# Effects of Ultra-violet Light on the Survival and Nuclear Division of a Dinoflagellate

By

**J. D. Dodge**

Department of Botany, Birkbeck College, London, W. C. 1

With 11 Figures

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## Introduction

This paper records one aspect of a detailed investigation of the nuclear structure and division in some flagellated members of the Dinophyceae (= division Pyrrophyta). It has been shown (Dodge 1963) that these organisms possess an unusual nucleus with the chromosomes condensed throughout the mitotic cycle. The mitosis appears to take place without the aid of normal spindle and centromeres. When a Dinoflagellate was treated with x-rays (Dodge and Godward 1963), as might be expected it reacted in a unique manner. The chromosomes were readily fragmented but the pieces were still able to segregate to the daughter nuclei. The size of the chromosome fragments was found to be roughly proportional to dose. As regards survival these organisms appear to be more resistant to x-rays than most higher organisms but by no means as resistant as most other micro-algae (Godward 1962).

In the present study, *Prorocentrum micans* Ehrenberg was irradiated with ultra-violet light (U. V.). Although a number of other algae have been treated with U. V. this is the first time its effects upon a member of the Dinophyceae have been investigated.

## Material and Methods

Clonal cultures of *Prorocentrum micans* Ehrenberg (strains 7 A and 7 E) were grown in supplemented sea water under artificial illumination by the methods already described (Dodge 1963). The cultures originated from the Plymouth laboratory of the Marine Biological Association. Freshly innoculated cultures in the logarithmic phase of growth were used for the experiments.

Two ultra violet sources of the low pressure mercury type have been used. In each case the material was placed centrally beneath the tube and 20 cms. away from it.

Source A. Vitreosil T/M 5/369 giving approximately 760,000 microwatts/sq.cm. at 20 cms.

Source B. Hanovia Model 11 giving approximately 70,600 microwatts/sq.cm at 20 cms. Both lamps produced over 99% of their radiation at the 2537 Ångstrom wavelength.

For irradiation 5 mls. aliquots of cell suspension in the normal sea water medium were placed in sterile 9 cm. petri dishes (without cover) under the source. As the cells are motile no shaking was given. After the required exposure the material was transferred by means of a sterile pipette to a conical flask containing about 40 mls of fresh culture medium. For cytological examination 5 mls. samples were removed at intervals of 2–3 days, fixed, stained and examined by the standard procedure. The number of mitotic stages and any abnormalities were scored.

For establishing the survival curve, 1 ml. samples were withdrawn, diluted and a sub-sample placed in a counting chamber with Lugol's iodine. After sedimentation had taken place the number of surviving cells was counted by means of an inverted microscope.

## Results

### A. Effects on survival and ability of cells to divide

As individual cells could not be plated out survival was estimated by sampling the cultures at intervals after irradiation. As cell division had often taken place the percentages of survivors are often more than 100. The curve obtained after various doses of UV is shown in Fig. 1. Here one set of points (open spots) represents the mean percentage survival after assessing the cell population at 3, 7, 13 and 24 days after irradiation with Source B. The other set of points (solid spots) represent the surviving percentage at seven days after irradiation. The survival curve shows the normal exponential relationship between survival and dose which has previously been obtained for many micro-organisms (cf. Hollaender 1955).

The progressive changes in the cultures used for survival estimation are shown in Fig. 2. It will be seen that 3 minutes exposure to U. V. scarcely affected the cells and the normal type of growth curve (Kain 1960) was obtained, with the logarithmic phase giving way to a more or less stationary phase after about one week. After a dose of 13 minutes the picture was rather different. There was a delay of several days during which time no divisions took place but then the cells increased in number and eventually achieved a total only slightly lower than that reached following the five minutes dose.

The material exposed for 30 minutes gave a very different response. For the first few days there was a rapid decline in cell numbers, this was followed by a small increase as a few cells divided but eventually most

died and after 24 days the survivors represented only 8% of the original irradiated population.

Another aspect of survival is the ability, or otherwise, of the cells to divide following irradiation. In an experiment using source A the cells in mitosis and those exhibiting abnormal divisions were counted at intervals after irradiation. The data obtained are summarised in Fig. 3. The per-

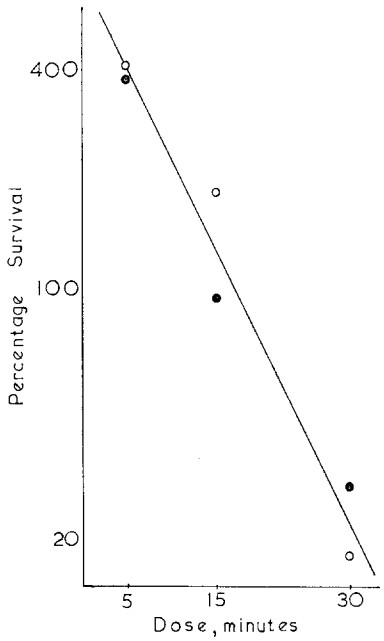


Fig. 1.

