COUNTERACTING THE RETARDING AND INHIBITORY EFFECTS OF STRONG ULTRAVIOLET ON FUCUS EGGS BY WHITE LIGHT*

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INTRODUCTION

It has been shown that moderate dosages of unilateral ultraviolet light cause rhizoids to form on the non-irradiated sides of Fucus eggs (Whitaker, 1941; Whitaker, 1942). Thus 50 ergs per mm.² of λ 2537 Å cause more than 98 per cent of the eggs in a population to respond (Whitaker, 1942). Dosages of this order of magnitude have no appreciable effect on the developmental rate, but dosages of 20,000 to 50,000 ergs per mm.² very considerably retard and inhibit development (Whitaker, 1942).

The addition of β-indole acetic acid to the medium after eggs have been irradiated with 20,000-50,000 ergs per mm.² λ 2537 Å does not revive the eggs or cause them to recover from the retarding and inhibiting effects of the ultraviolet light. If the sea water medium in which eggs are strongly irradiated and then reared is acidified from pH 8.0 to pH 6.0, the sensitivity of the eggs to the ultraviolet is decreased (Whitaker, 1942). The present experiments were undertaken to test the effect of white light upon the sensitivity of the eggs to strong dosages of ultraviolet.

Material and Method

Fucus furcatus was collected at Moss Beach and at Pescadero Point, California, during the months of April, May, and June, 1941. Gametes were obtained and fertilized in a manner previously described (Whitaker, 1942; Whitaker, 1936). Filtered sea water (specific gravity 1.025-1.027) at pH 7.9-8.1 was used as the medium throughout. The eggs were fertilized and reared in a constant temperature room at 15 ± 1/4°C., and were shielded from all light except the experimental exposures, and brief exposures to dim red light.

The eggs were irradiated with ultraviolet light in 4 clear fused quartz rectangular culture vessels, 50 × 25 × 15 mm. These vessels were made of polished stock 1 mm. thick and they have good optical properties. The eggs were arranged on plate glass slabs within the quartz vessels so that they were in two rows. One row

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was above and behind the other so that there was no eclipsing, and eggs were at least 5 egg diameters apart in the row. The radiation passed through 9 ± 1 mm. of sea water before reaching the eggs.

A Westinghouse sterilamp was used as the source of ultraviolet. It is a gas tube mercury resonance lamp, and more than 90 per cent of the radiant energy is of the wave-length 2537 Å. An appreciable amount of λ1800 Å is produced in the gas space, but almost all of this ozone producing frequency is absorbed in the corex glass tube of the lamp. Small amounts of λ130 and 3660 Å are emitted, as well as dim visible light of bluish color. Practically no heat is produced. The intensity of ultraviolet wave-lengths shorter than 3200 Å was measured by means of a Hanovia ultraviolet meter. The intensity of the lamp did not vary more than 6 per cent at most during a run. Intensity measurements were made periodically during each run, and the duration of the exposure was adjusted to compensate for variations in intensity, to give the desired total dosage. The eggs were placed 6 inches from the tube of the sterilamp and the rate of application of the ultraviolet energy to the eggs was approximately 525–550 ergs per mm.² per minute. The absorption of the ultraviolet in passing through 9 mm. of sea water to reach the eggs is minor and has been neglected in calculating dosage. The mid-point of the period of exposure to ultraviolet occurred in all cases very nearly at 8 hours after fertilization. The duration of the exposure to ultraviolet was very nearly 36 minutes for 20,000 ergs, 66 minutes for 35,000 ergs, and 95 minutes for 50,000 ergs.

Due to the delay in development caused by the ultraviolet it was necessary to extend observations over a number of days. Therefore, in order to free the quartz vessels for starting new experiments, after irradiation with ultraviolet the eggs were transferred on the glass slabs from the quartz vessels into rectangular Petri dishes for rearing with or without white light. Although this transfer was carried out with great care, the eggs may have moved somewhat, and therefore little consideration has been given in these experiments to the direction of rhizoid formation. Incomplete evidence suggests that eggs do not necessarily form rhizoids on the sides away from strong doses of λ2537 Å.

