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SENSITIVITY TO LIGHT AND THE REACTIONS TO CHANGES IN LIGHT INTENSITY OF THE ECHINOID *DIADEMA ANTILLARUM* PHILIPPI

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(Plates 15 and 16)

CONTENTS

The reactions of *Diadema antillarum* to directional illumination and changes in light intensity are described, and the responses of the spines, tube feet and pedicellariae are reviewed, and shown to depend on a process of adjustment, so that light- and dark-adapted phases can be recognized. The responses of the spines to decreases in light intensity are examined in detail and shown to be reflexes, the receptive surface for which extends over the entire surface of the test but not the spines. The degree of sensitivity is correlated with the degree of dispersion of the pigment in superficial melanophores. The areas with most melanin (tube feet and ambulacral margins) are most sensitive, the so-called 'eyes' appearing to be least sensitive. The radial nerve cords and their branches appear to be affected by changes in light intensity. No morphologically or histologically differentiated receptors have yet been found, and it is suggested that the nervous system may be influenced directly by light. The findings are reviewed in relation to existing knowledge of the similar phenomena in other animals, and a comparison made between the entire surface of *Diadema* and certain features of the photoreceptive surface in highly differentiated eyes.

I. Introduction

It has long been known that a wide variety of echinoderms are sensitive to light. Among the echinoids, certain forms, such as *Centrostephanus longispinus* and *Diadema setosum*, show striking responses to changes in illumination and attracted the attention of von Uexküll (1897 *a*, *b*; 1900) and Hess (1914). The subject clearly requires reinvestigation, however, in the light of modern concepts of nerve action and behaviour, especially since echinoderms have received relatively little attention from comparative physiologists in the last thirty years, and the light-sensitive echinoids of warm waters virtually none.

These forms offer a new and promising approach for determining the fundamental processes of light perception, since in most cases the photoreceptive areas appear primitive, being neither highly differentiated structurally, nor localized in image-forming eyes, but widely scattered over the surface. Also, the site of photoreception in echinoderms is a matter over which there has been much controversy. Among the *Diademas* especially, inferences as to sensory function have been made from histological appearances, and based on studies which were inadequate.

Having to hand numbers of the light-sensitive urchin *D. antillarum*, it was decided, as a beginning, to examine some of the responses to light and to determine something more of the photoreceptive areas. Since we have little reliable knowledge concerning the histology of *Diadema*, no attempt has been made to define the photoreceptive structures in histological terms, and for the moment it has proved necessary to confine the investigation to attempts at determining the distribution of the photoreceptive areas and discovering some of their properties, by simple experiments. Some of the results of this preliminary investigation have already been referred to (Millott 1950).

II. The nervous system

The structure of the nervous system of *Diadema* has not been studied in detail and an account of the histology is reserved for the future. The general anatomy of the nervous system has been described by Delgado y Núñez (1917). It does not differ essentially from that of typical regular echinoids as described by Cuénot (1891, 1948) and Delage & Hérouard (1903).

The peripheral portion of the system has special significance in this study, and the nerve supply to the spines, tube feet and integument will be clear from figures 1 and 2 to 4, plate 15. From each radial nerve (*r.n.c.*) a series of ambulacral nerves arise on either side which pass through the test and branch to supply each tube foot by a podial nerve (*p.n.*) and the integument by an integumentary nerve. The latter nerve is broad and it spreads out distally to pass into the superficial nerve layer (*s.n.l.*). In the thin sections shown in figures 1 and 3, only the inner (*i.m.n.*) and outer (*o.m.n.*) edges of the integumentary nerve appear in the plane of section, but an idea of its width can be gained from the stippled area in figure 1 and the section passing through a different plane shown in figure 2. The superficial nerve layer extends between and beneath the epidermal cells over the entire surface of the test, being thickened in parts, for example, around the spine base where it forms a ring (*n.r.*) and at the points where it is joined by the integumentary nerves from the radial nerve cords. As will be obvious from figures 2 to 5, plate 15, the most striking character of the system is its superficial position.

III. Preliminary account of the responses to light

D. antillarum is markedly sensitive to light, showing responses which fall into two main categories, namely, those which are produced by steady directional illumination and those which are provoked by changes in intensity. Only some of the latter have been studied in detail, but it is proposed in the first place to review briefly some of the former.

1. *Responses to directional illumination*

These take the form of an orientation of the whole animal to directional sunlight, and it is shown most clearly by urchins kept in aquaria lighted by a window facing east, for, in the early morning, clusters of them can sometimes be seen orientated with their aboral surfaces directed toward the east light. In large tanks the uniformity of the response is most striking, and sometimes the entire population can be seen orientated in this way

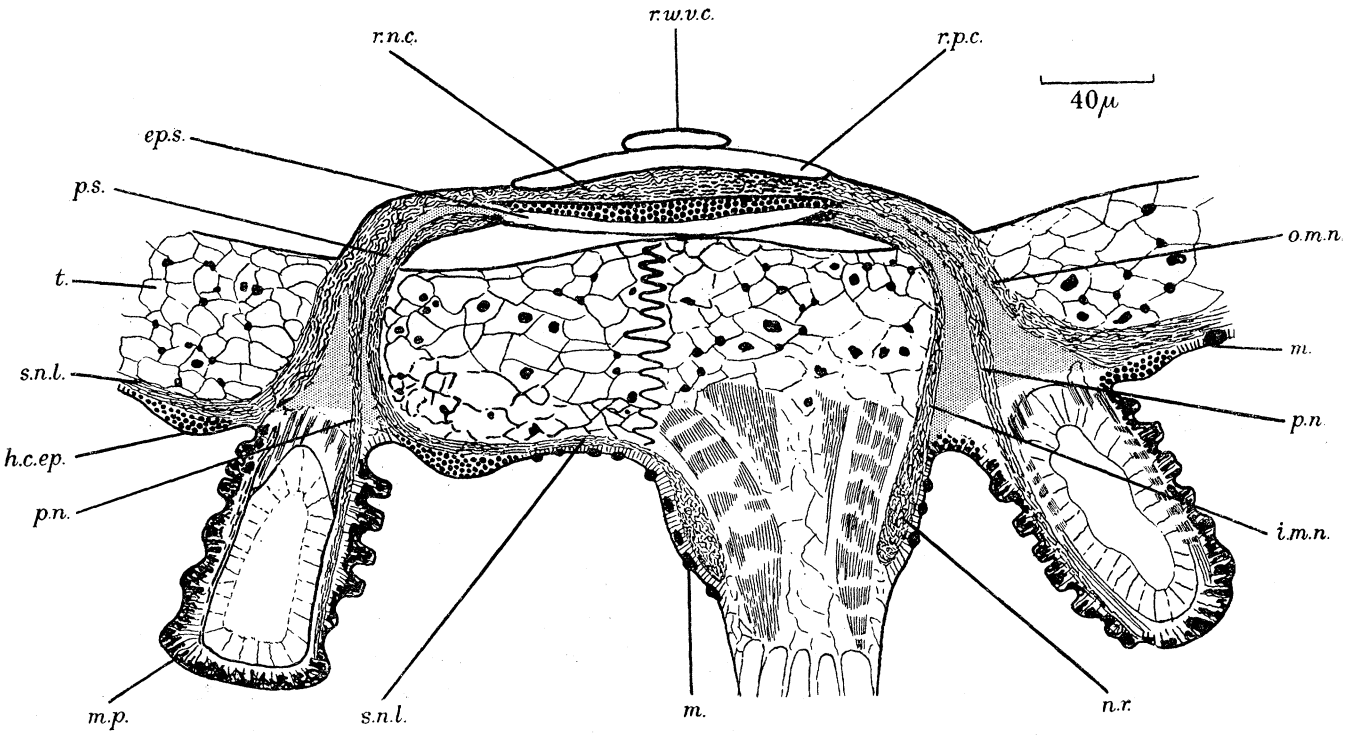

FIGURE 1. Transverse section through a radius of a young individual of *Diadema antillarum*, showing the branches of the radial nerve which supply the tube feet, integument and spines. The section is composite and somewhat diagrammatic. Since the section is thin, only the lateral edges of the integumentary nerve appear; the intervening portion, which is outside the plane of section, is indicated by mechanical stippling. *ep.s.* epineural sinus; *h.c.ep.* hillock in covering epithelium; *i.m.n.* inner (radial) margin of the integumentary nerve; *m.* melanophore; *m.p.* heavy deposit of melanin in tube foot; *n.r.* portion of nerve ring around spine base; *o.m.n.* outer (interradial) margin of the integumentary nerve; *p.n.* podial nerve; *p.s.* prolongation of epineural sinus; *r.n.c.* radial nerve; *r.p.c.* radial perihaemal canal (subneural sinus); *r.w.v.c.* radial water vascular canal; *s.n.l.* superficial nerve layer; *t.* decalcified test.

