Retardation of Division of Three Ciliates by Intermittent and Continuous Ultraviolet Radiations at Different Temperatures

A. C. GIESE, B. McCAW, and R. CORNELL
From the Department of Biological Sciences, Stanford University, Stanford

ABSTRACT The same dosage of ultraviolet (UV) radiation retards division of several protozoans more effectively when the light is intermittent than when it is continuous, and especially at temperatures of 25–35°C. At lower temperatures the difference between the effects of intermittent and continuous radiations is less marked. Somewhat similar results were obtained with the ciliates Paramecium caudatum, Blepharisma japonicum, and Colpidium colpoda, the disparity between intermittent and continuous light decreasing in the order given. The data are taken to indicate that thermochemical dark reactions succeed the absorption of UV radiations by the cells. In Blepharisma, besides initial delay in division, the cells stop dividing after one or two divisions, a “stasis” ensuing. Stasis is marked when the cells are irradiated at higher temperatures but is slight when they are irradiated at low temperatures, as if the temperature-sensitive reaction involved stasis (in all cases cultures are grown at 25°C). The data are related to findings in the literature.

INTRODUCTION
In a previous report it was shown that a given dosage of intermittent ultraviolet (UV) radiation delays division of the ciliate Didinium nasutum more than continuous UV radiation, especially when the cells are irradiated at higher than at lower temperatures. Intermittent UV radiation is presumably more effective than continuous because there is time for completion of the thermochemical (dark) reactions during the dark period following a flash of light and higher temperatures accelerate these dark reactions (Giese et al., 1956). However, in a study of mutation induction in the fungus Ophiostoma multianulatum intermittent UV radiation was no more effective than continuous radiation of the same dosage, even at higher temperatures (Zetterberg and Giese, 1962). To determine whether perhaps Didinium represents an isolated instance, the experiments on the comparative effects of the same dosages of continuous and intermittent UV radiation at low and high tem-
peratures (within the viability limits of cells) were extended to the ciliates *Colpidium colpoda, Blepharisma japonicum,* and *Paramecium caudatum.* Positive findings are reported below.

**MATERIALS AND METHODS**

Since the experiments have been done over a period of almost 6 years the methods varied. The data presented here, however, were gathered chiefly in the last 2 years and the methods described below apply only to these.

The ciliates were all grown under rather similar conditions on 0.05 per cent lettuce infusion buffered at pH 7.0 with 0.005 M phosphate buffer, and the infusion was inoculated with *Pseudomonas ovalis* 12 to 24 hours before adding the protozoans (see Giese and Taylor, 1935 for details). *Paramecium* and *Colpidium* were isolated from local pools. *Blepharisma japonicum* (Suzuki, 1954, Bhandary, 1962) race Rao A (obtained from India) rather than the local race previously used in our studies was used because of its greater size and vigor. Usually cultures were ready 2 to 3 days after inoculation, at which time numerous vigorous individuals fairly similar in interdivisional phase were available. Usually at several week intervals, fresh isolations were made and the ones dividing in small isolation tubes (Giese, 1945 a) at a maximal rate were chosen as stock for the next series of mass cultures. In this way some degree of synchrony in division was attained. However, for experiments it is necessary to use interdivisional cells—those dividing or recently divided, and to avoid large ones getting close to division. The test of the method is indicated by the usual synchrony of the divisions of the controls.

The cells used were sometimes irradiated in lettuce infusion which absorbs about 20 per cent of the incident radiation in the short UV range, or more commonly, washed beforehand and exposed in buffered, balanced salt solution which absorbs very little. When irradiated through lettuce infusion, a correction for the incident dosage had to be made if the intensity of incident light was measured through distilled water or salt solution; if the integrating dosimeter is used, no correction is necessary since this records total dose through whatever solution is used, including the organisms (see Giese and Lusignan, 1961).

The source of radiations was either monochromatic UV radiation of wavelength 2537A (or 2654A) obtained from a quartz mercury arc and a monochromator of natural quartz, or a sterilamp (about 85 per cent 2537A) filtered only through a thickness of several centimeters of water held in a quartz cell (after preliminary tests with a solution of acetic acid to absorb ozone-forming short UV radiations disclosed no difference between the two; see Cook, 1956). The intensity was measured either with a thermopile (calibrated against United States Bureau of Standards standard lamps) or a Westinghouse integrating UV meter (containing a tantalum photocell) calibrated against the thermopile at 2537A. In most experiments the intensity was set at 25 ergs/mm² sec., adjusting the distance from the arc to the cells being irradiated. Flashing was obtained by rotating a black disc, usually with 90 degrees cut out of the 360, and the motor was run at 640 RPM after preliminary trials showed other discs and speeds less effective (see Giese et al., 1956). The intensity of the UV radiations was
kept as close as possible to 25 ergs/mm² sec. even when intermittent UV radiations were used; the dosage in all cases was monitored by a Westinghouse integrating UV meter. The motor speed was measured by a strobotac and adjusted by rheostat to stay at this rate. It was found that even though a voltage regulator was used the motor tended to speed up and slow down. Observation with the strobotac and manual regulation by a rheostat were therefore necessary at times, but the speed variation was never more than a few per cent.

