STUDIES OF CHLOROPLAST DEVELOPMENT IN *EUGLENA*

XIV. Sequential Interactions of Ultraviolet Light and Photoreactivating Light in Green Colony Formation

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ABSTRACT Photoreactivation (PR) of green colony–forming ability in *Euglena* is pH-insensitive from pH 6.0 to 8.0 and temperature-sensitive with a maximum rate at 35°C. There is no PR at 0°C. The rate of PR varies with the growth stage of the cells; PR of exponential phase cells is slower than that of stationary phase cells. The reciprocity rule holds for PR over a 6-fold range of intensity. The shape of PR curves is a function of the UV dose; there appears to be a progressive increase in multiplicity until a limiting multiplicity is reached as indicated by the fact that curves for high doses are superposable. Dark-grown and light-grown cells give the same PR response for comparable UV doses. UV inactivation of cells which have been treated with UV and then with PR light shows that, if the PR dose is sufficiently large, the same UV-inactivation curve is obtained as for nonpretreated control cells. Doses of PR lower than the saturating dose produce UV-inactivation curves, the ultimate slopes of which are parallel to the slope of the control curve, but which show reduced multiplicity. The multiplicity of these curves increases with increasing PR dose. The UV inactivation of *green colony*–forming ability in *Euglena* is completely photoreactivable at the doses studied, in contrast with the UV inactivation of *colony*–forming ability, which occurs at considerably higher UV doses and behaves like most other photoreactivable systems, showing a photoreactivable sector of 0.32.

INTRODUCTION

Previous work has defined the effects of ultraviolet (UV) inactivation and photoreactivation (PR) on the green colony–forming system in *Euglena* (1). The UV inactivation of green colony formation is virtually completely photoreactivable in the sublethal range. UV is lethal to the cells at doses which are about 25 times higher than doses which inhibit 99% of green colony formation. The action spectrum for photoreactivation indicates that light of wavelengths between 3000 and 4000 A is effective, with a broad peak at about 3800 A; the nature of the

chromophore is unknown. In most systems, not all of the UV damage is photoreactivable (see references 2, 3, and 4), the *Euglena* green colony–forming system is unique in being completely photoreactivable over a very wide dose range of UV.

This paper further details the conditions for photoreactivation as well as the sequential interactions of UV and photoreactivating light on the ability of cells to form green colonies.

METHODS

Euglena gracilis var. *bacillaris,* its growth and conditions of UV irradiation and of plating have been described previously (1, 5).

For PR, two 15w black lights (Westinghouse G15T8BL) mounted 1 in apart were employed. All of the output of these lights is between 3000 and 4000 A with a peak at 3500 A. The dose rate could be varied by changing the distance between the source and the cells. For most experiments, however, the lamps were as close to the Petri dish containing the cell suspension as possible. The technique for PR experiments was essentially the same as that used for UV-inactivation experiments as described in the papers cited above.

The temperature optimum for PR was determined by diluting a stock culture into resting medium (6) and treating this suspension with UV. The suspension was then transferred to a Carrel flask and was incubated in the dark for 5 min in a water bath set at the desired temperature. An aliquot was plated at this time to test for heat reactivation. None was noted. The suspension at the desired temperature was then exposed to 5 min of PR light and plated.

The effect of pH on photoreactivation was determined by irradiating the stock culture with UV and then diluting it into 0.067M potassium phosphate buffers of different pH values. The various aliquots were then exposed to a 5 min dose of PR light. A control suspension was diluted into resting medium after UV and similarly irradiated with PR light.

RESULTS AND DISCUSSION

Conditions Affecting PR. For the first series of experiments, light-grown cells were irradiated with UV at room temperature, brought to the temperature indicated for PR and, after receiving a photoreactivating dose of light, were then plated under nonphotoreactivating conditions. Fig. 1 shows that PR in *Euglena* is temperature-dependent, as it is in other systems, with an optimum in the region of 35° C. This temperature optimum is different from that observed by Kelner (7) for PR of *Escherichia coli* viability and probably represents the temperature optimum of the photoreactivating enzyme involved (8). Although not shown on the curve, PR was essentially zero at 0° C.