RESULTS

In the first series of experiments, the eggs in half the vessels were exposed to white light from an ordinary frosted 75 watt bulb at 1 meter distance. This exposure to white light began immediately after termination of the irradiation with ultraviolet and transfer of the eggs to the Petri dishes, and continued until the end of the experiment. The same sides of the eggs were exposed to ultraviolet and to white light, except for any movement of the eggs during transfer (see Method). The white light passed through a water cell, and a fan caused air to flow from the eggs toward the light to minimize temperature effects.

The object of this first series of experiments was to see whether the white light would to some extent revive the eggs or cause them to recover from the retarding and inhibitory effects of the ultraviolet, when applied only after termination of the ultraviolet treatment. A part of the results is shown in
Fig. 1. Typical results showing effect of white light in accelerating rhizoid formation after eggs have been exposed to retarding and inhibiting dosages of ultraviolet. The white light was turned on after termination of the ultraviolet treatment at about 8.4 hours after fertilization. In A, the eggs received 20,000 ergs per mm.² ultraviolet; 446 eggs were reared in the light and 393 in darkness. In B, the ultraviolet dosage was 35,000 ergs per mm.²; 398 eggs were reared in the light and 344 in darkness.
Fig. 1. Fig. 1A shows the results of a typical experiment in which 20,000 ergs per mm.² ultraviolet were applied. Two other similar experiments involving 201 and 300 eggs reared in the white light, and 296 and 270 eggs reared in the dark, respectively, gave results very much like those shown in Fig. 1A, but the differences between the rates of development in the white light and in the dark were somewhat greater. Fig. 1B shows the results of an experiment in which 35,000 ergs per mm.² ultraviolet were applied. The retardation of development is greater in this case, but the effect of the white light is very marked in reducing the retardation. Another similar experiment, involving 320 eggs reared in the dark and 280 reared in the white light, gave similar results, although the effect of the white light was not quite so great. After 50,000 ergs per mm.² ultraviolet, the developmental delay was very great. Most of the eggs did not develop at all before cytolizing some days after the irradiation with ultraviolet. However, in two experiments with 50,000 ergs per mm.² ultraviolet, more eggs formed rhizoids in the white light than in the dark, and they formed them sooner.

It is clear from these results that the rhizoids form much sooner in the white light after the eggs have been exposed to ultraviolet. However, it has already been shown that normal Fucus eggs form rhizoids somewhat sooner in the light than in the dark (Whitaker, 1936). In the present experiments it is therefore of interest to find out how much acceleration of rhizoid formation can be attributed to the effects of the white light quite aside from the effects of ultraviolet. For this purpose, in a second series of experiments, eggs were illuminated with white light from the 75 watt bulb at 1 meter distance beginning at 8.4 hours after fertilization and continuing to the end of the experiment. The white light was also turned on at about 8.4 hours after fertilization in the first series of experiments. No ultraviolet was used in this second series of experiments. Two experiments were carried out, involving 4 vessels each, and the eggs in half the vessels served as dark controls. The rhizoids formed sooner in the light, but the speed up is not more than 2.5 hours at most, under these conditions. The speed up in the light is probably somewhat exaggerated as measured, since the light came from one side and caused the rhizoids to form on the opposite sides of the eggs where they could be seen from above at the very earliest stages of formation. In the two experiments, the time at which 50 percent of the population formed rhizoids was earlier in the light by 1 ¾ hours in one case, and by 1 ½ hours in the other. Half of the eggs had formed rhizoids in the dark at 16 hours after fertilization. This acceleration is small compared with that caused by the white light after strong dosages of ultraviolet.