Thermal control was attained by running water from a thermostated water bath around the exposure cell for from half an hour to an hour before starting the experiment. The temperature of the water just around the cell was measured with a thermometer. The temperature in the cell may vary as much as 1° from the cited temperature (higher at low temperatures, lower at high), but since conditions were the same time after time (the work being done in a constant temperature room), it is likely that the variation was in the same direction each time. In any case, the relative effects are still meaningful when a comparison is made among temperatures as different as 10, 25, 30, and 35°C.

Once irradiated, the cells were pipetted into small isolation tubes in the manner described for *Paramecium* (Giese, 1945 a), placed at 25°C, and examined several times daily for divisions. When growth was much retarded by the radiations it was necessary to transfer the cells to new culture medium every few days to avoid possible deleterious influence upon division of aging bacteria and altered medium.

In all cases the specimens were handled before and after irradiation in a constant temperature room (25°C) illuminated with yellow (insect-repellent) lights. The yellow portion of the spectrum is ineffective in producing photoreactivation in protozoans (Giese *et al.*, 1952, 1953).

It has been shown that starvation increases the sensitivity of *Paramecium* to UV radiations (Giese and Reed, 1940) and the same has been found for *Blepharisma* (Hirshfield and Giese, 1953) and for *Colpidium* (Giese *et al.*, 1953), and corroborated in the present study. However, it is important to call attention to the changes in sensitivity of cells to radiations related to their nutritional condition as a possible explanation for the observed variability in radiation sensitivity of samples of cells from day to day, although there is no proof that this is the cause. Other factors beyond our control may also play a part in the daily variations in radiation sensitivity of cells. Experiments are, therefore, performed in such a way that all factors, the effects of which are to be compared, are studied on the same day on the same population of cells in the same physiological condition. A higher dosage is always more effective than a lower dosage for a given sample of cells, but the absolute delay in division induced by any given dosage of UV is not the same day after day.

No essential difference was observed for the same dosage of monochromatic radiation delivered from the monochromator at 2537Å and from the sterilamp. Since the sterilamp proved much more adaptable and manageable than the monochromator and could be easily moved from room to room, it was used for most of the experiments reported. It also has the advantage that the intensity can be varied at will by changing the distance between the sterilamp and the exposure cell containing the cells to be irradiated.
EXPERIMENTAL RESULTS

1. Colpidium colpoda

Data on the effects of various dosages of UV radiations upon Colpidium have already been reported (Giese et al., 1952, 1953). The original data plotted in previous papers indicate that control and experimental data are generally reproducible. In a few series of the experiments, for unknown reasons, a long lag preceded divisions in both controls and experimental series; these data are not averaged for comparison of the action of continuous and intermittent radiations.

Data for nineteen series of experiments comparing the effects of different dosages of continuous and intermittent UV radiations upon the third division after irradiation are plotted in Fig. 1. The data for the first and second divisions after irradiation are omitted because the results are similar for all three divisions, but the effect is most clearly developed after the third division.
It is evident that no clear difference exists between continuous and intermittent radiations at the lowest dosage, the effect of which is slight as seen by comparison with division time of controls, there being an overlapping of points. While at higher dosages the sets of points are spread over a section of the ordinate, the clump of points for continuous radiations is distinct from that for intermittent radiations with only one exception (after 3000 ergs/mm²), and for any one run (as indicated by characteristic symbols) the separation is always good.

Since the effects of intermittent and continuous radiations are about as distinct after 3000 ergs/mm² as after larger dosages—and more convenient for determinations—comparison of the relation of temperature to the effectiveness of a 3000 ergs/mm² continuous and intermittent dose of UV radiation upon division of *Colpidium* was next tested several times. The experimental data shown in Fig. 2 indicate more marked radiation action during irradiation when the cells are kept at higher than at lower temperatures. After irradiation...
tion in all cases the cells are incubated at 25°C. The increase in effectiveness of UV radiations with increase in temperature is, however, about equal for continuous and intermittent radiation, contrasting with the situation previously found for *Didinium* (Giese et al., 1956) where only the intermittent light showed a marked temperature effect. The temperature coefficients for retardation of division by radiation are given in Table I.