(figure 6). The response does not persist, however, for within an hour many of the urchins are seen to have moved indiscriminately around the tank, and in glass-walled aquaria readily expose any part of their surface to light. It is thus possible that the preceding prolonged sojourn in darkness may be a significant factor, particularly as the animals become remarkably sensitized to light after a period in darkness (see below).

In their natural surroundings, however, orientation by light appears to play little part in their distribution, for though many are to be found in crevices, an equally large number exist in a few inches of water, fully exposed to the tropical sunlight, the effect of which they appear to be able to tolerate indefinitely.

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2. *Responses to changes in light intensity*

D. antillarum responds clearly to changes in intensity. The responses are made both to increases and decreases, and may involve the whole animal, or isolated parts such as spines, pedicellariae and tube feet. The movements of the spines and tube feet are most striking; they may be directional with respect to the light source which shows the intensity change, or non-directional, and they may be co-ordinated or unco-ordinated.

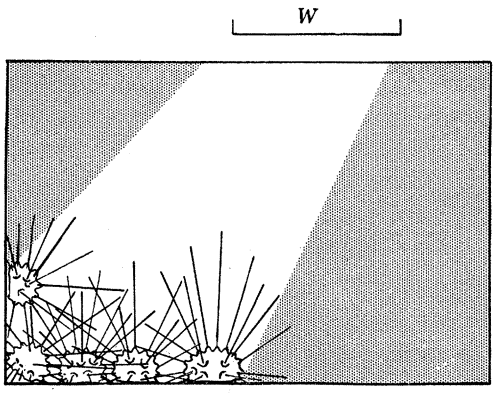

FIGURE 6. The orientation of *Diadema antillarum* to directional sunlight. The figure shows the position assumed in the early morning (about 8.30 a.m.) by the population of a large aquarium with opaque sides and floor, placed immediately below an open window (*W*) facing east. The most brightly illuminated area is shown unshaded.

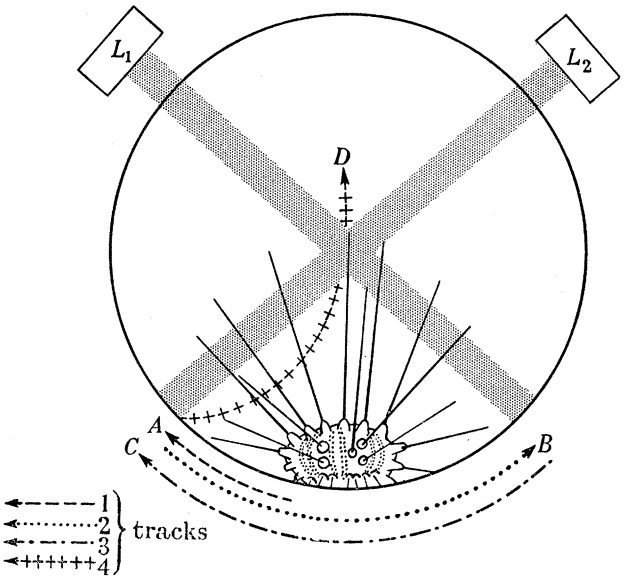

FIGURE 7. The behaviour of a dark-adapted individual when placed between two light beams. The approximate width of the beams is indicated by the shaded areas. L_1, L_2 = light sources in form of 50 c.p. microscope lamps with lenses and diaphragms, producing approximately parallel beams, of width shown. *A*, point at which the urchin arrested its locomotion along track 1; *B*, point at which the urchin arrested its locomotion along track 2; *C*, point at which the urchin arrested its locomotion along track 3; *D*, point at which the urchin arrested its locomotion along track 4.

Movements of the whole animal

These do not appear so regularly as those of the isolated organs such as the spines, etc. They were investigated in young forms with an average ambital diameter of 2 cm and appeared in about half of the cases examined. It must be mentioned, however, that many urchins were not fresh, having been in captivity for several days, a factor which may have affected the proportion showing the responses.

The movements were co-ordinated, but not directional with respect to the light source, a rapid change in the light intensity of the environment provoking local co-ordinated locomotory movements, which resulted in the urchin moving into either light or shade.

This depended on the light intensity to which the urchin had been subjected before the experiment, but in either case it came to rest in the area of the environment which had shown the least change. Thus urchins which had been confined in aquaria in darkness for about an hour responded to a light beam projected on them from a 50 candle-power lamp, at a distance of 3 ft., by moving briskly out of it into the darkest part of their environment. This is strikingly shown by using two beams as in the experiment described below and illustrated in figure 7.

A young urchin which had been kept in darkness in a glass dish was observed to have climbed the side and remained stationary. Two intersecting light beams from 50 candle-power lamps were projected across the dish so as to strike the sides about 3 cm on either side of the urchin. Stimulated by the change in intensity, the urchin moved over the side of the dish (track 1), until the edge of the test entered the light beam (at point *A*). It then stopped and reversed its movements, following track 2, until the edge of the test entered the other beam (at point *B*). Again it stopped and retraced its tracks (track 3), until the test entered the first beam again (at point *C*). Here it halted and then moved downward, passing into the shade beneath the light beams, where it remained.

No visible reaction resulted when the long aboral spines entered the light beam and they remained fully illuminated as the test gradually moved towards the beam. Progression ceased momentarily, prior to reversal of direction, only when the edge of the test entered the light beam.

The complementary type of experiment, performed by keeping urchins in a tank beneath an electric lamp and then altering the lighting quickly so that only a portion of the tank remained illuminated and the urchins were in the shade, showed that some of them quickly foregathered in the lighted area.

Again, urchins which had been stationary in a light beam, with their aboral surface illuminated, could often be induced to move by shining a light beam on to the oral surface, which hitherto had been in the shade.

Finally, it is most significant that the same urchin was often found to move into or out of the light, depending on whether it had been kept in bright light or in darkness.

It thus appeared that some of the urchins were responding to changes in the intensity of the light falling on any part of the surface of the test, by co-ordinated locomotory movements which persisted until the animal regained an area of the environment lighted to the intensity to which it had become accustomed. Such an interpretation of the observations presupposes that a process of adjustment or accommodation occurs, an assumption supported by the fact that urchins are found in their natural surroundings, as well as in aquaria, resting in situations of widely differing illumination, yet they are unquestionably responsive to light.

The existence of such a process can be shown by other simple experiments. Thus when an attempt was made to keep responsive urchins, which had become accommodated to darkness, continually moving by transferring a beam from a 50 candle-power lamp on to them, and then following them with the beam, they adapted and ceased to move out of the light spot after about four consecutive attempts. This was not the result of fatigue, however, for when the light beam in which they had come to rest was moved aside, some of the urchins moved back again into it. Again it appears that responses are determined

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by antecedent lighting conditions affecting a photoreceptive surface that is undergoing adaptation. Though the speed of the process was not studied, it may be mentioned that adaptation to darkness occurred after 15 min sojourn in the dark, in the young forms used in the above experiments, which had previously been kept under a 40 W tungsten lamp.

The process of adaptation appears related to the physiological colour change already reported (Millott 1952), for individuals which were observed to have adapted themselves to bright light were invariably dark in colour, and those which had adapted themselves to dim light or darkness were invariably pale. Moreover, individuals which were seen to have reversed their reactions, towards change in intensity, had also changed their colour. It is thus possible to distinguish light- and dark-adapted phases, corresponding, respectively, to the dark- and light-coloured phases already described (Millott 1952).

These preliminary experiments afford some indication of the areas that are light sensitive, for, as noted in the experiment described above, employing two light beams, the surface of the test and not the spines appeared to be light-sensitive. This conjecture is confirmed by denuding young urchins of their spines, when it was found that the foregoing reactions to changes in light intensity were not affected, apart from the direct effect on locomotion of the loss of spines.

