PHOTOREVERSAL OF NUCLEAR AND CYTOPLASMIC EFFECTS OF SHORT ULTRAVIOLET RADIATION ON PARAMECIUM CAUDATUM*

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Since Kelner's (1949 a) suggestion of the general nature of photoreversal by visible light of injurious effects of ultraviolet radiation (UV), a variety of effects have been shown to be photoreversible, such as: inactivation of bacteria (Kelner, 1949 b); inactivation of fungi (Kelner, 1949 a; Norman, 1951); inactivation of bacteriophage (Dulbecco, 1950); mutation in bacteria (Novick and Szilard, 1949); cleavage delay in sea urchin eggs (Blum *et al.*, 1950; Wells and Giese, 1950); division delay in ciliate protozoans (Kimball and Gaither, 1951; Giese *et al.*, 1952); the inhibition of adaptive enzyme formation in yeast (Swenson and Giese, 1950); and the spheration of the nucleoli of the grasshopper neuroblast (Carlson and McMaster, 1951). It has been suggested that since so many different effects are photoreversible some primary action of UV which induces widespread effects on the cell is subject to photoreversal (Kimball and Gaither, 1951; Dulbecco, see Hollaender, 1955, chapter 12). Nucleoprotein, which is present in the cell in fair amount, has been suggested as a probable primary chromophore for absorption of UV leading to photoreversible effects (Giese *et al.*, 1952; Kelner, 1953; Dulbecco, see Hollaender, 1955, chapter 12). Indeed, action spectra resembling absorption by nucleoprotein or nucleic acid are found for many UV-induced effects which are photoreversible: the bactericidal effect (Gates, 1929 a, 1929 b, 1930; Ehrismann and Noethling, 1932; Wyckoff, 1932; Hollaender and Claus, 1936); the fungicidal effect (Oster, 1934; Hollaender *et al.*, 1945); the killing of virus (Hollaender and Duggar, 1936; Hollaender and Oliphant, 1944; Zelle and Hollaender, 1954); mutagenic effects in microorganisms (Stadler and Uber, 1942; Hollaender *et al.*, 1945; Hollaender and Emmons, 1946; Kaplan, 1952); cleavage delay in sea urchin eggs (Giese, 1946, 1947); division delay in ciliate protozoans (Giese, 1945 b); the inhibition of adaptive enzyme formation (Swenson, 1950); and spheration of the nucleoli of the grasshopper neuroblast (Carlson and McMaster, 1951). Blum *et al.* (1951), Norman (1951, 1954), Kelner (1953), and Saracheck (1954) have presented evidence that the pri-

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mary locus of photoreversible UV-induced effects is the cell nucleus. It seems likely, therefore, that photoreversible UV-induced effects probably result from absorption of the ultraviolet by nuclear nucleoprotein (or nucleic acid). It should follow, also, that UV-induced effects showing action spectra similar to absorption by nucleoprotein will probably be photoreversible; conversely, UV-induced effects showing action spectra different from nucleoprotein absorption will probably be non-photoreversible.

A means of testing the foregoing idea was suggested by the observation (Kimball *et al.*, 1952; Hirshfield and Giese, 1953) that doses of ultraviolet wave lengths below 248 mμ, comparable to those which produce division delay in these protozoans at longer wave lengths, produce a striking immobilization of some ciliate protozoans, and that animals immobilized with wave length 238 mμ either died within 24 hours or recovered with little division delay. Giese (1945 b) has shown that the action spectrum for 50 per cent immobilization of *Paramecium caudatum* at wave lengths longer than 248 mμ resembles the absorption spectrum of albumin-like protein, and that the action spectrum for division delay in starved paramecia resembles the absorption spectrum of nucleoprotein. Since division delay in some ciliate protozoans is photoreversible (Kimball and Gaither, 1951; Giese *et al.*, 1952; Christensen, 1953), a comparison of the degree of photoreversal of division delay with that of immobilization might provide the desired test, the former probably being localized in the nucleus, the latter in the cytoplasm. Photoreversal of immobilization implies the reversal or inhibition by visible light of the process (or processes) *leading to* immobilization of the animal and not an effect on the animal's *recovery from* immobilization. The present paper reports the results of such a comparison in *Paramecium caudatum* of immobilization and division delay induced by wave lengths 226, 233, 239, 248, and 267 mμ in addition to a description of visible changes pertinent to an analysis of the locus of action of the radiations.

