
UV dose, 3000 ergs/mm.²									
	hrs.	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*		
10	19.5	34.5	15.0	29.8	30.3	10.3	10.8	31.3	28.0
12.5	15.2	49.2	34.0	25.7	26.3	10.5	11.1	69.2	67.4
25	15.2	49.2	34.0	22.8	21.6	7.6	6.4	77.7	81.2
	15.9	47.2	31.3	24.5	21.5	8.6	5.6	72.5	82.1
	15.9	47.2	31.3	23.5	21.6	7.6	5.7	75.7	81.7
50	15.2	49.2	34.0	19.6	18.6	4.4	3.4	87.1	90.0
	15.9	47.2	31.3	23.4	22.0	7.5	6.1	76.1	80.5
	15.9	47.2	31.3	22.2	23.1	6.3	7.2	79.8	77.0
100	15.2	49.2	34.0	20.8	18.6	5.6	3.4	83.5	90.0
	20.0	39.7	19.7	23.0	21.5	3.0	1.5	84.8	92.4
	18.6	33.4	14.8	22.0	19.6	3.4	1.0	77.0	93.2
200	18.9	39.6	20.7	19.7	19.5	0.8	0.6	96.1	97.1
400	20.0	39.7	19.7	22.8	20.8	2.8	0.8	85.8	96.0
UV dose, 4000 ergs/mm.²									
25	17.5	45.5	28.0	29.2	29.7	11.7	12.2	58.2	56.4
	18.8	36.8	18.0	25.9	26.5	7.1	7.7	60.6	57.2
50	18.8	35.9	17.1	22.3	23.1	3.5	4.3	79.5	74.9
	17.5	45.5	28.0	23.9	23.6	6.4	6.1	77.2	78.2
	18.8	36.8	18.0	22.6	22.7	3.8	3.9	78.9	78.4
100	18.8	35.9	17.1	20.7	20.5	1.9	1.7	88.9	89.9
	17.5	45.5	28.0	23.2	20.0	5.7	2.5	79.7	91.2
	18.8	36.8	18.0	22.1	20.8	3.3	2.0	81.7	88.9

sistant to UV. During this brief time the protoplasm of paramecium must have been digested and incorporated into the protoplasm of the didinium. After this time the resistance of the didinium remains constant for about an hour, then falls as the animal begins to starve. The experiments indicate that not mere physical screening but a chemical change after feeding increases the resistance of *Didinium* to UV.

3. Photoreversal of UV Injury to Didinium with Blue Light.—UV injury to *Didinium*, as measured by the increased time to the fourth division, is reversed by blue light to a considerable extent, in some cases a reversal of 86 to 96 per cent being observed. For these studies didinia were irradiated with UV, one-half were each fed a single paramecium just before, and a half just after photoreversal with blue light. The data for both sets of experiments are given in Table III. For both fed and unfed animals the degree

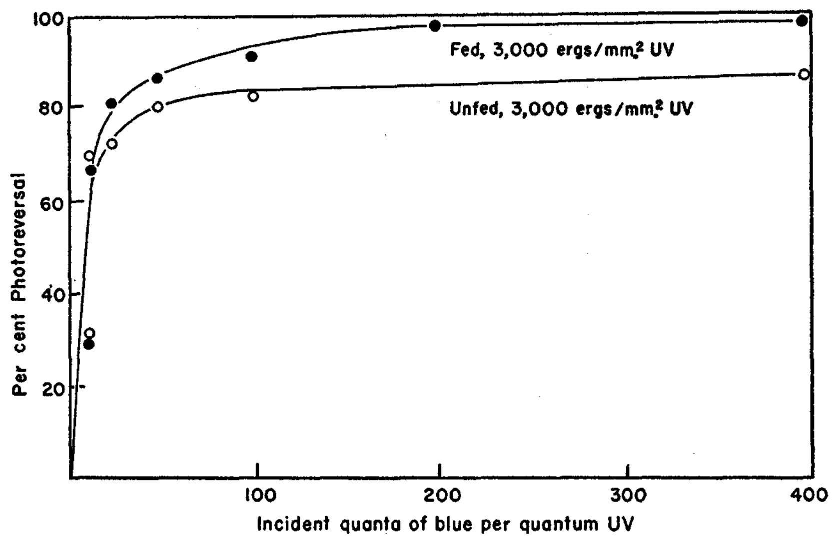

FIG. 3. Photoreversal of ultraviolet injury to *Didinium*. Average photoreversal determined as indicated in Table III, columns 9 and 10, plotted against the number of quanta of blue per quantum UV.

of photoreversal depends on the number of quanta of blue light delivered per quantum of UV as seen in Table III. Saturation occurs in both cases when approximately 100 to 200 quanta of blue have been delivered per quantum of UV (Fig. 3). The difference in photoreversal between fed and unfed animals is probably not significant, although the advantage always lies with the fed animals except when the dosage of photoreversing light is low.