For the next experiment, light-grown and dark-grown cells at various points in their growth cycles were exposed to comparable inactivation doses of UV followed by photoreactivating light. They were then plated under nonphotoreactivating conditions as before. Fig. 2 shows that, for each type of cell, photoreactivation is more rapid for cells in the stationary phase than in the exponential phase. The differences

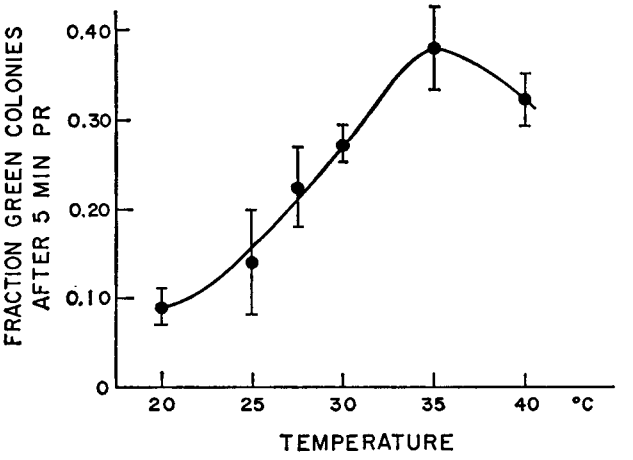

FIGURE 1 The effect of temperature on PR in *Euglena*. Light-grown cells. UV dose, 144 ergs/mm². Fraction green colonies with no PR < 0.01. For other details, see text.

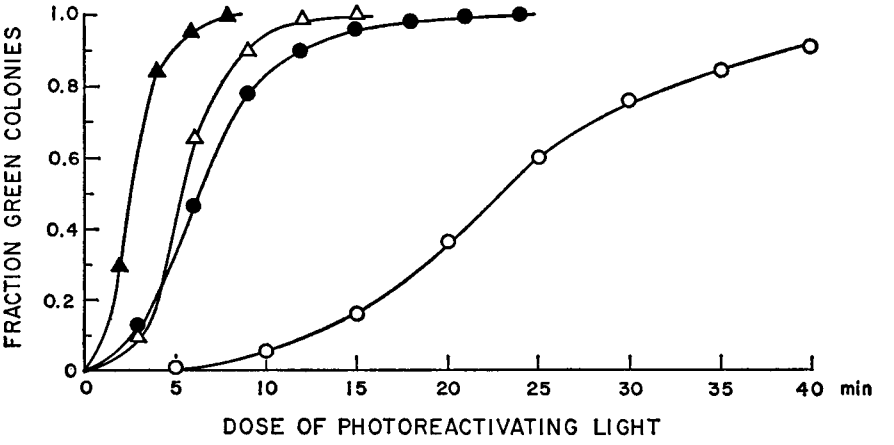

FIGURE 2 PR at different stages in the growth cycle of *Euglena*. ● Dark-grown cells, exponential phase, ▲ dark-grown cells, stationary phase. ○ light-grown cells, exponential phase, △ light-grown cells, stationary phase. Dark-grown cells received a UV dose of 110 ergs/mm² and light-grown cells, 192 ergs/mm².

between light-grown and dark-grown cells appear to be variable from experiment to experiment and the two types of cell should probably not be compared with each other on this basis. Since stationary phase cells are more easily photoreactivated, these were used in the subsequent experiments to be described.

Fig. 3 shows that the Bunsen-Roscoe reciprocity law holds in this system over the range of doses and times employed.