Methods and Materials

Clones of *Paramecium caudatum* were grown at 24°C. in 20 × 150 mm. soft-glass culture tubes containing 20 ml. of culture medium. The culture medium consisted of autoclaved 0.05 per cent lettuce infusion set at pH 7 with Sorensen buffer and inoculated 24 hours previous to use with *Pseudomonas ovalis* (Giese, 1945 *a*). Subcultures of the clones were made every 48 hours. Single organisms were manipulated with mouth pipettes. Prior to an experiment, four sterile Syracuse dishes contained in deep Petri dishes were half-filled with a 48 hour culture of paramecia, an equal volume of fresh bacterized medium was added, and the culture was incubated for 12 hours at 24°C. Then twelve animals in the "dumbbell" stage of division were picked from each of the four dishes during a 15 minute period, put into a common isolation tube of bacterized medium, and allowed to complete division and feed for 6 hours before being used in an experiment. Isolation tubes were made from 4 mm. soft-glass tubing (Giese, 1945 *a*). Subsequent to picking dividing paramecia, they were handled and observed in a room lighted only by ruby dark room bulbs; the

100 watt microscope lamp was filtered through 4 cm. of 0.05 per cent $CuSO_4$ and a Corning red filter number 2418 transmitting only light of wave lengths longer than 610 mμ.

After the paramecia had finished dividing, each isolation tube contained approximately ninety-six animals which were considered to be within 30 minutes of the same interdivisional age. For large numbers of paramecia within 60 minutes of the same interdivisional age individuals from three isolation tubes were pooled at the time of washing. After the 6 hour incubation period they were transferred with pipettes through three washes of sterile balanced salt solution (Taylor and Strickland, 1935) and pipetted into the quartz irradiation cell. The latter has an optically polished well 1 mm. deep and 5 mm. in diameter in the center of a small quartz plate 2 mm. thick.

A large natural quartz prism monochromator (lenses and prisms 8.5 cm. in diameter) using an atmospheric pressure mercury arc operating at 2.2 amps. and 220 v. D. C. served as the source of the short UV wave lengths: 239, 233, 226 mμ (for details see Brandt, 1955). The UV light passed from the monochromator into an irradiation chamber where it was focussed with a curved first-surface aluminized mirror onto the bottom of the quartz cell. The intensity of the light passing through the reaction cell was measured by a thermopile calibrated against a standard lamp. Visible light for photoreversal studies was obtained from either of two sources. For routine work the 435 mμ mercury line from a super-high pressure capillary mercury arc (Ames type) resolved by a monochromator was focussed on the cell and its intensity was measured by a calibrated thermopile. In the immobilization experiments visible light from a G. E. CH_4-SP mercury spot lamp, filtered through a 4 cm. layer of 0.05 per cent $CuSO_4$ and two Corning filters, No. 3850 (ST) and No. 5113 (1/2ST), to remove the infrared and to isolate a narrow band of visible between 360 mμ and 500 mμ with a maximum at 410 mμ was applied concurrently with the UV by focussing it onto the top of the reaction cell. The intensity of the light was measured prior to use by a photometer (photovolt No. 200m) previously calibrated against the known intensity of the 435 mμ mercury line from the monochromator (for details see Brandt, 1955).

The paramecia were observed for immobilization during irradiation under magnification (× 40) through a dissecting microscope focussed on the image of the reaction cell reflected by a mirror. The paramecia were observed in the slight amount of visible light which passes the monochromator (less than 6 per cent), and by their fluorescence. After irradiation, individual organisms were further observed with the higher magnification of a Reichert phase contrast microscope, light from the 100 watt microscope lamp being filtered as indicated previously when exposure to visible light had to be avoided.

Subsequent to irradiation, the paramecia were left in the reaction cells in individual damp chambers or were transferred singly to isolation tubes of medium and incubated in the dark at 24°C. Twenty paramecia receiving the same treatment were placed singly in isolation tubes for division determinations. The number of paramecia present in each isolation tube was determined three times daily under a dissecting microscope (× 6.6) and the division rate computed. Effects of different doses of UV were compared on the basis of division delay which is the difference between the average time necessary for all the clones in a given experimental group

and those in the control group to reach the fourth division. This end-point was chosen because preliminary experiments on division rate indicated that paramecia treated with sublethal doses of 267 mμ recovered their normal division rate by the time they had completed the fourth postirradiation division.