4. Effect of Irradiated Food on Division of Didinium.—Didinia were found to feed upon irradiated paramecia provided the paramecia were not cytolyzing. This presented an opportunity to determine whether UV produced in the paramecia a diffusible photoproduct toxic to *Didinium* in the same way as UV. Paramecia were accordingly irradiated with UV until seriously injured and fed to unirradiated unfed didinia. They were attacked and eaten in much the same way as unirradiated paramecia. The fourth division of didinia fed irradiated paramecia occurred on the average after a lapse of 19.2 hours and after 19.6 hours in fed unirradiated controls. When the number of paramecia provided in experimental and control is rather sparse, the didinia thrive on irradiated paramecia since they are more readily captured. The general conclusion may be drawn that irradiated paramecia contain no photoproduct injurious to didinia.

5. Resistance of Didinium to UV with Lapse of Time after Excystment.—Following early indications of variability of a *Didinium* stock with lapse of time after excystment, periodic tests were made for division of controls and UV-treated animals of a single clone of *Didinium* kept for a period of several

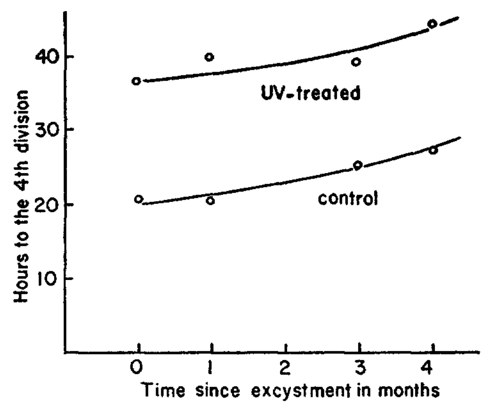

FIG. 4. Ultraviolet resistance of *Didinium* after lapse of time since excystment. Note that division rate declines but that didinia are similarly affected by exposure to ultraviolet radiations (3000 ergs/mm.^{2} at 2654 A). Points are averages of three or more series of experiments.

months after excystment. The data are summarized in Fig. 4. A decline in vigor of the stock after several months is evidenced by the increasing time lapse between divisions. On the other hand, the sensitivity of didinia to UV did not change significantly since the curves for division of the UV-treated animals and controls are parallel. The delay in division due to UV injury is just added to the time for the fourth division of the control in each case. Therefore it is apparent that the decrease in vigor of the stock is not accompanied by a corresponding increased sensitivity to UV, at least for the period of time tested.

DISCUSSION

All cells manifest a capacity for recovery from environmental injuries such as exposure to UV. A didinium recovers from UV injury more readily if it ingests food before exposure to UV provided time is allowed for digestion and incorporation of the food into the protoplasm. The present experiments indicate that this period is quite short. As soon as 15 minutes after ingestion, resistance is increased. In as short a time lapse as 90 minutes, the resistance decreases again. Presumably some valuable nutrients which render didinia more resistant to UV have been used up.

Incorporation and use of food in *Didinium* is known to be very rapid. A large vacuole containing an ingested paramecium is formed in about 1 minute or less and many small vacuoles, in which disintegration of the structures of the prey is apparent, are formed in 20 minutes. It is believed that the dipeptidases of the paramecia are used to digest their own bodies (Doyle and Patterson, 1942). *Didinium* divides more than six times daily at 26°C. provided adequate food is present. Starvation of more than 4 hours results in such weakness that a didinium may fail to engulf a vigorous paramecium.

Active metabolism stimulated by intake of food has been seen to favor resistance to UV in other species as well, *e.g. Paramecium* (Giese and Reed, 1940) and *Colpidium* (Giese *et al.*, 1953, 1954). In all three protozoans experiments indicate that physical screening cannot account for the increased resistance of fed animals to UV, since ingestion is not immediately followed by increased resistance to UV, but only after a lapse of time during which, presumably, the nutrient is incorporated into the protoplasm.