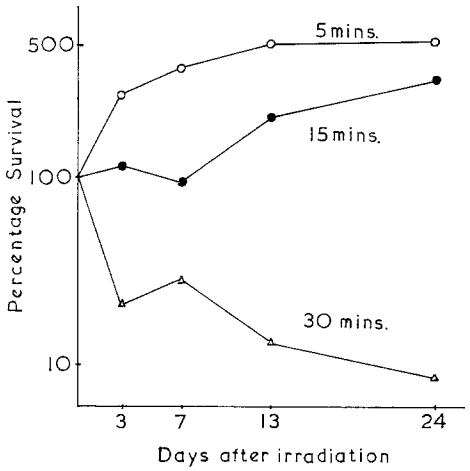


Fig. 2.

Fig. 1. The survival of *Prorocentrum* after various doses of U. V. The open spots (O—O) represent the mean percentage of survivors at 3, 7, 13 and 24 days after the irradiation and the solid spots (●—●) the survivors at 7 days. (U. V. source B).

Fig. 2. Progressive survival curves for *Prorocentrum*. Here are plotted the same data as used for Fig. 1 but shown against time after irradiation.

centage of cells in mitosis (open spots) is seen to decrease with dose although the nine minutes exposure gave an unexpected increase. The percentage of cells exhibiting abnormal divisions of the chromosome bridge type (solid spots) is roughly similar for all doses and there was not a great increase with dose as happened after x-ray treatment (Dodge and Godward 1963). The totals of abnormal divisions (bridges and cleavage failures) increased slightly with dose.

Progressive data from the same experiment (as for Fig. 3) are illustrated in Fig. 4. Here the percentage of normal or abnormal divisions are shown by the height of the histograms at various times after irradiation. This illustrates two points. Firstly that after higher doses (6 and 9 minutes) there is a delay before any division takes place. This was over a week in

the case of the highest dose. Secondly it shows the time of appearance of the chromosome aberrations after the various doses. In each case they are confined to a short period preceded and followed by normal mitoses. The significance of this fact will be discussed later.

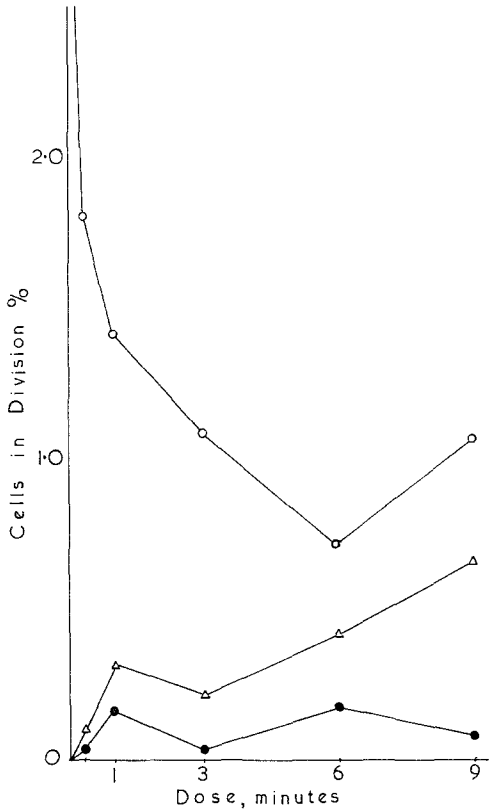


Fig. 3.

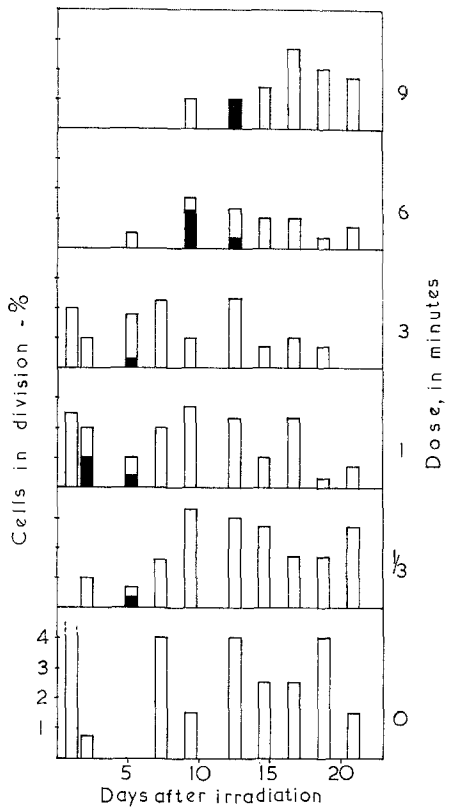


Fig. 4.