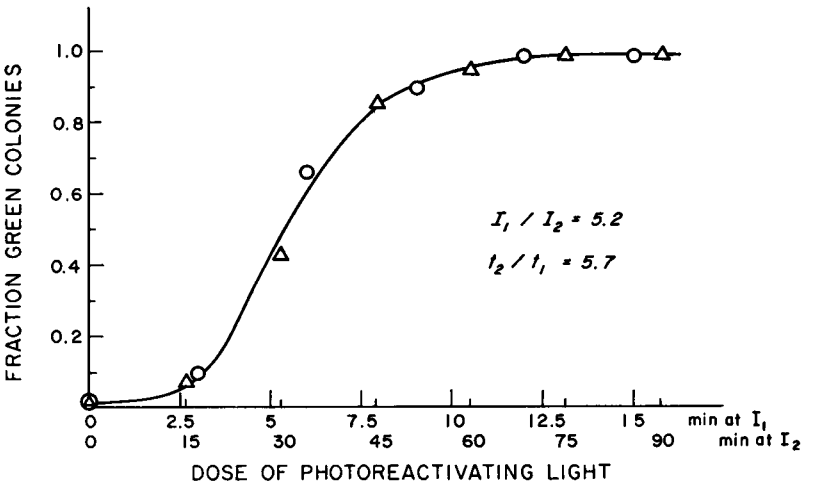

FIGURE 3 Reciprocity and PR. Light-grown cells were irradiated with a UV dose of 192 ergs/mm². One aliquot was exposed to a range of PR light at an intensity giving a light meter reading of 36.5 ma (○, I_1), the other at an intensity giving a meter reading of 7 ma (△, I_2).

Over a pH range of 6 to 8, there was no influence of hydrogen ion concentration on PR.

PR as a Function of UV Dose. For the following experiments, dark-grown cells received various doses of UV followed by increasing amounts of photoreactivating light. The cells were then plated as above. Fig. 4*a* shows the results of such an experiment. The kinetics of PR change with UV dose. Two effects can be noted; as