EXPERIMENTAL

1. Comparison of Visible Effects of Wave Lengths 226 mμ and 267 mμ on Paramecium.—Since the visible effects of UV wave lengths below 248 mμ on organisms have not been described previously in detail, some observations comparing the action of UV of 226 mμ and 267 mμ are recorded, especially those which are pertinent to the main objective of this study.

Only the side of a paramecium directly exposed to 226 mμ fluoresces blue-green; whereas the entire paramecium exposed to 267 mμ fluoresces. This is visual evidence of major difference in mode of action of these two wave lengths upon larger cells such as paramecium. Unilateral fluorescence in 226 mμ indicates only superficial penetration of the radiation, whereas fluorescence of the entire animal in 267 mμ demonstrates penetration of the radiation throughout the protoplasm.

According to reports in the literature ciliates recoil when entering the part of a cell illuminated with UV and accumulate on the dark side (see Giese, 1953, for references). To test this reaction at wave length 226 mμ, two-thirds of the bottom of the reaction cell exposed to UV was covered with black paper but the paramecia accumulated on neither the dark nor the light side. The experiment was repeated with visible light striking from the top to improve visibility, but the paramecia seemed to remain equally distributed on either side of the cell. The observed difference in phototaxic action between longer UV such as 254 or 267 mμ and 226 mμ may perhaps rest on the unilateral action of the short as against more general action of the somewhat longer wave lengths of UV.

Immobilization and killing of paramecia by short UV (226 mμ) are described in the next few paragraphs. Whereas unirradiated paramecia placed in balanced salt solution in the quartz reaction cell characteristically swim around the circumference of the circular well, after a few minutes of irradiation they become randomly distributed. Upon continued exposure their movements become noticeably slower, until the circumferential swimming once again is resumed, but at a slower rate. The rate of movement continues to decrease and the paramecia become randomly distributed again. From time to time they temporarily cease forward movement and rotate, the anterior end describing a large circle about the posterior end as a pivot. The number of individuals rotating becomes larger as irradiation proceeds, and some settle to the bottom of the well where they eventually become immotile. Immobilized paramecia are sometimes observed to jerk suddenly as if they had been struck. It is

possible that this movement is recoil from a unilateral discharge of trichocysts in the unilaterally exposed immobilized individual. Such unilateral discharge was observed under higher magnification.

Paramecia appear normal when first immobilized by exposure to 226 mμ, and if the irradiation is discontinued most of the organisms recover complete motility after several hours. Higher magnification of these immobilized animals reveals the body cilia to be completely inactive; the cilia in the oral groove often continue to beat, although too feebly to move the animals. Continued observation of such immobilized paramecia reveals that a large proportion of the immobilized cilia subsequently disappear; whether they are resorbed or shed is difficult to determine with certainty.

In paramecia immobilized with a minimal dose of 226 mμ, cyclosis and contractile vacuole function are slower than in controls. Trichocysts appeared to remain intact for the most part, although unilateral loss of them is occasionally observed under higher magnification (× 400). If paramecia are irradiated further after immobilization by 226 mμ, irreversible changes ultimately lead to their death. They gradually shorten and assume the shape of an egg with a protuberance at the anterior end. Two types of degenerative changes are observed preceding death. The most common is the accumulation of fluid in the contractile vacuoles which eventually swell to fill almost the entire cell. The vacuolar membrane then disrupts internally, and the entire distended animal takes on a uniform appearance as if the cytoplasm had been homogenized. At this point cytoplasmic cyclosis ceases and no sign of life remains. Animals dying in this manner often remain intact for more than 12 hours, appearing "fixed" as if by cytological fixatives except that no internal structure is discernible. The surface of such paramecia is hardened since a cutting needle slides off instead of indenting and cutting it into two parts, as occurs in non-irradiated controls. Death may also occur by vesiculation, vesicles forming on the surface of the paramecia simultaneously with enlargement of the vacuoles. The protoplasm is then forced into one of these vesicles, followed by rupture of the surface and extrusion of the contents of the cell into the surrounding medium. Such cytolysis leaves behind a "shell" which remains intact for many hours afterward. Often extrusion of the internal contents in this manner releases vacuoles which remain intact in the balanced salt solution. Such vacuoles appear to be contractile vacuoles; on disruption of a vacuole with a glass needle a liquid of different refractive index than the surrounding fluid is released.