Fig. 3. The percentage of cells found in division after various doses of U. V. (source A). The open spots (O—O) represent the total percentage in division and the triangles (△—△) show the proportion of these divisions which were abnormal (chromosome bridges or cleavage failures). The lower line (●—●) shows the percentage of chromosome bridges found.

Fig. 4. The percentage of dividing cells at various times after irradiation with different doses (source A). Note the delay before any divisions took place after the higher doses. The portions shaded in black represent the divisions showing chromosome bridges.

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Figs. 5–6. Two focal levels of a chromosome bridge between the two daughter nuclei following treatment for six minutes (×1,600).

Figs. 7–8. Another bridge formed after a one minute exposure (×2,000).
Figs. 5–8: Aceto-carmine stained. U. V. from source A.

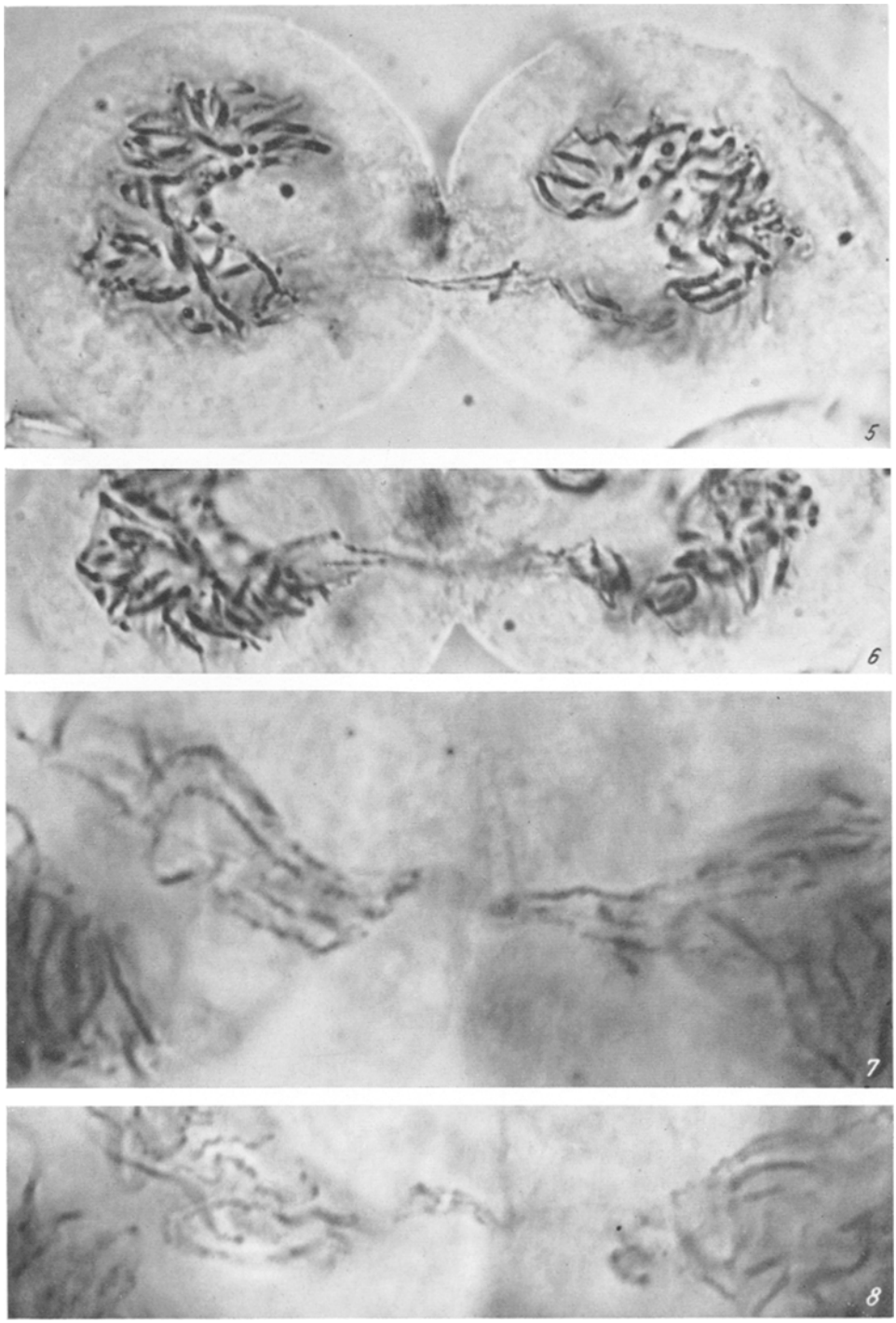


Fig 5–8.