<table>
<thead>
<tr>
<th>Species</th>
<th>Interval</th>
<th>Continuous UV</th>
<th>Intermittent UV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First Second Third</td>
<td>First Second Third</td>
</tr>
<tr>
<td>Colpidium</td>
<td>15-25</td>
<td>1.19 1.11 1.2</td>
<td>1.2 1.1 1.3</td>
</tr>
<tr>
<td>colpoda</td>
<td>25-33</td>
<td>1.67 1.98 1.77</td>
<td>1.71 1.71</td>
</tr>
<tr>
<td>Blepharisma</td>
<td>10-25</td>
<td>1.08 1.11 1.26</td>
<td>1.20</td>
</tr>
<tr>
<td>japonicum</td>
<td>25-35</td>
<td>1.64 1.50 1.48</td>
<td>1.46</td>
</tr>
<tr>
<td>Paramecium</td>
<td>13-25</td>
<td>1.25 1.31 2.63</td>
<td>2.32</td>
</tr>
<tr>
<td>caudatum</td>
<td>25-33</td>
<td>2.12 2.44 2.97</td>
<td>2.32</td>
</tr>
</tbody>
</table>

* Exposure to the temperature indicated was only for the duration of irradiation. Before and afterwards cultures were maintained at 25°C.
† Impossible to calculate since values for the third division are not available for the high temperatures. If infinity is taken as the value (no division) the $Q_{10}$ calculated would be very high.
§ The values are about 1.00.

2. *Blepharisma japonicum*

Twenty-eight series of experiments were performed with intermittent UV radiation using this species. As a set of illustrative experiments or original data have already been published (Giese and Lusignan, 1961), only average points are given in Fig. 3 for the effects of continuous and intermittent UV radiations (each 4000 ergs/mm², intensity 25 ergs/mm²/sec.) upon division of *Blepharisma* irradiated at various temperatures spanning the range 10 to 35°C, and incubated thereafter at 25°C.

It is evident from Fig. 3 that intermittent radiation produces greater division delay at higher temperatures than at lower during irradiation (the cells being incubated in culture medium at 25°C immediately thereafter). The effect of a given dosage of continuous radiation is also increased considerably with rise in temperature over the range 25 to 35°C, although increasing the temperature from 10 to 25°C has little effect. The temperature coefficients for the radiation effects over the intervals studied and for the various postirradiation divisions are given in Table I.
3. *Paramecium caudatum*

Data for the effects of various dosages of continuous and intermittent UV radiations upon division of *Paramecium* are given in Fig. 4. While these data compare the action of radiation upon only the second subsequent division, the difference between the effects of continuous and intermittent radiations upon the first and third divisions after irradiation was found to be as distinct as that upon the second division (e.g., Fig. 5). Intermittent radiation seems much more effective than continuous radiation at all dosages studied, the difference being only slightly less at lower dosages. Such data suggest that a thermal effect follows the absorption of UV radiations. The effect of temperature changes upon the action of UV radiations is, therefore, of interest.

The increased effectiveness of UV radiation of 3000 ergs/mm² (at an intensity of 25 ergs/mm²/sec.) with rise of temperature during irradiation (but incubation thereafter at 25°C) is evident in the curves of Fig. 5. At 13°C both intermittent and continuous radiations have about the same division-retarding effect, producing a relatively slight delay in the first and second division times. However, when temperature is raised to 35°C, the division retardation due to intermittent radiation is much greater than that due to continuous radiation. The increased effectiveness of intermittent radiation at 35°C as compared with 13°C is also evident in the curves of Fig. 5.

**Figure 3.** A. Average data for experiments (from twenty-eight series) comparing the effect of exposure to a 4000 ergs/mm² dosage of continuous and intermittent UV radiations at 10, 25, and 35°C (and subsequent culture at 25°C) upon the division of *Blepharisma japonicum* (sterilamp radiations, intensity 25 ergs/mm² sec.). The temperature coefficients for the increase in the effect are given in Table I. Average division times for the second and third division of controls at 25°C are indicated by arrows below the curves. B. Effect of a 4000 ergs/mm² dose of UV upon regeneration of *Blepharisma*. Irradiation at 10, 25, and 35°C; subsequent culture at 25°C.
divisions after irradiation. By the time of the third division after irradiation the greater effectiveness of intermittent radiation appears to have become evident even at 13°C. The intermittent radiations are much more effective than the continuous at 25°C and become strikingly more effective at 37°C.