Description of plate 15

Figure 2. Photomicrograph of portion of a transverse section through a radius of *Diadema antillarum*, showing the integumentary nerve, (*i.n.*) emerging at the surface to join the superficial nerve layer (*s.n.l.*) (see p. 188). Note that at the point where the integumentary nerve joins the superficial layer (=ambulacral margin), the covering epithelium is thickened (*h.c.ep.*). The extensive deposition of melanin (*m.p.*) in the walls of the tube foot is also evident. Prepared from a young individual, measuring about 1·3 cm across the ambitus. Fixed in Bouin's fluid; stained by Masson's method for the argentaffine reaction; counterstained in Mallory's triple stain $10\mu \times 660$.

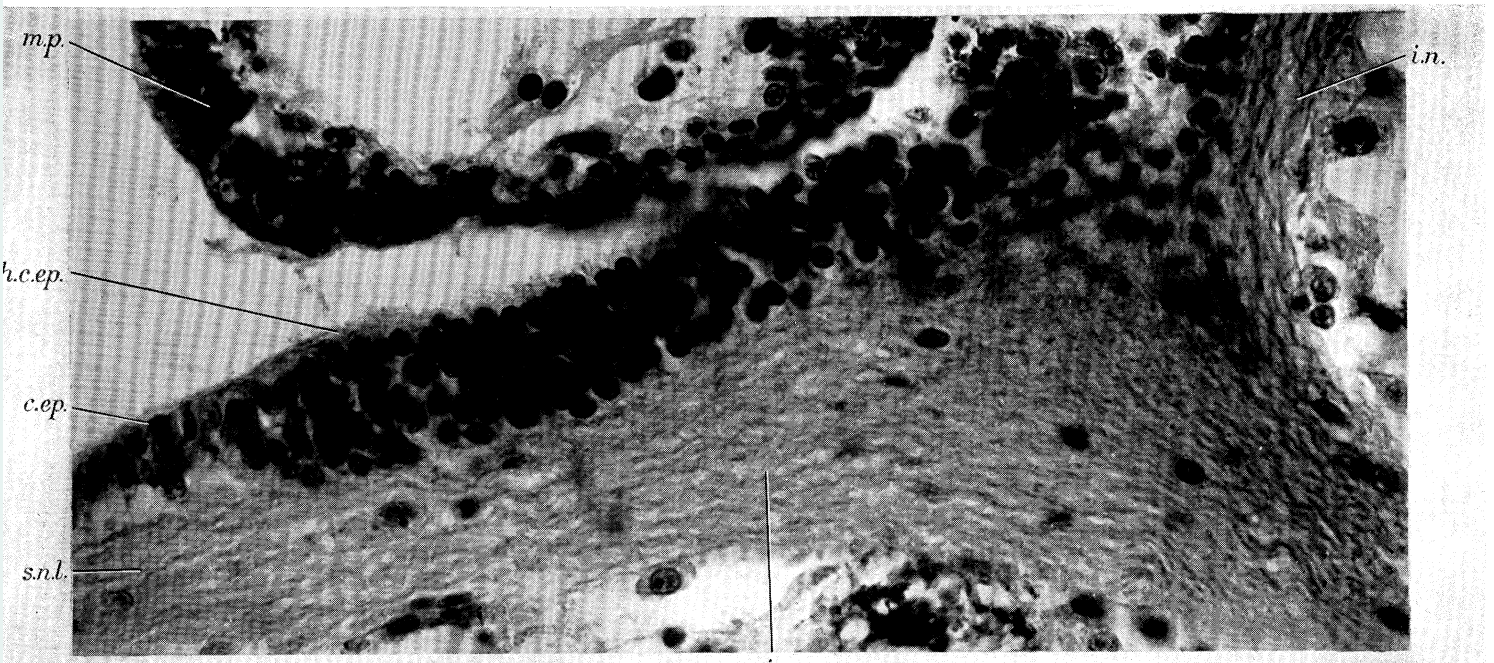
Figure 3. Photomicrograph of a portion of a transverse section through a radius of *Diadema antillarum* showing the origin of the podial (*p.n.*) and integumentary nerves from the radial nerve (*r.n.c.*). The integumentary nerve whose radial and interradial margins are shown respectively by the letters *i.m.n.* and *o.m.n.*, joins the superficial nerve layer (*s.n.l.*) (see p. 188), which is thickened to form a ring (*n.r.*) around each spine base. Prepared from a very young individual measuring 2·5 mm across the ambitus. Fixed in Bouin's fluid; stained in Delafield's haematoxylin and eosin. $5\mu \times 500$.

Figure 4. Photomicrograph of portion of a section through the base of a spine, showing the relations between the superficial nerve layer (*s.n.l.*) and the melanophores (*m.*) in the covering epithelium (*c.ep.*) (see p. 204). From a young individual measuring about 1·0 cm across the ambitus. Fixed in Bouin's fluid; stained in Mallory's triple stain. $10\mu \times 660$.

Figure 5. Photomicrograph of a section through an iridophore (=so-called 'eye') of *Diadema antillarum* (see p. 200), cut in a plane normal to its outside surface (*o.s.*). From a young individual measuring about 1·3 cm across the ambitus. Fixed Bouin's fluid; stained by Masson's method for the argentaffine reaction, counterstained in Mallory's triple stain. $10\mu \times 660$.

Lettering

c.ep. epithelium covering surface of test.
o.s. outside surface of iridophore.
Other letters as in figure 1.



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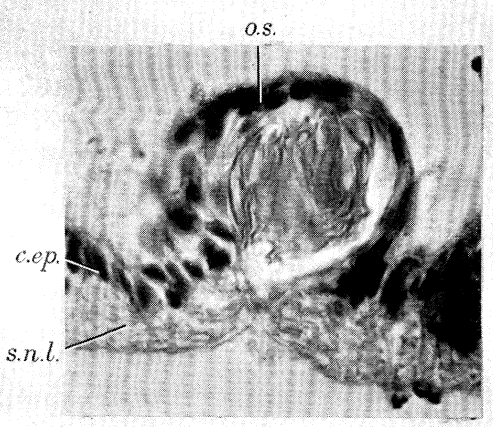

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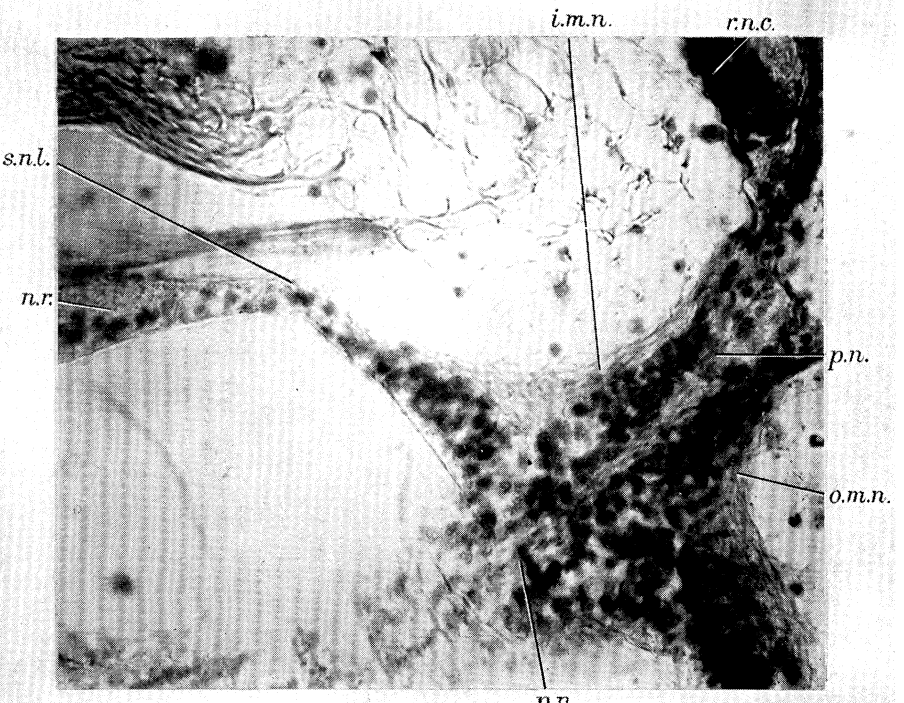

