ON THE LIGHT RESPONSE OF THE CHROMATOPHORE OF THE SEA-URCHIN, *DIADEMA SETOSUM* (LESKE)

By MASAO YOSHIDA

Misaki Marine Biological Station, Misaki, Kanagawa-ken, Japan

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The responses of chromatophores to light stimuli have been reported in various kinds of animals. In almost all cases, the stimuli have been regarded as not affecting the chromatophore directly, but through nervous or hormonal mediation. Little is understood about chromatophore behaviour as a primary response to light.

In *Diadema*, as Millott has already reported (1950, 1952), the chromatophores distributed over the interambulacral zones expand or contract following a suitable period of light or dark treatment. According to Millott, a local expansion can be elicited following illumination by a light spot. This seems to indicate a direct response of the chromatophore to light. In Millott's experiment, however, since relatively large areas were illuminated (0·5–1·5 mm. in diameter), there remains the possibility that peripheral nerve endings may have been involved in the photic response. The present experiment is made in an attempt to investigate whether or not the chromatophore reaction may be really direct (i.e. intracellular).

MATERIALS AND METHODS

Sea-urchin species *D. setosum*, available at Misaki, seems to comprise two forms. Urchins used in these experiments were limited to the form which had 'globular-shaped' blue spots along the border between the ambulacral and the interambulacral zone and five 'white' patterns at the equatorial plane of the test in the interambulacral zones.* An area convenient for observation was between the genital pore and the white pattern. Since colour changes can be induced by dark-treatment independently of the central nervous system, as Millott reported, this area (about 0·5 cm. wide and 1·5 cm. long) was cut off from the test. It was then placed in a glass vessel and observed under the microscope.

For stimulation, light from a microscope lamp was thrown on the material by the following arrangement. The light, after passing through a water filter (2·5 cm. in thickness), was made to converge to 3μ in diameter by an optical system consisting of a diaphragm, a prism and four lenses. These components were arranged in the following order.†

* The other form has 'rod-shaped' blue spots and 'red' patterns at the corresponding places; the chromatophore distributions in the first group used in the experiment are sparser and the reactions to the light clearer than the second form.

† Author's thanks are due to Mr T. Nakatsubo of the Olympus Optical Company, without whose co-operation this apparatus could not have been prepared.

(1) An aperture (0·3 mm. in diameter) in the diaphragm served as the image, 150–200 Lux in intensity.

(2) A high-power lens ($f=2$ mm.) reduced the size of diaphragm image.

(3) Two low-power lenses of long focal distance picked up the small image and formed its virtual image at a conjugate focus of an objective lens (5).

(4) Along the course of (3), a prism was inserted to bend the ray at right angles so as to make the beam enter the optical axis of the microscope.

(5) An objective lens (× 10), which was being used for observation, picked up the virtual image formed by (3) and focused its real image of 3μ in diameter at the plane of the focus of the microscope.

If chromatophores are generally illuminated the complete dispersion of the pigment from the concentrated state takes 20–30 min.; when kept in darkness its complete concentration from the dispersed state requires 1–1½ hr. Therefore, when a light spot was thrown on a part of a chromatophore with fully concentrated pigment (whether it was applied on the pigment-containing or the pigment-free part of the cell) the illumination was continued for 20–30 min. When the light spot was thrown on a part of a chromatophore when its pigment was dispersed, the illumination was continued for 1–1½ hr.

RESULTS AND DISCUSSION

Description of the reaction will be given with reference to Fig. 1 (A–F and G–I).

(A) The initial condition of the experiment. The whole field has been generally illuminated for some time and the pigment is largely dispersed. The spot of light is now placed on the middle part of the branch of no. 4 chromatophore in which the pigment is incompletely dispersed (marked with a cross) and the general illumination is cut off.

(B) The whole preparation has been in darkness for 1½ hr. except for the spot of light on no. 4 chromatophore (now marked with a circle). It is significant that the pigment remains dispersed in the illuminated area of the chromatophore. In one branch of no. 5 chromatophore the pigment remains dispersed, and since this occurs nearest the light spot, it is probably due to light scattered from the spot.