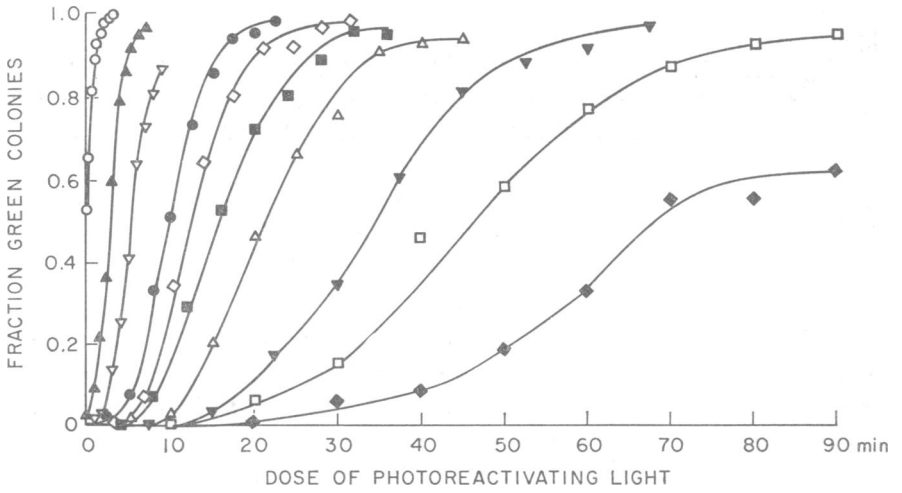

FIGURE 4*a*

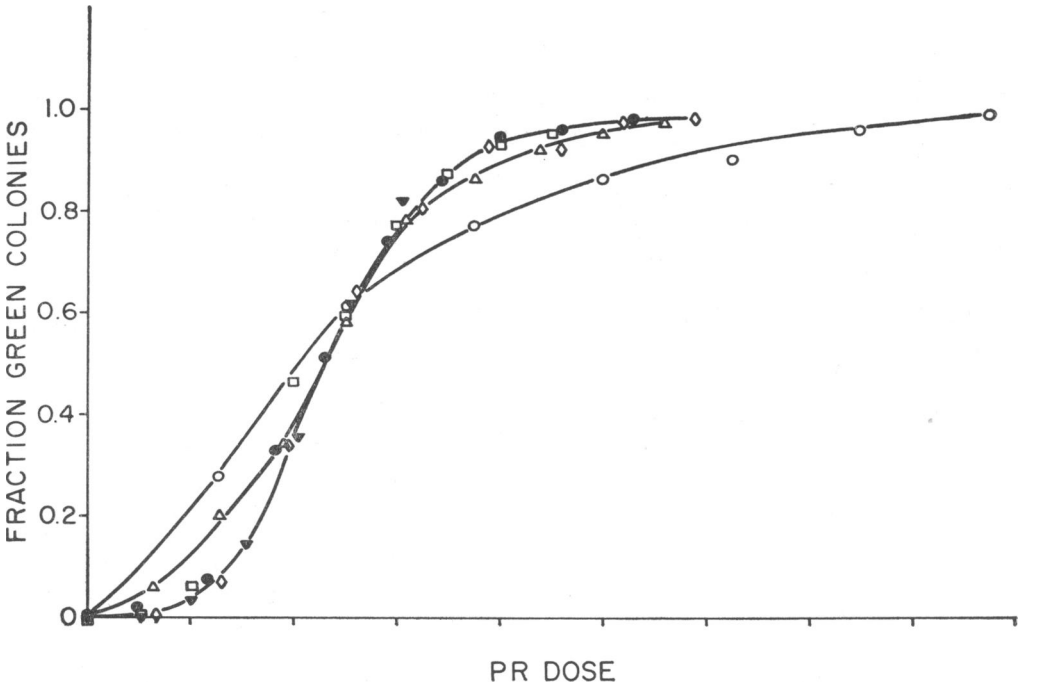

FIGURE 4*b*

FIGURE 4 PR of green colony–forming ability in dark-grown *Euglena*. (*a*) ○ UV 48, ▲ 80, ▽ 110, ● 160, ◇ 208, ■ 255, △ 320, ▼ 480, □ 640, and ◆ 960 ergs/mm². (*b*) The curves in (*a*) have been adjusted by a scale factor in an attempt to superpose them.

the UV dose increases, a pronounced lag in the kinetics becomes increasingly apparent, and the rate of PR decreases as the UV dose is increased. At high UV doses, lag and rate change concommitantly with increasing UV dose so that, when these data are normalized by arithmetic factors in the dose, all curves coincide. This is shown in Fig. 4*b*, where curves for high UV doses superimpose, while those for lower doses do not. (The low dose curves have been corrected, when necessary, to give 0 fraction green colonies at 0 PR dose.) This has important consequences for the development of a model to be presented later (9).

The same effects can be reproduced with light-grown cells (Fig. 5*a*, *b*, and *c*, representing separate experiments). This is emphasized in Fig. 6 where data from many experiments have been pooled, the dark-grown cell data being made comparable to that from light-grown cells by using the difference in UV sensitivity shown previously to be 1.7 (5, 10) as a means of normalizing the effective UV doses given. The curves were further normalized for PR dose as described above. As may be seen from Fig. 6, comparable UV effective doses yield superimposible PR curves when corrected for rate of PR thereby emphasizing the similarity in shape of