## B. Effects on the chromosomes

Irradiation of *Prorocentrum* with U. V. produced chromosome aberrations in a small percentage of cells. At the anaphase following treatment the aberrations were observed as either chromosome bridges or fragments. The bridges were composed of two (Fig. 9) or four (Figs. 5–8) closely associated chromosomes. No single chromosome bridges and no fragment bridges (see Dodge and Godward 1963) were discovered. From the fact that pairs of chromosomes were apparently involved in all the bridges it is likely that the original aberration, which made possible the chromosome exchange seen as a bridge, was of the chromosome break type. If

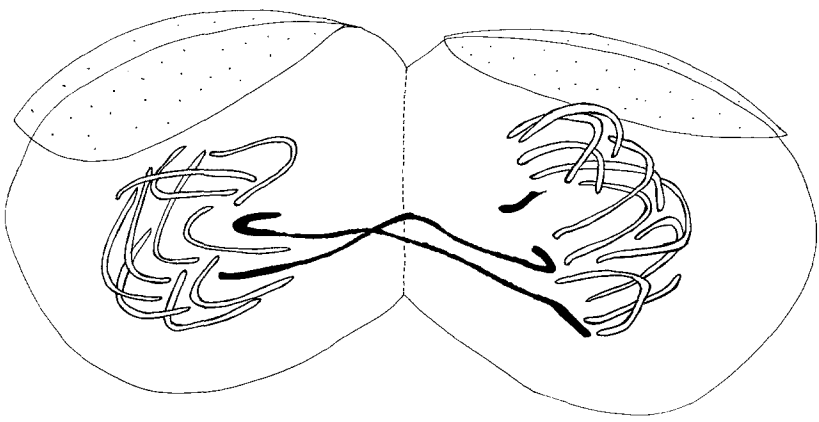

Fig. 9. Drawing of a nuclear division with chromosome bridge and fragment. Only about one sixth of the normal chromosome complement is shown. The dotted oval bodies at the top of the picture are the two halves of the wall of the parent cell.

chromatid breaks occurred these should give rise to single chromosome bridges.

Chromosome fragments were found occasionally (Fig. 9). They invariably had one end drawn out as a tail and this suggests that they were often the remaining part of a chromosome bridge which had been broken by the tension applied during anaphase. On one occasion a small fragment was seen in an interphase nucleus.

## C. Mutation

Ultraviolet light is much used for the production of mutants in microorganisms. Generally these are biochemical mutants but morphological mutants have been obtained in fungi and algae. *Prorocentrum* cannot as yet be grown in a defined medium so it has not been possible to screen for biochemical mutants. However, certain morphological changes apparently resulting from the effects of the irradiation, have been observed. Generally these take the form of gross aberrations which prove lethal; the cell being unable to divide again. One non-lethal mutant has been observed (Fig. 11). This appeared some months after a culture was treated with the sub-lethal

dose of 30 minutes irradiation (Source B). Of the surviving population five months after this irradiation 17% of the cells were found to have the form illustrated, with the right anterior corner of the cell missing. Figure 10 shows a cell of normal shape from the same culture. Cytological examinations (Fig. 11) showed that the nucleus appeared normal and no gross chromosome aberration could be seen.

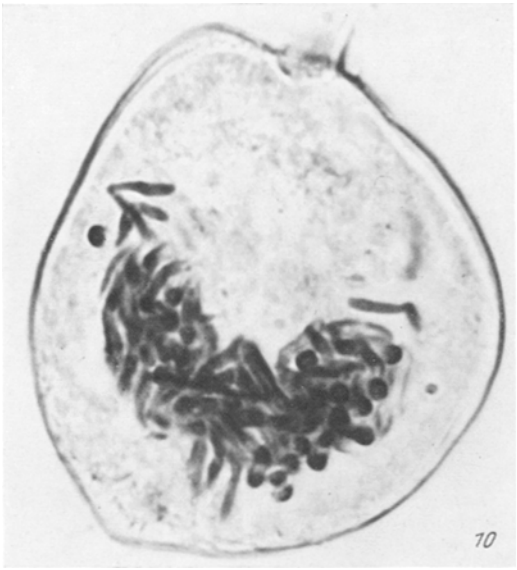


Fig. 10.