Immobilization and killing of paramecia by UV of 267 mμ, in general similar to that described by Giese and Leighton (1935), are briefly described for comparison with the effects of 226 mμ. Like the shorter radiations, UV of 267 mμ induces random swimming of the paramecia instead of circumferential. Like the shorter wave length, UV of 267 mμ induces a gradual loss of motility. On continued exposure to 267 mμ as with 226 mμ, the contractile vacuoles cease

to function and the animals become shorter and broader. The vacuoles increase in size until they fill almost the entire animal. About this time some of the paramecia are becoming immotile and vesicles appear on their surface. Cytolysis after exposure to 267 mμ usually occurs by rupture of the protoplasm into the blisters and thence into the surrounding medium, followed by complete disruption of the paramecia rather than in the two ways observed following treatment with 226 mμ.

Immobilization of paramecia by exposure to UV of 226 mμ requires a dose of 2.0×10^{14} quanta/mm.^{2} (12 minutes to administer), while at 267 mμ even a dose of 30.0×10^{14} quanta/mm.^{2} (2 hours and 30 minutes to administer at an intensity comparable to that of the 226 mμ used above) immobilizes only about 75 per cent of the animals, the remaining 25 per cent of the animals still moving about slowly or cytolyzing on continued irradiation without being immobilized. Furthermore, whereas most of the animals immobilized by a dose of 226 mμ later recover motility, those immobilized by a dose of 267 mμ never recover motility and within 1½ hours they all cytolyze.

All the lines of evidence described above indicate a marked visible difference in action of UV of 226 mμ as compared to that of 267 mμ. These data suggest that these two wave lengths act in different ways, the first superficially and perhaps primarily on the cytoplasm, the second passing through the paramecia and affecting the nucleus as well. The relatively greater effects of equivalent doses of 267 mμ than of 226 mμ upon division delay, described in subsequent sections, strengthen the likelihood of a more potent deeper (nuclear) action of the longer than of the shorter wave length and of a more superficial action of the latter.

2. Tests for Photoreversal of Immobilization and Division Delay Produced by Short UV in Paramecium.—85 per cent of a sample of paramecia immobilized with a dose of 226 mμ recover motility if allowed to remain in balanced salt solution, and they subsequently divide when placed in bacterized nutrient medium. It is therefore possible to measure the division rate of animals which have recovered from such an immobilizing dose of UV, and to compare photoreversal of immobilization with photoreversal of division delay in the same sample of animals.

In making this comparison, onset of immobilization was determined visually during irradiation through a dissecting microscope, the end-point being defined as the complete loss of motility of all the animals (at least one hundred) in the sample. The reaction cell was then removed from the irradiation chamber and placed in a damp chamber to allow the organisms to regain motility. Experience showed that after 5 hours approximately 85 per cent of the sample would recover; the others cytolyze. Therefore, individuals were transferred singly 5 hours after immobilization to isolation tubes of bacterized medium to determine the division delay produced by the immobilization dose.

To compare photoreversal of immobilization with photoreversal of division delay, visible light was applied concurrently with the UV since light applied after the immobilization could not be expected to have any effect on the process leading to immobilization. If immobilization is photoreversible by visible light, then a larger dose of UV should be necessary to immobilize the animals when they are concurrently illuminated than when they are treated with UV alone. Illumination concurrent with irradiation was shown by Whitaker (1942), and

TABLE I

Photoreversal of Ultraviolet (226 mμ)-Induced Immobilization and Division Delay in Paramecium caudatum by Concurrent Visible Light

Experiment	Intensity of UV	No. of paramecia immobilized		Immobilization dose in (Q/mm.^{2})$\times 10^{14}$		Difference*	Division delay to the fourth division		Photoreversal* of division delay
		UV	UV + visible	UV	UV + visible		UV	UV + visible	
	ergs/mm.^{2}/sec.					*per cent*	*hrs.*	*hrs.*	*per cent*
A	7.03	160	—	1.70	—	—	5.0	—	—
	7.03	—	158	—	1.59	−6.5	—	5.0	0
B	4.62	110	—	1.95	—	—	—‡	—‡	—
	4.60	—	102	—	1.90	−2.6	—	—	—
C	2.66	122	—	2.15	—	—	4.0	—	—
	2.63	—	101	—	1.89	−12.0	—	5.3	−33
D	6.34	192	—	1.87	—	—	14.8	—	—
	6.27	—	212	—	1.89	+1.0	—	4.8	+73
E	2.49	115	—	2.26	—	—	13.3	—	—
	2.46	—	110	—	1.78	−21.0	—	7.0	+54

* A plus sign indicates a difference in the direction of photoreversal, a negative sign the opposite.