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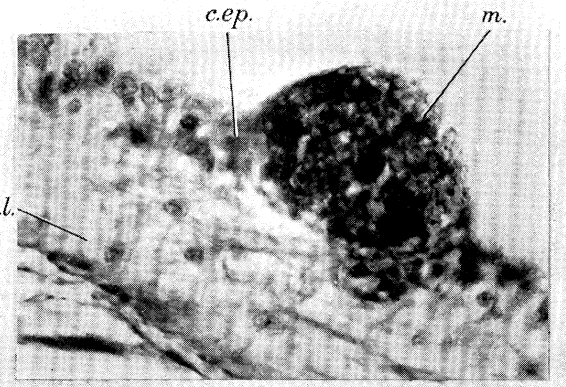

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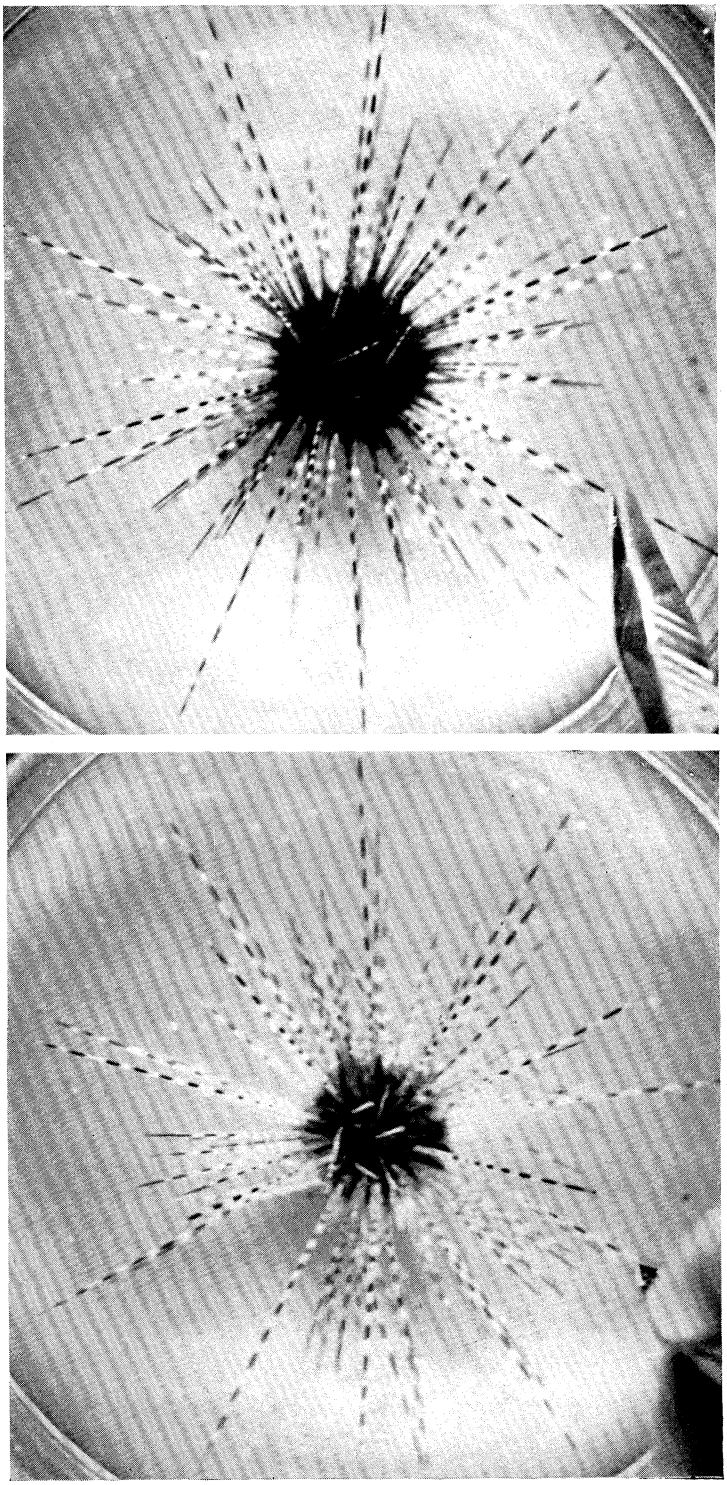

A

B

FIGURE 12. Photographs of the aboral aspect of the young individual used in experiment E 12 A (see p. 203) showing the light adapted (*A*) and dark adapted (*B*) phases. Approx. natural size. The urchin was photographed in each case in identical artificial lighting, on the same type of plate, using identical exposures and settings of the same camera. The plates were developed simultaneously in the same tank, and printed under exactly the same conditions. In the dark-adapted phase, note the white pattern developed on the test. The intervening regions of the test are also conspicuously paler than in the light-adapted phase, with the exception of the ambulacral margins (see p. 205) which stand out prominently by virtue of their persistent blackness.

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Movements of isolated organs

Technique. Simple means were used to change the light intensity. In most cases it was achieved by projecting a beam from a 50 candle-power tungsten lamp on to the surface of urchins kept in small shallow aquaria. Intensity was decreased either by casting a shadow over the urchin by means of a convenient opaque object, or by switching off the lamp. To obtain smaller differences in intensity, urchins were illuminated by tungsten lamps, with a total wattage of 240, mounted about 1 ft. from the surface of the water, and one additional 40 W lamp was switched on or off. To prevent abnormal temperature changes, experiments were always of short duration, and the final temperature of the water was never allowed to exceed 28·5° C, nor to differ from the initial temperature by more than 6° C. This range was well within that experienced under natural conditions, where, as previously reported (Millott 1953*a*), the water in which many urchins are found may reach a temperature of 32° C shortly after mid-day. No attempt was made to achieve standard conditions of temperature or light intensity.

Spines, pedicellariae and tube feet all showed responses to changes in intensity, but only those of the spines were studied in detail. The responses of the other two will be mentioned briefly however.

Tube feet. When a shadow was cast on an urchin, the tube feet that were attached by suckers quickly shortened, pulling the urchin down firmly to the substratum and halting locomotion. Any extended, but unattached, tube feet with suckers were shortened and flexed quickly toward the substratum. The ambulacral gills were flexed quickly oralwards, usually without shortening. All quickly recovered their former positions. These responses were always the same, whether the shadow was cast on the oral or aboral surface, or on the ambitus.

When the light intensity was increased some tube feet began the co-ordinated stepping movements of locomotion, while others performed movements which seemed unco-ordinated. The latter appeared to have no significance in locomotion. They extended outwards in all directions, and rapidly and successively attached and detached themselves by their suckers so long as the urchins remained in the light.

Pedicellariae. The responses to shading were much less well defined than those of the tube feet, both triphyllous and tridentate forms showing no reaction, or bending sharply towards the oral side, irrespective of the direction of the shading, or sometimes bending down the head repeatedly in a kind of 'nodding' movement, swaying in all directions, or merely contracting their stalks. Often these movements were accompanied by a vigorous snapping of the jaws. The precise relationship of these movements to changes in light intensity was not always clear, partly because the pedicellariae would occasionally perform swaying and snapping movements which appeared unrelated to any known stimulus. Again, shading caused vigorous jerks in the surrounding small spines (see below), which frequently collided with the pedicellariae, thereby not only making observation difficult, but also providing a mechanical stimulus for the pedicellariae which might have been the cause of their movement. The latter factor, however, could be eliminated by cutting short the shafts of the spines, when it was observed that the pedicellariae still responded.