The light spot is then moved to a new position indicated with a cross.

(C) One hour after B. In chromatophores nos. 1, 2 and 6, the pigment disperses as far as the light spot (circle), while in the branches of chromatophores nos. 4 and 5 the pigment has become fully concentrated.

The light spot is again moved to a place shown by a cross at the end of C.

(D) One hour after C. The pigment in nos. 5 and 6 chromatophores is apparently contiguous at the point of illumination.

The light is then moved slightly toward the centre (cross-mark) of no. 6 chromatophore.

(E) One hour later. After the shift of light, granules of no. 6 chromatophore remain on the stimulated point, while in no. 5 cell it has become concentrated again; as in C.

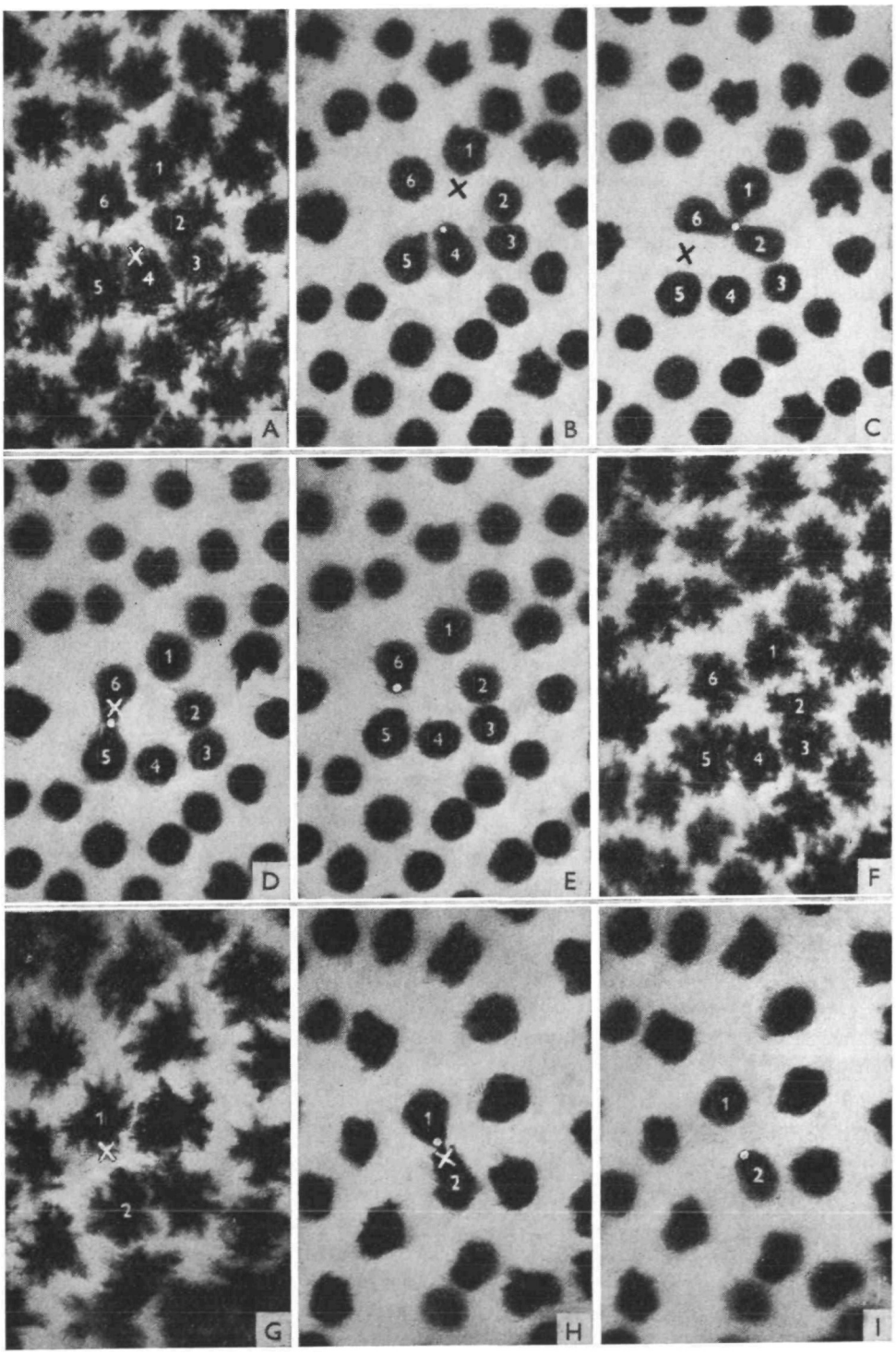

Fig. 1. Two series of experiments (A–F and G–I) in which spots of light are projected on various parts of chromatophores; temperature 16–18° C.; for explanation, see the text.

(F) Forty minutes after E—20 min. in darkness and 20 min. under illumination. The same condition as in A is resumed, with no sign of injury or fatigue. This is taken as evidence that the cells have been maintained in healthy condition, although the experiments from A to E lasted for 5 hr.

The next series:

(G) The initial condition after general illumination. The spot of light is then thrown on the distal end of the branch of no. 1 chromatophore in which the pigment is partly dispersed (marked with a cross).

(H) One and a half hours after G. Branches of nos. 1 and 2 chromatophores are apparently in contact at the stimulated point.

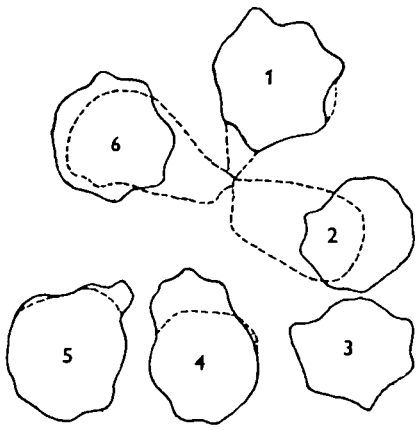

Fig. 2. Superposition of the outline of chromatophores of B (solid lines) and C (broken lines); for explanation, see the text.

The light spot is now shifted to a place shown by a cross.

(I) One hour after H. The light is at the margin of no. 2 chromatophore. In no. 1 cell the pigment is fully concentrated.

Another series of experiments shows that if the centre of the chromatophore is illuminated by the spot of light, the pigment does not disperse, but remains fully concentrated, regardless of its initial state.