The $Q_{10}$ values for the intervals 13 to 25°C and 25 to 37°C, given in Table I for Paramecium, are greater than those for Colpidium and Blepharisma, as well as those for Didinium previously reported (Giese et al., 1956). This indicates a marked thermal component in the UV radiation effect upon the division of Paramecium.

![Graph showing effects of various dosages of continuous and intermittent UV radiations.](image)

**Figure 4.** Effects of various dosages of continuous and intermittent UV radiations at 25°C upon the second postirradiation division of Paramecium caudatum (sterilamp radiations, intensity 25 ergs/mm² sec.). The same wide separation was obtained for effects of continuous and intermittent radiations upon the first and third postirradiation divisions, but the data are omitted for lack of space. The time for the second division of the control at 25°C is indicated by the arrow.

4. **Effects of Intermittent Light upon Regeneration of Blepharisma**

Several series of experiments comparing the effects of intermittent and continuous UV radiations (4000 ergs/mm²) upon the rate of regeneration of the mouth parts removed from a Blepharisma are summarized in Fig. 3B. Here the difference between intermittent and continuous light is less marked than is the effect on cell division. Since the time required for regeneration in the control at 25°C is only 5 hours, it is possible that there is insufficient time for expression of the effects. The special problems of regeneration following UV irradiation are considered elsewhere (Giese and Lusignan, 1961).
5. Effects of Intensity Variation

If intermittent radiation is more effective than continuous radiation because it allows for completion of thermal reactions in the dark intervals between light flashes, one might expect that a low intensity of UV radiations would be more effective than the same dose delivered at a high intensity, because in this case also, more time would be allowed for completion of dark reactions between successive absorption of radiations by given cell components. Without documenting the results in detail, it was found in all cases that a given dosage of radiation applied at a lower intensity was more effective than the same dosage at a higher intensity, at least when the difference in the two intensities

![Graph](image_url)
was large. Marked effects were found, especially with *Paramecium* and *Colpidium*. The problem is complex and has already been considered in the case of *Didinium* for which considerable data are available (see Giese et al., 1956).

Still remaining to be studied in detail, however, is the relative effectiveness of extremely low intensities of UV radiations. A given dose at extremely low intensity (*i.e.*, fractions of an erg/mm²/sec.) appears to be less effective in retarding division of *Blepharisma* than radiation delivered at a somewhat higher intensity (5 to 25 ergs/mm²/sec.). Lack of confidence in the accurate measurement of such low intensity radiations with the equipment available makes these conclusions tentative.

**DISCUSSION**

Division delay is caused by a wide variety of agents, both physical and chemical, and it is quite probable that many different reactions in the cell are affected—sometimes localized in the nucleus—sometimes in the cytoplasm (Mazia, 1961). Radiations appear to have their most potent action on the nucleus (Giese, 1947). However, UV radiations have differential effects upon cytoplasm and nucleus, and division delay shows an action spectrum comparable to absorption by proteins in some cells and comparable to absorption by nucleic acid or nucleoproteins in small cells such as sperm (Giese, 1938),

![Figure 6](image_url)
indicating that the action of the radiations is multiple and not upon a single process in the cell. Short UV radiations preferentially affecting the cytoplasm have little effect upon division and the effects of UV radiations upon nucleic acid systems in the cell (possibly in the nucleus only) appear to be subject to photoreactivation while the effects upon protein systems do not (Brandt and Giese, 1956). However, a component of injury to nucleic acid substances is always evident, at least during recovery reactions, even when the action spectrum for the immediate effect suggests an effect upon proteins (Giese, 1945 b). Evidence in the literature therefore points to retardation of division of a cell by radiation action upon many sites in an animal cell.

Although mutation rate is accelerated by UV radiations and mutation almost certainly involves alteration of DNA (free or combined, Kornberg, 1960), the effect of a given UV dose of continuous and intermittent radiations at various temperatures on the mutation rate of the fungus, Ophiostoma multiannulatum is comparable in all cases, regardless of the manner of application of radiations and the temperature at the time of irradiation. Zetterberg and Giese, 1962). Somewhat similar results with a different manner of fractionation of the light are also reported for mutations resulting from UV irradiation of the polar cap cells in Drosophila (Altenberg and Browning, 1962). Presumably, then, one can exclude DNA changes in the cells as the locus of the thermosensitive reactions.