Spines. To an increase in light intensity, the spines of all sizes usually responded by a jerk

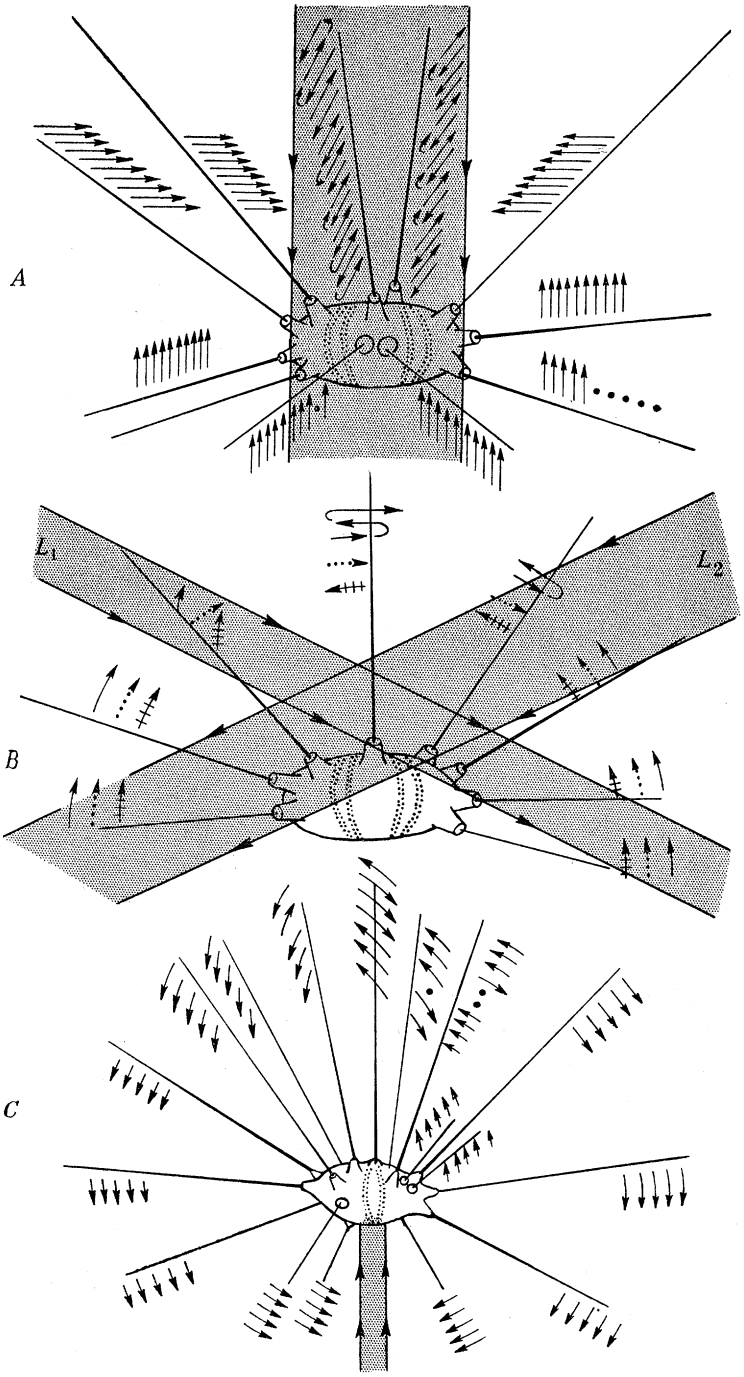

FIGURE 8. The responses of the spines to cutting off a parallel light beam projected on to the test of young individuals. The approximate boundaries of the beams are indicated in each case by the shaded area and the direction of the light, by arrows on the edges of the beam. The direction of movement of each spine in response to shutting off the beam by a shutter, is shown by the arrows alongside. The individual arrows show the results of successive experiments. Where no spine movement was observed, a dot is shown alongside. A few only of the spines are shown.

A shows the results of interrupting a beam projected on to the aboral surface of a dark adapted individual. Note the erratic movements of the spines with their axes more or less parallel to the light beam.

B shows the effect of interrupting beams directed on to the test of a light adapted young

toward the oral or aboral side, followed by a quick recovery and a more or less continuous waving, which might continue for as long as 20 min. This was sometimes followed by spasmodic waving affecting most of the spines, or merely isolated groups. Many variations were observed; thus a single jerk might be shown, or sometimes prolonged waving.

The response was not elicited in all individuals, but appeared in the majority; thus in experiments performed on thirty-two young individuals, the response appeared in twenty-seven. In general, the response was most striking in dark-adapted forms. Usually it was not possible to obtain a clear response several times in succession, and so in many cases it appeared only in the first of a succession of experiments on the same individual, though it reappeared after several hours in individuals which had not been subjected to further stimulation. No attempt was made to determine the minimum time for its return.

Finally, there was no clear-cut orientated response toward an increase in intensity of directional lighting.

The responses of the spines to a decrease in intensity of illumination were the most striking, vigorous and constant of all the responses. It was immaterial whether a sharp shadow was cast, or whether the intensity of light was merely reduced, the result was always the same, namely, a vigorous jerk of the spines on all sides towards the shadow or the light source whose intensity was diminished, followed, in some cases, by a vigorous waving of the spines, occurring continuously, or in short spasms, for as long as 60 s. The response always appeared unless the urchins had been subjected to repeated changes in light intensity.

In sharp contrast with the responses to increased lighting, they could be elicited repeatedly, and appeared after each of thirty shadows cast consecutively at 15 s intervals, and with the exception noted below, they were consistently orientated with respect to the light source. It was immaterial whether the shadow was cast on the oral, aboral or ambital region.

Spines with their long axis parallel to the beam either did not respond or, much more commonly, responded with a jerk that was erratic in orientation, swinging either to one side or the other, or making a circular movement (figure 8*A*). This figure shows that, in general, the directional uniformity of the spine response increases with the angular separation of the spine axis from that of the incident light beam, as can also be shown by observing the movement of one spine and then shifting the light source with respect to it in the manner described later.

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urchin from the right and left sides, both separately and simultaneously. The light sources are indicated by L_1 and L_2 and three successive responses of a few spines only are recorded.

The crossed arrows show the responses to interruption of the beam from lamp L_1, the dotted arrows to interruption of the beam from lamp L_2, and the solid arrows to the simultaneous interruption of both beams. Note the erratic responses of the spines situated in the path of both beams, when the latter are interrupted simultaneously.

C shows the effect of interrupting a narrow light beam directed on to the peristome of a young urchin suspended in a dish by means of a clamp. The results of five consecutive experiments are shown, except where the movement observed was erratic, in which case more responses were recorded. Only the spines whose movements were recorded are illustrated. Unlike previous illustrations, the movements of some small spines are also shown.

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That the erratic response is due to the change in intensity acting more or less equally on opposite sides of the spine was shown by a simple experiment in which an urchin was illuminated by two crossed beams of equal intensity (figure 8*B*), so that opposite sides of the central aboral spines were equally lighted. When one beam was interrupted the spines moved towards it, but when both were simultaneously interrupted the same spines moved erratically, first to one side then the other. The other spines, which were still unevenly illuminated, contained to orientate precisely.

The responses to a light beam directed on to the peristome (figure 8*C*) are particularly noteworthy, because the aboral spines showed a clear response. Since these spines were on the side remote from the light source, which emitted a very narrow parallel beam, and since due precautions had been taken to eliminate reflexions, the change in light intensity could not have affected them directly. The effect must therefore have been due to the spreading of excitation from the oral surface, or brought about by a change of light intensity inside the test. The latter is clearly possible, since the peristome is translucent in young forms. That this factor is significant may be deduced from experiments in which a narrow light beam was directed on to the ambitus of an urchin. When the light beam was interrupted, spines on all parts of the test responded. If the outer surface of the test on the side nearest the light source was scraped bare, then the spines on the opposite (intact) side still responded when the light beam was focused on to the naked area. Conduction over the outside surface of the test thus cannot be responsible for the spread of excitation, and therefore the effect appears to be due to a change in light intensity occurring inside the test, a possibility which is re-examined below.

IV. Analysis of the spine responses

Since the responses of the spines to a decrease in light intensity are so vigorous, well defined and regular in occurrence, they are the obvious choice for analysis.

Echinoderm organs such as tube feet, pedicellariae and spines have been described as showing a considerable measure of autonomy by such authors as Romanes & Ewart (1881), von Uexküll (1899), Cowles (1911), Jennings (1907) and Smith (1945). One may therefore inquire whether the change in intensity acts directly on the effectors, or indirectly via the nervous system, possibly in conjunction with a specially differentiated photoreceptive surface. In the last event, it is important to discover whether the receptive surface is localized or widespread.

The simplest method of attacking the problem is to discover the effect of eliminating the nervous system. This was done in two stages: first by eliminating the central nervous system, and secondly by eliminating the peripheral.