The reason for the decrease in resistance of *Didinium* to UV immediately after ingestion of *Paramecium* is not clear, but the experiments are consistent and numerous enough to indicate clearly that the phenomenon is real (Fig 2). Whatever is liberated during digestion which benefits *Didinium* and increases its resistance to UV is obscure.

Surprisingly enough photoreversal of UV injuries to *Didinium* by blue light is not markedly influenced by the variation in nutritional state tested. For example, injury to didinia fed just after exposure to UV but before illumination with blue light, is photoreversed only slightly more (Table III) than in those fed only after exposure to blue, in each case a half-hour being allowed for incorporation of the nutrients into the protoplasm. Colpidia fed after UV but before photoreversal were also little affected. However, for photoreversal of UV injuries to starved colpidia eight times as many quanta of blue/quantum UV are required than for fed animals (Giese *et al.*, 1953, 1954). For both fed and unfed *Didinium* the same number of quanta are needed to achieve maximal photoreversal (Table III).

The differences in the latter results between colpidia and didinia are perhaps resolvable in terms of their feeding habits and nutritional states. *Colpidium*, a bacterial feeder, takes up bacteria even after a prolonged period of starvation. *Didinium*, on the other hand, being a selective carnivore, can be starved for no more than 4 hours. If starved for a longer time, didinia are unable to cope with vigorous paramecia. Consequently photoreversal of UV injury on thoroughly starved didinia cannot be tested. The experiments with colpidia probably show better the effects of extreme inanition on photoreversal.

The data on photoreversal in *Didinium* are interesting, none the less, because they show that inanition affects resistance to UV much more than the capacity for photoreversal of the UV injury by visible light. The UV effect and its photoreversal are separable to this extent at least. It would appear that some nutrient essential to UV resistance is more rapidly exhausted than the nutrient essential to photoreversal.

The change in vigor of didinia with time lapse after excystment (Fig. 3) as indicated by slower division is interesting in view of the fact that Beers (1928) saw no nuclear reorganization in his strain of *Didinium* and multiplication occurred at a constant rate for prolonged periods of time. However *Didinium* has a ravenous appetite, eating 8 to 10 paramecia per day, the number eaten depending upon the size and condition of the paramecia and upon the temperature. Possibly growth of paramecia on a single strain of bacteria, as in the present study, fails to supply some essential nutrient to *Didinium* owing to its lack in the paramecia grown in this manner. Study of the literature on *Didinium* has not singled out the reason for the difference (Mast, 1917; Mast and Ibara, 1923; Beers, 1937, 1945, 1947). However the constancy of UV action on the declining didinia indicates that this change in state neither predisposes nor protects didinia from UV.

While UV has been considered by some to be injurious because of the production of a toxic diffusible photoproduct, possibly a peroxide (Stone *et al.* 1947; Novick and Szilard, 1949), didinia fed upon UV-treated paramecia grow at the same rate as controls, no delay being observed at the fourth division after such feeding, in fact in some experiments the didinia divide faster than controls. Yet didinia fed upon paramecia treated with hydrogen peroxide and washed divide more slowly than controls or they refuse to eat the paramecia at all. The data gathered here, therefore, cast doubt upon interpretation of UV action by toxic diffusible photoproducts.

SUMMARY

1. The effect of the nutritional state of *Didinium nasutum* on its resistance to short ultraviolet (UV) radiation (2654 A) and its recovery from the injury following illumination with visible light (4350 A, blue) was studied.

2. The resistance of a didinium to UV is considerably increased by feeding it a paramecium 15 to 60 minutes before exposure to UV. If fed just before exposure to UV, the resistance is less than that of an unfed control.

3. Photoreversal is only slightly greater in didinia fed after irradiation with UV but before exposure to visible light as compared to those fed after exposure to visible light.

4. Irradiated paramecia are eaten by didinia, provided they have not

started to cytolyze. Didinia fed on irradiated paramecia divide at about the same rate as controls or slightly faster.

5. The available stock of *Didinium* declines in vigor with lapse of time after excystment, as measured by the time required for division. The sensitivity of *Didinium* to UV did not change essentially during the 5 month period over which tests were made.

6. The theoretical implications of the results are considered.

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