The chromatophore behaviour described above can be summarized as follows: (1) when part of a chromatophore is illuminated, the pigment in that part cannot be withdrawn from the illuminated area: (2) when the pigment-free area of a chromatophore is illuminated, the cell disperses its pigment so as to cover the illuminated area. It may be inferred from these facts that the photic response of the chromatophore is direct; and further, that this photosensitivity is a property of the hyaloplasm since the presence of the pigment granules is not necessarily required in the illuminated part. Thus, the pigment granules have a capacity to move through the cellular fluid medium toward the illuminated point of the cell.

This interpretation may be more clearly illustrated if the above photographs of B and C are superimposed (Fig. 2). The darkward boundary of no. 2 chromato-

phore, which is reacting to the light spot placed far from its resting state, moves toward the light. Such is also the case for no. 6 chromatophore. These phenomena are observable in many other instances. The boundaries of nos. 2 and 6 chromatophores under any conditions, however, are indicated by areas of the pigment when fully dispersed. The shift of the chromatophore, described above, is apparent rather than real, and is due to a migration of pigment toward the lighted region of the cell.

It may be concluded that the chromatophores in *D. setosum* are photosensitive cells. However, it remains to learn whether the granules may themselves move toward the light or whether some other factor within the cell may initiate cytoplasmic movements which carry the granules.

SUMMARY

1. The nature of the photic response of chromatophores distributed over the test of *Diadema setosum* was investigated.

2. By applying a minute light spot to the various parts of single chromatophores, it was observed that the chromatophore can react locally to the light stimulus.

3. It is concluded that the photosensitivity resides within the chromatophore itself.

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