‡ Division delay not measured in this experiment.

recently reported by Kelner (1953), to be effective in reducing the UV injury; in fact, Whitaker's results with *Fucus* eggs indicate that visible light concurrently applied is *more* effective than visible light applied after UV treatment. A similar conclusion was drawn from preliminary experiments on division delay in paramecia done here, using 267 mμ for irradiation, the CH_4 spot lamp for photoreversal concurrently with UV, and 435 mμ for photoreversal immediately after UV.

To determine whether immobilization by 226 mμ is photoreversible the washed paramecia were divided into two groups, one of which was observed while being irradiated to immobilization under UV alone, and the other to the

same end-point with visible light applied concurrently with the UV. They were then allowed to recover for 5 hours in balanced salt solution and subsequently transferred to isolation tubes for division rate determinations.

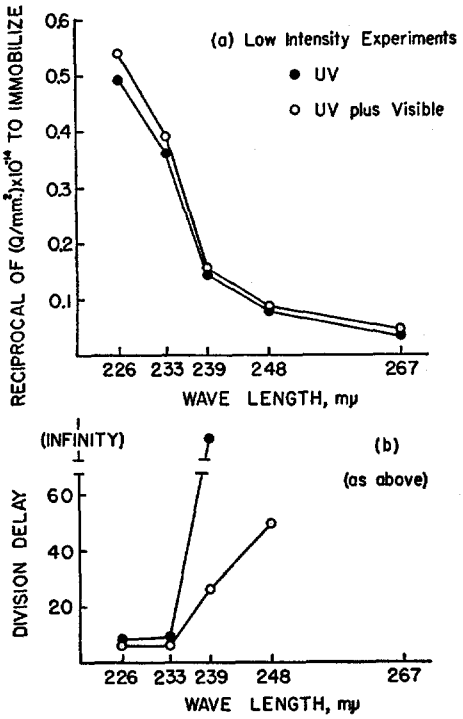

FIG. 1 *a*. Action spectrum for immobilization of *Paramecium caudatum* determined with UV alone and UV plus visible light applied concurrently. All UV wave lengths were adjusted by non-selective filters to an intensity of approximately (3×10^{11} quanta)/mm.²/sec. Ordinate is the reciprocal of the UV dose in (quanta/mm.²) $\times 10^{-14}$ necessary to immobilize. Each point for wave lengths below 248 mμ represents the average of at least three determinations, and for wave lengths 248 mμ and 267 mμ the average of at least two determinations.

FIG. 1 *b*. Hours' delay to the fourth division of the same paramecia whose immobilization doses are given in graph Fig. 1 *a*.

Paramecia treated in the same way except for irradiation or illumination served as controls. Table I presents the data from five such experiments. The data indicate no photoreversal of immobilization. Division delay produced by immobilizing doses of 226 mμ was slight, in two cases being within the range of variability of controls. Where a greater degree of division delay was observed, as in the last two experiments listed in Table I, photoreversal is clearly apparent, the average of these two experiments being 63 per cent.

3. Photoreversal of Immobilization and Division Delay Produced by Other Short Wave Length UV.—The effects of wave lengths 226, 233, 239, 248, and 267 mμ on immobilization and division of paramecia were next studied to determine