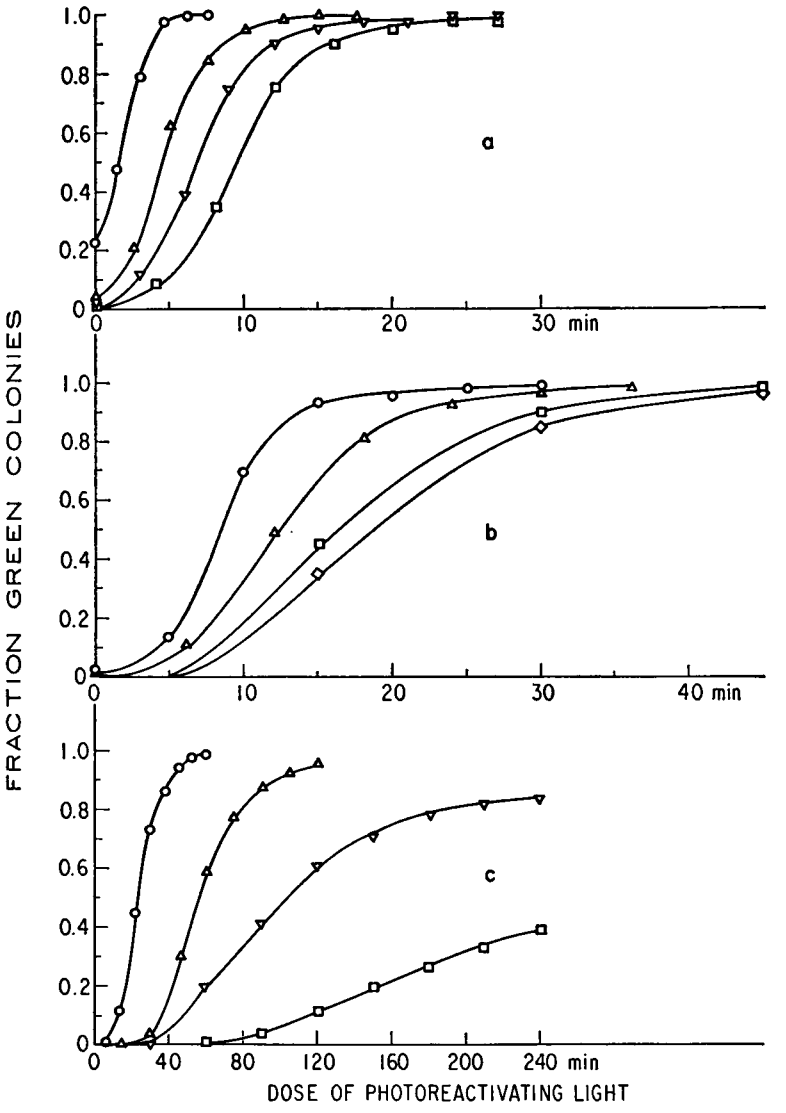

FIGURE 5 PR of green colony-forming ability in light-grown *Euglena*. (*a*) Experiment 1, ○ UV 96, △ 144, ▽ 192, □ 240 ergs/mm². (*b*) Experiment 2, ○ UV 288, △ 384, □ 480, ◇ 576 ergs/mm². (*c*.) Experiment 3, ○ UV 480, △ 960, ▽ 1920, □ 2400 ergs/mm².