A third series of experiments was undertaken to see if a strong white light applied during the time of exposure to ultraviolet would exert a protective effect upon the eggs. An ordinary 200 watt bulb was placed at a distance of 14 inches from the eggs in half the vessels. The white light passed through a water cell
to absorb heat, and the same sides of the eggs received both the ultraviolet and
the white light. The white light was turned on in all cases 4 minutes before the
ultraviolet, but the ultraviolet and the white light were turned off at the same
time. Thereafter the eggs developed in darkness except for brief exposures to
dim red light to make counts. In two experiments the eggs were exposed to
20,000 ergs per mm.² ultraviolet. In both cases the exposure lasted 36 min-
utes, its mid-point occurring at 8 hours after fertilization. The results of one
of these are shown in Fig. 2. The results of the other, involving 200 eggs ex-
posed to white light and 300 controls, are essentially similar although the rate
of rhizoid formation in the light was not quite so great. A third experiment
was carried out with 35,000 ergs per mm.² ultraviolet applied during 66 minutes
(33 minutes on either side of 8 hours after fertilization). The development
was very greatly inhibited by the ultraviolet in this experiment, but at 170
hours after fertilization rhizoids had formed on 7 per cent of 280 eggs that
received white light with the ultraviolet, and on 1 ½ per cent of 270 eggs that
received ultraviolet only. The concurrent white light thus appears to reduce
the inhibition as well as the retardation of rhizoid formation.
DISCUSSION

From the results of the first series of experiments (Fig. 1), it is clear that white light applied after strong dosages of ultraviolet counteracts the retarding action of the ultraviolet on development to a very considerable extent. Since white light tends to speed up the rate of rhizoid development in normal Fucus eggs that have not been subjected to ultraviolet light (Whitaker, 1936), it is of interest to compare the accelerating effect of white light with and without ultraviolet treatment. The results of the second series of experiments show that the white light does not speed up the rhizoid formation, in the absence of ultraviolet effects, more than about 2.5 hours at most, under the conditions of the experiments. After eggs have been retarded by heavy dosages of ultraviolet, the white light has a much greater accelerating effect than this, as may be seen in Fig. 1. In Fig. 1 B, for example, 20 per cent of the population reared in white light formed rhizoids more than 100 hours before the same percentage of the population reared in the dark. After the strongest dosages of ultraviolet (e.g., 50,000 ergs per mm.²), which completely inhibit rhizoid formation in most of the eggs, the white light appears also to increase the percentage of eggs that form rhizoids before cytolizing, although this effect is not as well established as the increase in rate of rhizoid formation.

The third series of experiments shows that strong white light shining on the eggs at the same time they are receiving heavy dosages of ultraviolet greatly protects the eggs from the retarding and inhibiting effects of the ultraviolet (Fig. 2). This protective effect appears to be even more marked than the recovery effect shown in the first series of experiments, although the white light was on so briefly that it cannot have had much of the type of effect shown in the second series of experiments. The white light was turned on 4 minutes earlier than the ultraviolet, in the third series of experiments, so that photosynthesis might be well established when the ultraviolet began to fall on the cells.

Until monochromatic bands or limited regions of the spectrum are tested, there is of course no basis for a definite opinion about what frequencies are effective or about the means of action of the white light. If the longer wavelengths of the visible spectrum are effective, it might be supposed that photosynthesis is involved.

SUMMARY

1. Strong dosages (20,000–50,000 ergs per mm.²) of ultraviolet light, predominantly of the wave-length 2537 Å, greatly retard and inhibit the development of rhizoids in Fucus eggs irradiated at about 8 hours after fertilization.

2. If white light shines on the eggs after the irradiation by ultraviolet is terminated, the white light causes a considerable degree of recovery from the retarding and inhibiting effects.
3. If strong white light shines on the eggs during the ultraviolet irradiation, its effect is even more marked in protecting the cells from the damaging effects of the ultraviolet.

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BIBLIOGRAPHY