For the purpose at hand, the central nervous system may be considered to consist of the circum-oesophageal nerve ring which can easily be eliminated by isolating it from the rest of the nervous system by cutting around the outer edge of the peristome. This exerted an immediate effect on the stance of the spines in the oral hemisphere, which were erected so that the mouth was lifted away from the substratum. The spines also became rigid and the righting reflexes were abolished, so that if turned over on to their sides the urchins remained in that position. Despite this, their responsiveness to shading was unimpaired in vigour, and sometimes appeared exaggerated. In some experiments the spines pointed

toward the shadow with normal precision, but in others their capacity for making consistently orientated movements was seriously diminished, sometimes for as long as 3 or 4 h.

The second type of experiment was more drastic in that the eviscerated test was broken up into fragments. It was found that when the fragments included both ambulacral and interambulacral regions, the response to shading was unimpaired in the spines of both regions. Where, however, the ambulacral portions were missing, the responses could never be elicited. The same was observed in fragments which had been specially sensitized to light changes by leaving them in darkness for several hours (see p. 203). On the other hand, the responses appeared with surprising clarity in the spines carried on pieces of test measuring only $\frac{1}{2}$ by $\frac{1}{2}$ in., provided that a portion of the ambulacrum was included. Thus when a beam from a 50 candle-power lamp, projected on to an isolated fragment of test (figure 9 I) bearing only five spines, was interrupted repeatedly, the spines responded to as many as ten consecutive stimuli. The orientation of the movement of some spines was as perfect as in the intact animal, and, further, showed the same increasing uniformity of response with increasing angular separation of the spine axis and the beam, as will be clear from the figure.

It is possible by means of this type of preparation to demonstrate the validity of this relationship in another way and to show that the effect is not merely due to differences between spines. Thus the large spine *X* in figure 9 I will be seen to have orientated its response ten successive times with perfect consistency. On shifting the light beam, so that its axis lay along that of the spine (figure 9 II), the movements in response to the same stimuli immediately became erratic, as previously reported in whole urchins (p. 195).

The necessity for the inclusion of a portion of the ambulacrum at once suggests that a portion of the radial nerve may be essential for the response. That this is so can be shown by scraping the inside of the test in the above type of preparation so as to remove the radial nerve. The response is forthwith abolished. It is essential to remove all traces of nervous tissue inside the test, otherwise a response may be observed in the spines near the region which has been imperfectly denervated.

The necessity for the ambulacrum was confirmed by other experiments on dark-adapted urchins from which the peristome, Aristotle's lantern and the circum-oesophageal nerve ring had been removed and the test illuminated from one side by means of a light spot projected on to the ambitus. Spines in all regions responded to interruption of the light beam. When the experiment was repeated after the radial nerve and associated structures below the illuminated region had been destroyed by scraping inside the test, the response of the spines on the illuminated side was abolished, but those on the opposite side of the test, where the radial structures were intact, continued to respond. Now it is significant in relation to the question raised on p. 196, that when a Plasticene plug was inserted inside the test so as effectively to prevent all light passing through the perivisceral coelom, interruption of the light beam no longer called forth any response in the spines. It thus appears that changes in light intensity occurring in the perivisceral coelom are able to elicit responses in the overlying spines, provided that these spines retain their connexion with the radial nerve. Such indications of the presence of light-sensitive structures inside the test are noteworthy in view of succeeding experiments designed to test this possibility more directly (see p. 210).

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However, the possibility of direct interference with the effectors must be eliminated, since removal of the radial nerve could affect the contractile mechanism of the spine muscles, so that even though all the nervous apparatus necessary for the response to

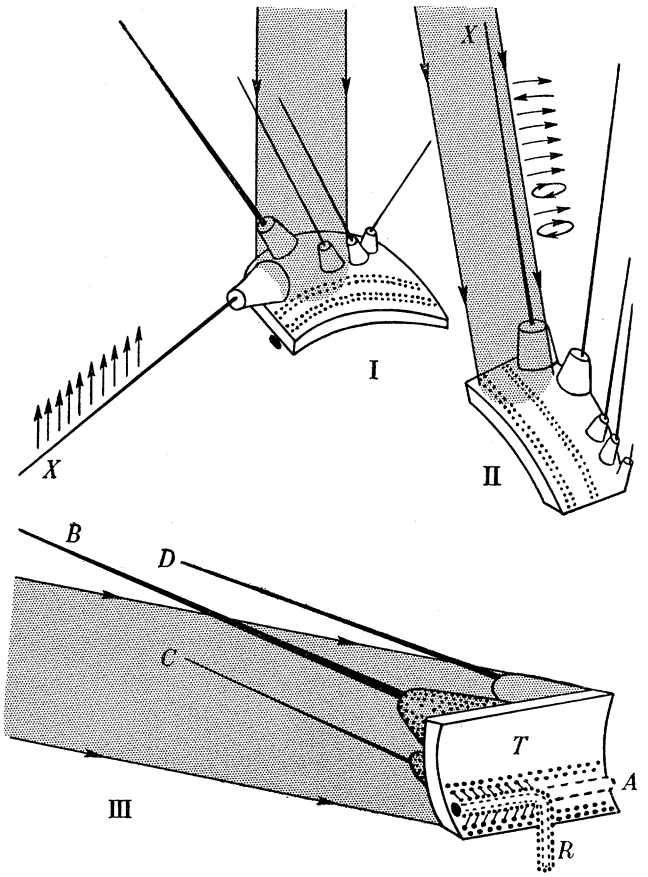

FIGURE 9. I and II. An experiment illustrating the directional responses to interruption of a narrow light beam, of a spine (X) borne by an isolated fragment of test which includes portion of an ambulacrum. The experiment also shows that the directional uniformity of the responses depends on the angle between the spine axis and the beam. The approximate width of the beam is indicated by the shaded area, and the response of the spine to ten successive interruptions is shown by the arrows alongside. In I the spine X subtends a wide angle with respect to the light beam and shows a uniform response; in II, the fragment of test has been re-orientated so that the axis of X lies along the beam, resulting in an erratic response. III. Diagram of an experiment showing that the radial nerve is necessary for the responses of the spines to shading. Interruption of a light beam (indicated by the shaded area), directed on to an isolated fragment of test (T), though producing responses in spines B and C overlying the region where the radial nerve and associated structures (R) are indicated, failed to elicit a response from spine D, situated in an area from which the radial nerve had been dissected away by cutting through the ambulacral nerves, so as to leave it hanging free as shown. A, ambulacrum, the margins of which are shown by a diagrammatic representation of the pore-pairs for the tube feet.

shading might be there, the response might not appear owing to the incapacitation of the effectors. That this does not occur is shown by the vigorous and apparently normal responses of spines to mechanical stimuli. A demonstration of this can be seen in the type of preparation shown in figure 9III, in which a small isolated portion of the test (T),

including both ambulacral (*A*) and interambulacral regions, was stimulated by interrupting a narrow light beam projected on to the outer surface. When first excised, all the spines responded normally, but when a portion of the radial nerve and associated structures (*R*) was dissected from the test as shown, by cutting through the ambulacral nerves, only the spines *B* and *C* (with stippled bases) in the area overlying the intact nervous system responded. When, however, a spine (such as *D*) in the region devoid of the radial nerve, etc., was stimulated mechanically, not only did it respond by a vigorous contraction, but the waving spread to all the other spines on the piece.

These experiments show, therefore, four things: first, that the response to shading is not the result of a direct action on the spine muscles, but involves the nervous system; secondly, that the response involves the ambulacral nerves and the radial nerves; thirdly, that although excitation following mechanical stimulation can spread over the surface of the test directly from spine to spine, excitation set up by a change in light intensity cannot spread in this way. Lastly, they show that the photoreceptive surface must be diffuse.

Since we have established that the response is at least partly nervous and involves the central nervous system, it is appropriate to apply the term 'shading reflex' to it, following in this respect the earlier terminology ('Beschattungsreflex') of von Uexküll (1900).

Two of these conclusions agree closely with what has previously been discovered in other urchins. Thus von Uexküll (1897*a*, 1899) and Holmes (1912) observed that the reactions of the spines to shading, in *Centrostephanus* and *Arbacia* respectively, are independent of the nerve ring, but dependent on the integrity of the radial nerves.