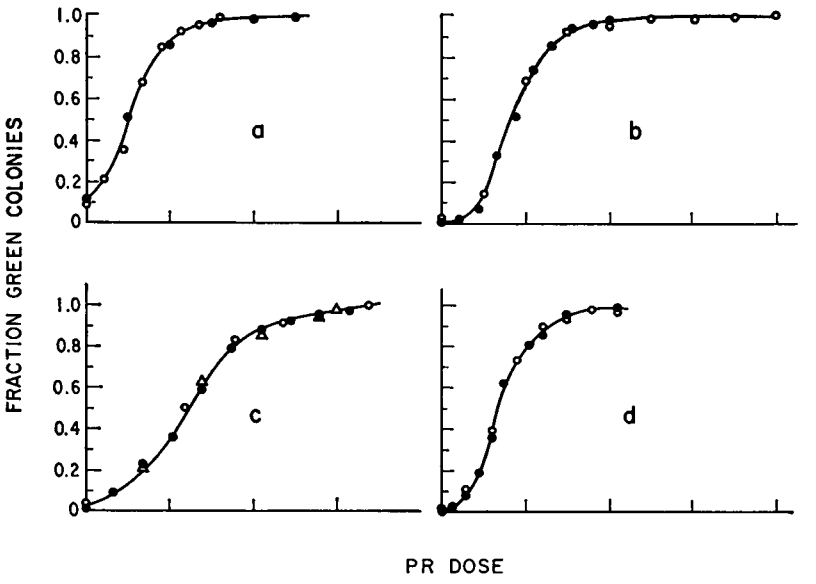

FIGURE 6 PR of dark-grown and light-grown cells compared. Curves are compared for UV doses giving the same fraction of gree colonies for both dark-grown and light-grown cells before PR. (*a*) Dark grown (●), UV 48, light-grown (○), UV 68 ergs/mm². Ratio of UV doses (dark:light), 1.4 (*b*) Dark-grown (●), UV 160, light-grown (○), UV 285 ergs/mm². Ratio 1.8 (*c*) Dark-grown (●), UV 80, light-grown (○ and △), UV 128 ergs/mm². Ratio 1.8. (*d*) Dark-grown (●), UV 110, light-grown (○), UV 190 ergs/mm². Ratio 1.7. Because of the variability in the rate of PR under different conditions, abscissas are in arbitrary units. The true abscissas are adjusted to make the curves coincide.

the curves. The shapes of these curves indicate multihit kinetics and are similar in behavior to PR of the T_3 bacteriophage system (4) in that the multiplicity is a function of the UV dose.

UV Inactivation after PR. To determine whether PR returns the system to the nonultraviolet condition, the kinetics of UV inactivation after PR were studied. For these experiments, dark-grown cells were treated with 192 ergs/mm² of UV, the controls remaining unirradiated. Those cells which received UV subsequently received various doses of photoreactivating light. Each of these were then further subjected to a range of UV doses before being plated.

Fig. 7 shows the results of a typical experiment. The fact that multiplicities for the second UV-inactivation curves increase as a function of photoreactivating dose indicates that increasing numbers of targets are being restored by PR until the original number characteristic of the unirradiated cells is reached. If all the targets are completely restored, the curve measured after saturating PR is achieved should be identical with the UV-inactivation curve of untreated cells. Fig. 8 shows the exact

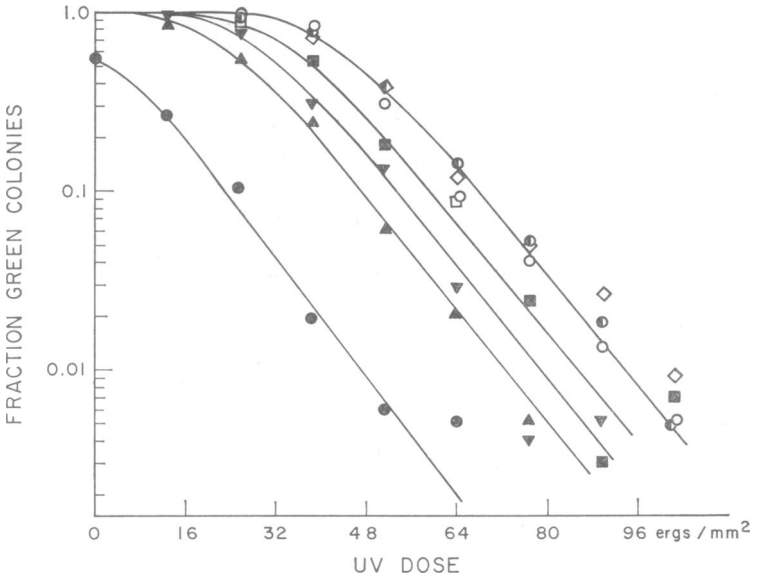

FIGURE 7 UV-PR-UV. Suspensions of dark-grown cells were treated with UV and then to different amounts of PR light. Following PR, each suspension was exposed to a range of UV doses. Control, no prior UV or PR, ●; UV 192 ergs/mm², PR 15 min, ▲; UV 192 ergs/mm², PR 30 min, ▼; UV 192 ergs/mm², PR 60 min, ■; UV 192 ergs/mm², PR 120 min, ◐; UV 192 ergs/mm², PR 180 min, ◇; UV 192 ergs/mm², PR 240 min, ○.