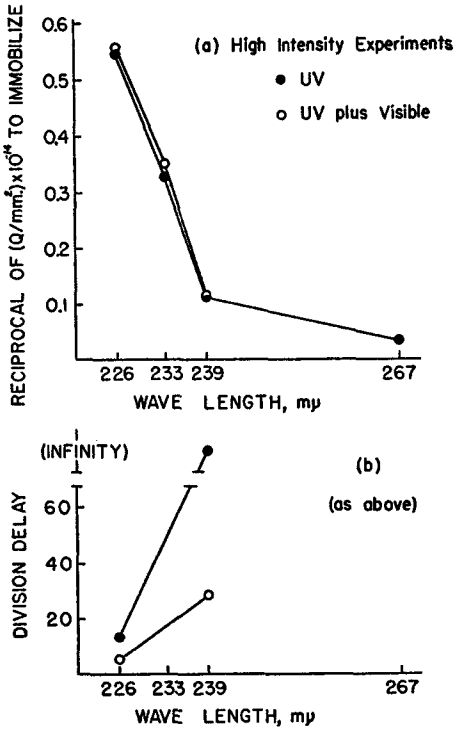

FIG. 2 *a*. Action spectrum for immobilization of *Paramecium caudatum* determined with UV alone and UV plus visible light applied concurrently. All UV wave lengths were adjusted to an intensity of approximately (8×10^{11} quanta)/mm.²/sec. Ordinate as in Fig. 1 *a*. Each point for wave lengths below 267 mμ represents the average of at least two determinations, and for wave length 267 mμ one determination.

FIG. 2 *b*. Hours' delay to the fourth division of the same paramecia whose immobilizing doses are given in Fig. 2 *a*.

wave length dependence of the effects. Loofbourow (1948) discusses the assumptions involved in measuring the wave length dependence (action spectrum) of a biological effect, and points out that the reciprocity law must hold for the intensities employed. Since the intensities used in this work with short UV are low initially, the validity of the reciprocity law could not be tested over a wide intensity range and only two intensities were tested for each wave length. The intensity (quanta/mm.²/sec.) of the shortest wave length, 226 mμ, was used as

a standard; the intensities of the other wave lengths were adjusted by the use of discs of black paper with holes of different diameter in front of the collimating lens or by neutral filters made of Nos. 80 and 120 bronze pump strainer cloth to give approximately the same rate of delivery of quanta as this wave length.

The effectiveness of the short UV wave lengths in producing immobilization and division delay in paramecium is shown in Fig. 1 for low intensity and in Fig. 2 for the maximal intensity available from a new mercury arc. Immobilization at wave lengths 248 mμ and 267 mμ is difficult to compare accurately to that at shorter wave lengths because some of the animals cytolyze during irradiation even before becoming immobile. The UV doses necessary to im-

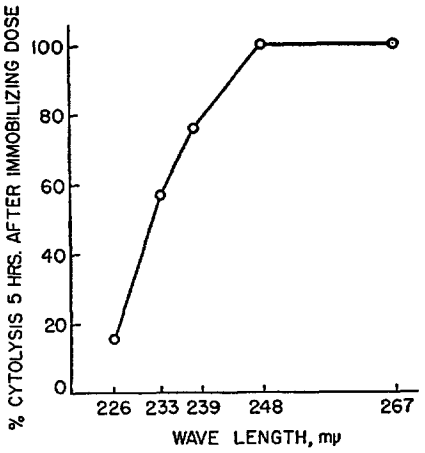

FIG. 3. Per cent cytolysis of *Paramecium caudatum* at 5 hours after an immobilizing dose of UV.

mobilize while under concurrent illumination with visible light are included in Figs. 1 *a* and 2 *a*. No evidence for photoreversal of immobilization is observed after dosage at any of the various wave lengths used whether low intensity (Fig. 1 *a*) or high intensity (Fig. 2 *a*) of UV is used. Since the data for high and low intensity of UV are essentially similar they were pooled and are plotted in Fig. 4 as the relative efficiency of the wave length in producing immobilization, setting as an efficiency of 100 the quantum dose necessary to immobilize at 226 mμ.

Data on the effectiveness of each of these wave lengths in producing division delay in the same animals after recovery of motility are given in Figs. 1 *b* and 2 *b*. These data indicate that as wave length increases in the series tested, the division delay becomes greater and photoreversal more detectable. For instance, whereas paramecia treated with UV (239 mμ) alone, divide several times, but

ultimately die, those in a sample subsequently treated with visible light recover and reach the fourth division with an average delay of less than 40 hours.