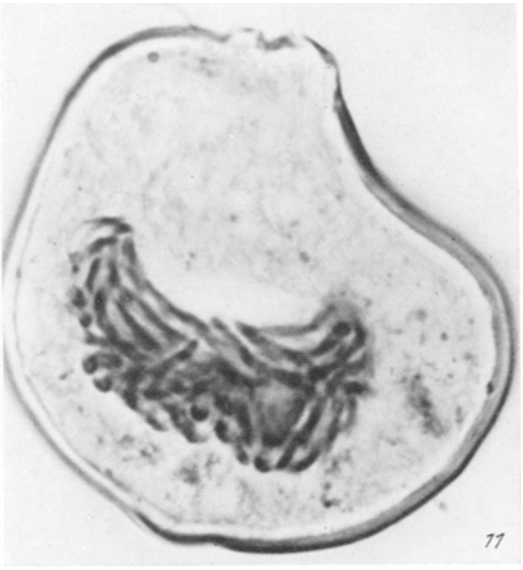


Fig. 11.

Figs. 10–11. Cells stained with aceto-carmine, photographs ×2,000. U. V. source B, 30 minutes exposure.

Fig. 10. *Prorocentrum micans.* Cell of normal shape but with nucleus in which some chromosomes at the top are slightly separated from the remainder.

Fig. 11. Cell of abnormal shape found after irradiation. The nucleus is normal here.

## Discussion

Although many algae have been treated with U. V. the effects of the irradiation have normally been assessed in different ways by workers interested in different effects, thus making comparisons very difficult. It may be of interest to outline the main discoveries. The first worker in the field was Meier (1932) who found that certain wavelenghts were lethal to *Chlorella.* Later Shettles (1938) found that large doses of U. V. at 2537 Å were lethal to *Peranema.* More recently it has been shown that in *Chlorella* (Redford and Myers 1951), *Chlamydomonas* (Nybom 1953) and *Eudorina* (Rayns 1961) increase in dose decreased the percentage of

survivors. Normally an exponential relationship of the type obtained here for *Prorocentrum* was observed.

The effects of large doses of U. V. on respiration and photosynthesis of algae have been examined by Holt, Brooks and Arnold (1951) and McLeod and McLachlan (1959). The latter found that diatoms were more sensitive than the members of the Chlorophyta which were irradiated.

Morphological mutants have been obtained in the desmid *Cosmarium* (Korn 1959) and in *Chlamydomonas* (Lewin 1954, Nybom 1953, Gowans 1960) mutations affecting the flagellar apparatus have been obtained. Non-motile mutants have been isolated in *Euglena* and *Pandorina* (Lewin 1960). A large number of biochemical mutants have been obtained in *Chlamydomonas* (reviewed by Levine and Ebersold 1960 and Ebersold 1962). A number have been obtained in *Chlorella* (Bendix and Allen 1962, Kvitko and Khropoua 1963). The morphological mutant described in the present paper is the first mutant recorded in the Dinophyceae or in any of the yellow or brown pigmented divisions of the algae.

*Prorocentrum* is also the first alga in which chromosome aberrations have been observed after U. V. irradiation. Such aberrations are never very frequent in higher plants (Swanson 1957) and in fact although mutants and perhaps chromosome fragments can be produced relatively easily, chromosome exchanges of the type necessary to give an anaphase bridge are very rare.

The time of appearance of the *Prorocentrum* bridges is of considerable interest. As normal divisions were observed both before (even in the case of considerably delayed mitosis) and after the aberrant divisions it would appear that the original lesion occurred only in a small percentage of cells which were in a particular phase of the mitotic cycle, presumably the stage of chromosome (or DNA) duplication. That this stage is highly sensitive to a small threshold dose of U. V. at 2537 Å is born out by the similar percentage of cells with aberrations no matter what dose was given. It is to be hoped that further work on this organism will shed some light on the replication of the chromosomes and on the whole question of the mechanism of radiation induced chromosome breakage.

## Summary

Irradiation of *Prorocentrum micans* with ultra violet light gave rise to the normal exponential survival-dose relationship. The number of cells able to engage in nuclear division also decreased with increase of dose. Some chromosome breaks and exchanges giving rise to anaphase bridges were observed and a morphological mutant (cell form) was discovered.

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