The nature of the photoreceptive surface

In considering this we may first review statements that have been made concerning it.

Sarasin & Sarasin (1887) described in *D. setosum* large numbers of blue spots on the surface of the test which they believed to be eyes, with a high level of organization, involving a lens ('lichtbrechenden Körper'), cornea and retina; but in spite of this they failed to find a nerve supply to the organs. In this interpretation they have been followed by several authors, notably Mortensen (1940) and Dahlgren (1916), and their ideas have appeared from time to time in standard zoological works. However, Cuénot (1891), on histological grounds, guardedly doubted their conclusions, while von Uexküll (1900), on experimental evidence, denied the existence of eyes in *Diadema*. Delgado y Núñez (1917) failed to find in *D. antillarum* eyes such as those described by Sarasin & Sarasin. Nevertheless, blue areas recalling the blue spots of *D. setosum* are present in *D. antillarum*, as already reported (Millott 1953*a*, *c*).

The observations of Dahlgren are especially interesting since they were made at Tortugas, on what he described as a *Centrechinus*, which, therefore, was presumably *D. antillarum*. Noting its capacity to make directional spine responses, he inferred that the blue areas were eyes, since they were the only eye-like organs present! As already indicated (Millott 1953*a*, *b*), these conclusions were reached without adequate experimental evidence, and, moreover, the histological evidence adduced by Sarasin & Sarasin is not wholly acceptable, as noted by Mortensen (1940), who, nevertheless, accepted their general conclusions. It should also be noted that Sarasin & Sarasin admitted that their histological evidence was

difficult to interpret. It is thus clear that further investigation is essential, as already indicated by Mortensen.

In so far as *D. antillarum* is concerned, the first step is to discover whether the sensitivity to light is localized in the blue areas. It must be emphasized, however, that apart from the remarks of Dahlgren noted above, it is the blue spots of *D. setosum* which have been described as eyes and I have never had the opportunity of examining them. Nevertheless, judging from the descriptions given by Mortensen (1910, 1940), the blue areas in the two species correspond closely in situation and disposition. Further, as already pointed out (Millott 1953*a*, *b*), so far as can be judged from Mortensen's description (1940), they correspond in minute structure, but it is to be regretted that Mortensen's figures of his sections do not show more, or bring out the characteristic fibrillar appearance he describes, which is so reminiscent of that seen in sections of the blue areas of *D. antillarum* (figure 5). In view of this it is reasonable to suppose that the blue areas in the two species correspond and are composed of organs of the same kind.

In determining the localization of sensitivity, the shading reflex has been used; it must be emphasized, however, that it is strictly sensitivity to changes in light intensity that is revealed by the occurrence of the reflex. Such sensitivity has been termed 'differential sensitivity' (Crozier 1918).

The experiments previously described show clearly that the photoreceptive surface is widespread, and assuming that it is superficial in position, it is appropriate in the first place to discover whether, like the blue areas, it is confined to the test, or whether the spines are sensitive.

The spines were easily eliminated by simple experiments of the type shown in figure 10, in which the spine to be tested was isolated under conditions as close as possible to normal.

The urchin was held in a clamp in a shallow dish through which sea water was circulated. The dish was blackened to minimize interference from shadows, sources of stray light, internal reflexions, etc. A large spine was introduced into a narrow-bore glass tube (*T*) as shown, which was just wide enough to admit it comfortably. The sea water which entered the dish was passed down the tube in a constant stream (dotted arrows) to prevent anoxaemia or abnormal temperature fluctuation. A 50 candle-power beam was focused by suitable lenses to a small spot occupying successive positions along the spine, starting at the tip and working towards the base. The light beam was allowed to play on one place for 30 s and then abruptly cut off. The other spines were watched carefully for a response. Each experiment was repeated three times.

It was soon evident that no response occurred unless the light spot was projected on to the base, where the spine articulated with the test, when a clear typical response was obtained each time. The experiment was repeated, using spines situated at various places over the surface, not only the normal black ones, but also the white spines of partial albinos that occur fairly frequently. The result was always the same.

There is thus no evidence to indicate that the spines are sensitive, except in the region where they articulate with the test. It may be argued, however, that clamping the urchin may well exert a powerful mechanical stimulus which could inhibit spine movement in response to shadows, unless the shading stimulus were strong enough to overcome it. Admittedly this is a weakness, but it is pointed out that interrupting a similar light beam

projected on to the naked spines of an urchin which was free elicited no response either, unless the beam fell very near the test or actually on to it.

Projecting a similar light spot on to the outer surface of the test produced an altogether different result, for wherever the spot was projected, interruption of the beam called forth a vigorous response of the spines.

It may be stated therefore that, in general, the surface of the test, but not that of the spines, is sensitive to changes in illumination, a conclusion which supports previous indications to this effect, and is fully supported by experiments described below. A similar distribution of sensitivity was also found by von Uexküll in *Centrostephanus*.

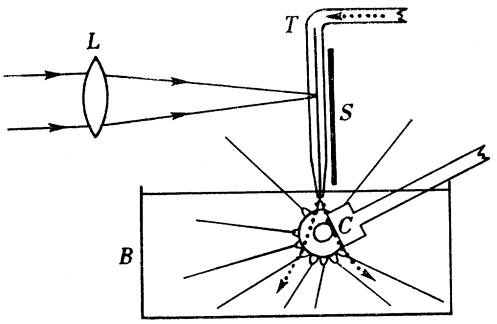

FIGURE 10. Testing the sensitivity of the spines. The lens system (*L*) concentrates a beam on a spine enclosed in a glass tube (*T*) through which sea water is circulated (dotted arrows) into the blackened dish (*B*) containing a young urchin held by a clamp (*C*). Light dispersion is prevented by the opaque screen (*S*).

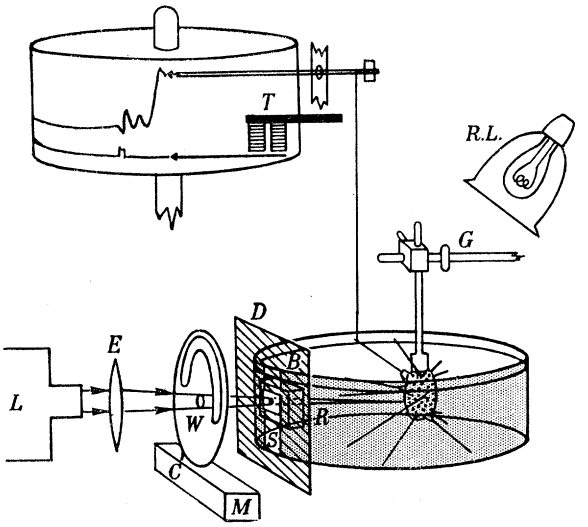

FIGURE 11. Diagram showing the technique used to obtain the localized change in light intensity and to record its effect. *B*, cork block carrying glass rod (*R*); *C*, spike making electrical contact in mercury bath (*M*) to operate signal marker on the kymograph; *E*, lens system; *G*, clamp, movable by rack and pinion and holding the urchin or portions of the test; *L*, light source; *M*, bath of mercury; *R*, glass rod; *R.L*, low wattage red lamp; *S*, transparent strip in wall of dish; *T*, signal marker.

The distribution of sensitivity over the test surface may now be examined more minutely. To do this a more refined method is necessary, since the use of a beam, such as that just described, permits too great a degree of light spread, especially as it must be passed through aerated sea water with suspended reflecting particles, air bubbles, etc.

The difficulty was overcome by using a polished glass rod to conduct the light to the surface of the urchin. The rod (figure 11, *R*) was tapered to a fine point and then cut so as to produce a flat tip. In some cases the rod was used in this form, but in others it was found preferable to cover the outside with black enamel dried and hardened by stoving, because of the exceptionally abrasive spines which sometimes damaged the polished surface, resulting in the escape of light.