Determination of the division delay at the longer wave lengths, 248 and 267 mμ, produced by an immobilization dose was complicated by the rapid death of the animals subsequent to such a dosage of irradiation. The data on the amount of cytolysis occurring by the end of the 5 hour recovery period subsequent to an immobilizing dose are plotted against the wave length in Fig. 3 and indicate that cytolysis increases with wave length. At wave lengths 248 and 267 mμ, following an immobilizing dose of UV, cytolysis was complete within 1½ hours and division rate measurements were impossible. Only about 15 per cent of the animals immobilized with 248 mμ under concurrent photoreversing light recovered sufficiently to allow measurement of the division rate (Fig. 1 *b*). At wave length 267 mμ even illumination with visible light concurrently with UV irradiation did not prevent cytolysis of the immobilized animals.

DISCUSSION

Data presented in this paper support the thesis that nuclear injuries produced by UV radiation are subject to photoreversal but that cytoplasmic injuries are not. The first statement is amply demonstrated by the data already in the literature and documented in the opening paragraphs of this paper. It is therefore only the second part of this thesis that requires amplification and discussion.

The nucleus is presumably not required for ciliary movement of paramecium since fragments of paramecia without a nucleus move about. Motility of paramecium therefore depends upon the cilia and the cytoplasm in which they are anchored. Immobilization could therefore result from injury to the cilia or to the underlying cytoplasm. Paramecia immobilized by exposure to UV recover motility a few hours after irradiation is stopped; therefore, they cannot be considered moribund. Failure to raise the dosage of UV required for immobilization, when paramecia are illuminated simultaneously with UV irradiation, seems to constitute a valid argument against photoreversal of the injury to cilia or underlying cytoplasm.

The nature of the chromophore which absorbs the radiation responsible for immobilization is suggested by the composite action spectrum for the effect, which approximates the absorption spectrum of albumin (Fig. 4). In the short end of the spectrum lipides absorb and here the action spectrum does not discriminate between lipides and proteins.

That we are dealing with wave lengths which can cause photoreversible injury is demonstrated clearly by photoreversal of the division delay induced by short UV in paramecia simultaneously with immobilization. Photoreversible and non-photoreversible effects are induced in the same specimens at the same time and by the same wave lengths.

It is true that short UV has a relatively slight effect on division rate, yet is most effective in immobilizing paramecia. This selective action is probably resolvable on the basis of relative transmissibility of short as compared to somewhat longer UV. Bovie (1918) has calculated that 10 μ of protoplasm will

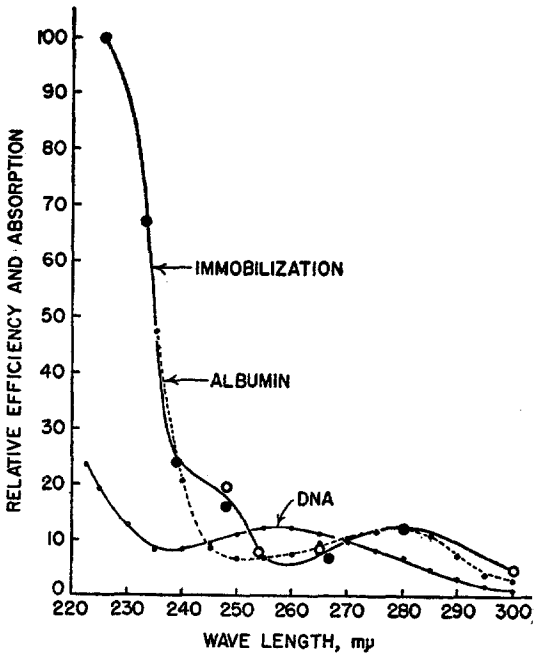

FIG. 4. Composite action spectrum for immobilization of *Paramecium caudatum* (large solid circles, this paper; large open circles, Giese, 1945 *b*) compared with the absorption of 0.1 per cent serum albumin (Giese, 1950) and 0.0035 per cent thymus nucleic acid (Kunitz, 1950). Ordinate for action spectrum is 1/immobilizing dose in quanta/mm.^{2} and for the absorption spectra is 1/optical density; units are arbitrarily chosen to make the relative efficiency of wave length 226 mμ equal to 100 and the absorption maximum of serum albumin at 280 mμ and of thymus nucleic acid at 260 mμ both equal to the immobilizing efficiency of wave length 280 mμ.