Kimball et al. (1952) have been able to separate two qualitatively different effects of radiations upon division of Paramecium aurelia, one a genic effect resulting in non-viability and the other a delay in division which manifests itself primarily as a lag before the first postirradiation division, followed by a stasis or cessation of division after one or two postirradiation divisions. The lag preceding the first postirradiation division is considered to be the result of an effect on the mitotic apparatus, while stasis is considered to be the result of blockage of synthases required for growth. Since growth processes of cells appear to be governed by RNA (Brachet, 1957), it has been postulated that stasis in division of irradiated cells might be the result of injury incurred by the synthetic machinery residing in the ribosomes of the cells (Giese and Lusignan, 1960).

The lag in division after radiation injury is general but a stasis period is marked in P. multimicronucleatum, P. aurelia, and Blepharisma japonicum, but not in P. caudatum (Giese and Reed, 1940) or in Colpidium colpoda. Perhaps recovery from ribosomal injury in the latter species is more gradual than in the former, complete recovery being indicated by a return to a normal rate of division. When cells recover from stasis they resume division at a normal rate.

It is interesting in this regard that when Blepharisma is irradiated at low temperatures the subsequent stasis period of division delay is either not noticeable or is very slight (Fig. 6), whereas at higher temperatures the
stasis period is increased. These experiments suggest that temperature perhaps selectively affects the reactions which cause division stasis. The secondary thermal reactions following absorption of radiation by cells may then be those which control stasis; these, as pointed out above, may be localized in the ribosomes.

Cutting Blepharisma after irradiation induces a quicker recovery of the normal division rate and a reduction of the stasis period. Cutting, which is known to induce macronuclear reorganization accompanied by reconstitution of structures missing in the cut cell, could result in quicker liberation of new ribosomes which bring about synthesis of materials essential for cell division (Giese and Lusignan, 1960).

Somewhat puzzling are data indicating equal action of the same dosages of continuous and intermittent UV radiations upon the rate of cleavage of eggs of the purple sea urchin, Strongylocentrotus purpuratus, and of the pink sea urchin, Allocentrotus fragilis (Giese, unpublished data). Cook and Rieck (1962) have also reported negative results with eggs of the sand dollar. However, such cells are presumably loaded with stores of DNA and other constituents needed for development of the egg into an embryo, cleavage serving to partition the chromosomes and the rapidly formed cytoplasm into new blastomeres. Synthesis from simpler precursors is probably minimal during cleavage, as witness the speed of cleavages (about an hour; see Giese, 1938) of marine egg cells—even at the low temperatures of sea water (e.g., 13°C) as compared to the much slower division (about 24 hours) of mammalian tissue cells at body temperature which have to synthesize their DNA, RNA, proteins, and other needs from simpler precursors (see Puck, 1957). If radiation-induced delay in cleavage of marine egg cells is localized in the components of the division processes rather than in the major synthetic reactions, the radiation effect might well be independent of thermal reactions.

Preliminary experiments indicate that intermittent UV radiation is more effective than continuous radiation of the same dosage upon yeast division, as measured by colony formation and this is also true for the formation of buds (R. Cornell, unpublished data). More extensive studies on action of intermittent radiations upon cells other than protozoans are desirable.

Why intermittent UV light is not more effective in producing mutations than continuous UV light when all genes are thought to express themselves by way of DNA-produced RNA messenger molecules directing synthesis of proteins (Zamecnik, 1960) is disturbing. However, in experiments reported only the rate of mutation production was tested for, not the rate of mutation expression (Zetterberg and Giese, 1962). The rate of mutation production presumably depends upon the rate of DNA alteration, which in turn depends upon the rate of absorption of UV radiations, not upon the temperature at the time of application and absorption of the radiations. It would be interesting
to study in the above manner, effects of delayed mutational change which are thought to involve chemical reactions (e.g., in Doudney and Haas, 1960).

We are indebted to Professor L. E. Moses of the Applied Mathematics and Statistics Department for discussion of some aspects of the work. Preliminary experiments on some of the problems reported were performed by Mrs. Molly Lusignan, Dr. Donald Prolo, and Mr. James Stanley. The work was supported in part by United States Public Health Grant No. C-3461.

Received for publication, November 29, 1962.

BIBLIOGRAPHY


DOUDNY, C. O., and HAAS, F. L., 1960, Some biochemical aspects of the postirradiation modification of UV-induced mutation frequency in bacteria, Genetics, 45, 1481.


GIESE, A. C., IVZERSON, R. M., SHEPARD, C., JACOBSON, C., and BRANDT, C. L., 1953, Quantum relations in photoreactivation of Colpidium, J. Gen. Physiol., 37, 249.