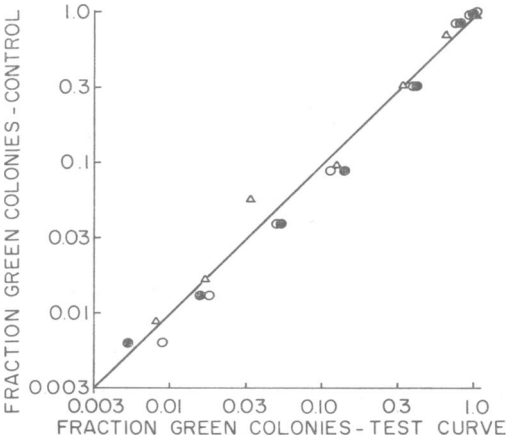

FIGURE 8 Identity test for UV-inactivation of untreated cells and photoreactivated cells. UV-inactivation curves after UV and high doses of PR light are compared, point for point, with control curves for untreated cells. ○ UV 192 ergs/mm, PR 180 min vs. control, ● UV 192 ergs/mm², PR 240 min vs. control (see Fig. 7); △ UV 64 ergs/mm², PR 60 min vs. control. If the curves are identical, all the points should fall on the line shown.

point by point correlation of the two types of curves. Therefore, PR appears to restore the system to the same condition that prevailed prior to UV inactivation.

It is important to note that physiological damage can be excluded as an explanation of UV effects since the asymptotic slopes of the UV-inactivation curves for cells which had previously been UV inactivated and photoreactivated are the same.

Most systems in which UV inactivation can be photoreactivated, can only be photoreactivated to the extent of about 60%. The PR of green colony formation in *Euglena* is atypical and shows virtually complete photoreactivation even for relatively large UV doses. This high efficiency could have at least three explanations. First, the high efficiency might be due to an inherent difference in the repair mechanism in *Euglena.* Second, it could be due to the fact that approximately 30 entities per cell are involved, with all the possibilities for mutual cooperation of partially damaged entities to restore the competence of an individual cell. Third, other systems may sustain photoreactivable and nonphotoreactivable damage while the *Euglena* green colony system may be sensitive only to the former.

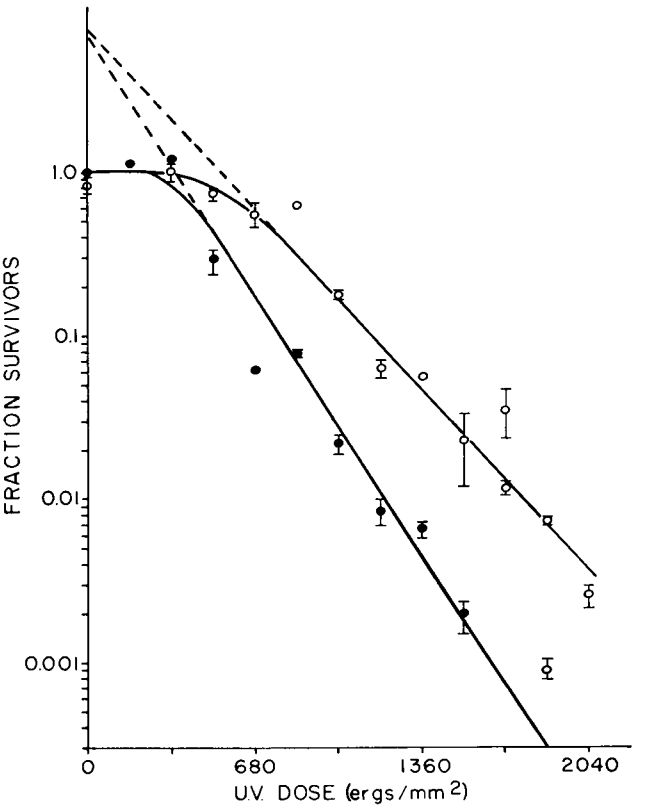

FIGURE 9 UV inactivation of colony-forming ability in *Euglena.* ●, Plates incubated in the dark; ○, plates incubated in the light.

The first explanation would seem unlikely, since the data presented in this paper indicate no anomalies in the response of the system to temperature, pH, previous history of irradiation, and stage in the growth cycle. Further, the PR of viability in *Euglena* proceeds to about 60% of maximum, as in other systems (Fig. 9). Thus the 100% photoreactivation of chloroplast-forming ability stands out even when compared with photoreactivation of viability in the same organism.

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