completely stop all radiation of wave length 214 mμ incident upon the cell. At 226 mμ greater penetration is to be expected than at 214 mμ, but still much of the radiation is absorbed superficially as indicated by the unilateral fluorescence of paramecia exposed to it. The powerful action of UV on the cilia may result from such superficial absorption. The slight effect of short UV upon division rate may result from the fact that little radiation actually reaches the nucleus. Nucleic acids absorb strongly in the short end of the UV spectrum, therefore such radiation as reaches the nucleus may be absorbed, producing the

photochemical damage resulting in division delay. Effects of very short UV of 160 mμ on ciliate protozoans have been described by Bovie (1915), and Hughes and Bovie (1918). Superficial action of such short UV is even more striking than that described here, as might be expected by extrapolation. At longer wave lengths such as 267 mμ penetration is more complete than at 226 mμ, as indicated by fluorescence of the entire paramecium. Concomitantly, injury to the nucleus indicated by division delay is profound because the radiation reaches it. At the same time immobilization is less marked and the cell may become moribund even before being completely immobilized, because of general injury to all its parts. The importance of penetration to the degree of injurious action of UV is suggested by the much greater susceptibility of starved paramecia, colpidia, and didinia to UV (Giese and Reed, 1940; Giese *et al.*, 1954; Brandt *et al.*, 1955).

Other cytoplasmic effects have recently been grouped in the "non-photoreversible" category, namely activation (artificial parthenogenesis), cytolysis, fixation, and jelly removal in *Arbacia* (Blum *et al.*, 1954). Permeability effects on erythrocytes (Green, unpublished) also fall in this category. It is probable that still other effects localized in cytoplasmic structures, such as ciliary reversal and sensitization to heat (Giese, 1945 *b*), will also prove non-photoreversible although adequate tests have not yet been made.

Why cytoplasmic effects should not be subject to photoreversal is an open question. The presence of chromophores is presumably required for photoreversal to absorb the visible light energy which in some way makes possible repair of the UV lesion, perhaps by destroying UV-generated cell "poisons." Such chromophores may be lacking in the superficial cytoplasm, or the poisons produced by UV in this part of the cell may be of a different nature. The cytoplasmic responses involve immediate reaction whereas the nuclear changes are slow in appearing. The time relations may underlie the difference in nuclear and cytoplasmic response to visible light following UV injury. Until more specific information is available, further speculation seems fruitless. However, if it becomes fully established that only UV injuries to the nucleus (nuclear nucleoprotein) are subject to photoreversal, ultraviolet radiation may be used as a tool for investigating nucleocytoplasmic function, since the maximum degree of photoreversal obtainable for a UV-induced effect will indicate the degree to which nuclear nucleoprotein absorption is responsible.

SUMMARY

1. Irradiation with three short ultraviolet (UV) wave lengths, 226, 233, and 239 mμ rapidly immobilizes *Paramecium caudatum*, the dosage required being smaller the shorter the wave length. 85 per cent of paramecia immobilized with wave length 226 mμ recover completely. Recovery from immobilizing doses is less the longer the wave length.

2. Irradiation continued after immobilization kills the paramecia in a manner which is markedly different for very short (226, 233, and 239 mμ) and longer (267 mμ) wave lengths.

3. An action spectrum for immobilization in *P. caudatum* was determined for the wave lengths 226, 233, 239, 248, and 267 mμ, and found to resemble the absorption of protein and lipide in the wave length region below 248 mμ. Addition of these data to those of Giese (1945 *b*) gives an action spectrum resembling the absorption by albumin-like protein.

4. Division of *P. caudatum* is delayed by doses of wave lengths 226, 233, and 239 mμ which cause immobilization, the longest wave length being most effective.

5. Immobilization at any of the wave lengths tested (226, 233, 239, 248, 267 mμ) is not photoreversible when UV-treated paramecia are concurrently illuminated.

6. Division delay resulting from immobilizing doses of 226, 233, and 239 mμ is photoreversible by exposure to visible light concurrently with the UV.

7. Division delay induced by exposure to wave length 267 mμ is reduced by exposure to visible light applied concurrently with UV or immediately afterwards.

8. The data suggest that the shortest UV wave length tested (226 mμ) affects the cytoplasm selectively, because it is absorbed superficially as indicated by unilateral fluorescence in UV. Consequently it immobilizes paramecia rapidly but has little effect on the division rate because little radiation reaches the nucleus.

9. The data support the view that nuclear effects of UV are readily photoreversed but cytoplasmic effects are not.

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