In view of this it was necessary to examine the enamelled glass rods from time to time and to repolish or re-enamel them as necessary. When enamel was used it was carefully removed from the ends of the rod so as to permit light to enter or escape. Three such tubes were made, the diameter of the tapered ends being approximately 2·0, 1·0 and 0·5 mm. One of these tubes, selected according to the size of the area it was desired to illuminate, was mounted and sealed in a cork block (*B*), carried by a clamp in a rack and pinion, so that the rod could be moved up and down the inside wall of the dish. The beam from the intense low-voltage lamp (*L*) was directed on to the polished end of the rod as shown, and the urchin, held in a clamp, was then positioned so as to bring the tapered end of the glass rod as close as possible to the area of surface to be tested. By moving the glass rod, or the urchin, it was possible to illuminate a minute spot on any part of the surface and thus, by interrupting the light beam, to change rapidly the intensity of illumination in this minute area. The resultant spine movement was observed or recorded on a smoked drum.

Interruption of the light beam was achieved either by operating a hand shutter, or by means of a disk (*W*), bearing a slit and revolved by clockwork. This was arranged so that the moment the light beam was interrupted or allowed to pass could be recorded on the smoked drum by means of a signal marker (*T*), operated when an electrical circuit was closed by the contact (*C*) dipping into a bath of mercury (*M*).

As the reactions studied must necessarily begin as photochemical ones, they will ultimately depend on absorbed quanta of light energy. It is therefore essential, in accordance with the Bunsen-Roscoe law, to ensure that both the length of time that the area is illuminated and the intensity are standardized. The intensity was standardized by ensuring that no part of the illuminating apparatus was changed or moved. The so-called 'presentation time' was fixed so that the light spot was continuously projected on to the urchin for 3 min (unless otherwise stated) in each experiment, before cutting off the light beam. To prevent disturbing effects due to light carried by internal reflexion in the wall of the dish, or to shadows resulting from movements outside the dish, etc., its internal surface was enamelled a dull black, a strip (*S*) being left transparent so as to receive the end of the glass rod. Stray light was minimized by using screens such as that shown at *D*.

With these precautions and provided that manipulation over the top of the dish was avoided, light changes at the surface of the urchin could be confined to those occurring at the tip of the glass rod. In view of the length of the glass tube and the circulation around it of water ensured by aeration, it was considered that heating effects were negligible.

By day the intensity of light in the laboratory was usually sufficient to permit the observation of spine movements, especially as the young forms have spines banded with white (figure 12, plate 16), but by night the urchin was illuminated by a dim, red lamp (*R.L.*), the tips of the spines being whitened to facilitate observation. It was found by experiment that, in general, urchins showed no responses in this red light unless they had been kept in total darkness for several hours, when some became more sensitive. Under these circumstances it was essential to avoid casting shadows, or moving the lamp, but such urchins were generally avoided.

When, by this method, the surface of the urchin was tested for sensitivity to localized changes in light intensity, it was found that the results depended on whether the animals were in a dark-adapted or a light-adapted condition. Thus when the surface was scanned

in normal laboratory daylight with the illumination used it appeared completely insensitive, but when it was scanned at night it was found to be sensitive to changes of the same magnitude. The following may be quoted as examples:

TABLE 1

experiment no.	diameter of urchin (cm)	time of day	lighting conditions	region scanned	result
SE1	2·25	9.30–10.30 a.m.	laboratory daylight	tested at twelve places around the ambitus extending over three ambulacra and two interambulacra	all places tested insensitive
		5.30–7.20 p.m.	fading daylight to darkness with red lamp	as above	six places sensitive and six insensitive
		9.05–10.00 p.m.	darkness with red lamp	as above	all twelve places sensitive
E6A	1·8	5.30–6.45 p.m.	fading daylight	tested at sixteen places extending across oral surface	three places sensitive
		6.50–7.30 p.m.	darkness with red lamp	as above	all places sensitive
E12A	*2·0	5.00–5.40 p.m.	fading daylight	tested in eleven places extending across the aboral region in an interambulacrum from the periproct to the ambitus	all places insensitive
		8.30–9.10 p.m.	darkness with red lamp	as above	all places sensitive
		6.50–7.30 p.m.	darkness with red lamp	tested in nine positions along the aboral region of an interambulacrum from the periproct to the ambitus	all places sensitive

* The light- and dark-adapted phases of this urchin were photographed and are shown in figure 12, plate 16.

The foregoing experiments show that sensitivity to localized changes in light intensity changes with the onset of darkness. Thus in experiments SE1 and E12 A, all the sensitive places were recorded in the half of the experiments which were conducted at night or when the daylight had considerably faded. Further, experiment SE1 shows that the number of sensitive places increases progressively as the urchin remains in darkness. Again, experiment E6 A shows that places which were insensitive to predetermined changes in intensity at 5.30 p.m. had become sensitive to such changes some 80 min later.

The cause of this is not clear. It might be found in the photoreceptor system, since such systems generally show enhanced sensitivity after a sojourn in darkness; moreover, Hess (1914) has shown that the tube feet of Astropectinidae show adaptive sensitivity, becoming more sensitive in darkness. Alternatively, it might be due, at least in part, to changes in the pigmentary effector system. The latter is clearly a possibility in view of the well-marked colour changes already described, resulting from changes in superficial melanophores (see figure 12 and Millott 1952). The suggestion is strengthened by the fact that the change in colour occurs at the same time as the increase in sensitivity. Thus in experiment E6 A above, it was observed that when the urchin began to respond it had become pale in

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colour. It is also significant that one of the pigments present in *Diadema*, already provisionally identified as a melanin (Millott & Jacobson 1951), when dispersed would certainly screen any subjacent light-sensitive elements with resulting loss in sensitivity, a matter which will be re-examined later.

This association of change in sensitivity and change in colour forms a striking parallel with that already described as existing between the latter and changes in phototactic response, which, furthermore, could be explained on a similar basis.

Two conclusions therefore emerge, first, that sensitivity is not localized in the blue areas and that the entire surface of the test is sensitive to changes in light intensity; secondly, that the degree of sensitivity varies inversely with the intensity of the background lighting.

It now remains to discover whether all parts of the test surface are equally sensitive.

Making the fundamental assumption that the reflex under study depends ultimately on a photochemical reaction, in accordance with the Bunsen–Roscoe law, the photochemical effect required to produce a threshold response (represented by E_t) will be a product of the time (t) that the light is allowed to act on the photoreceptive surface ('presentation time') and the intensity (i), viz. $E_t = it$.

Relative sensitivity can thus be determined on the basis of the minimum intensity or presentation time necessary to elicit a response. With the apparatus then available it was only possible to vary the time with accuracy. Accordingly, a series of experiments was performed to test the relative sensitivity of all parts of the surface by discovering the minimum presentation time necessary to elicit a spine response for each area. The method used was to illuminate each area by means of one of the glass rods already described for progressively lengthening intervals of time, starting at 0·1 s, until a response of the spines was obtained on suddenly cutting off the light.

In general, the apparatus was the same as that described in preceding experiments, but certain additional precautions were taken. The urchins used were all as nearly as possible the same size (about 4·0 cm across the ambitus) and showed similar depth of pigmentation under the same lighting conditions. The latter is especially important in view of the relation between colour and sensitivity already discussed, but the size is also significant in this connexion, since melanin accumulates outside the chromatophores as the urchins age. To eliminate as far as possible interference from melanin, all the urchins used were thoroughly dark-adapted by keeping them in darkness for $2\frac{1}{2}$ h before subjecting them to testing. A standard interval of 3 min was observed after cutting off the light beam in each experiment before beginning the next, in order to allow restitution and to obviate the effect of fatigue (such an interval would appear to be more than adequate in view of previous findings, see p. 195). The same illumination intensity and glass rod (0·5 mm in diameter at the tip) were used throughout, and by introducing the glass rod between the spines, the tip was placed as close as possible to the surface of the test without touching it.

Under such circumstances mechanical stimulation was unavoidable, and it was considered advisable to wait 3 min to allow recovery before starting an experiment.

The most significant point in favour of this method is that the use of dark-adapted urchins, and the precisely localized intensity changes made possible by the use of the glass rod, permitted strictly localized stimulation of the blue areas on the test, for it is singularly

fortunate that these areas become easily identified in dim lighting. As previously reported (Millott 1952, 1953*a*, *b*, *c*), the blue areas become white in such light, forming a characteristic pattern, which is conspicuous in young forms such as those used in these experiments. The results are shown in table 2.

TABLE 2. DIFFERENTIAL SENSITIVITY OF OUTER SURFACE OF TEST

presentation time (sec)	ambulacrum, between margin and centre experiment no.				ambulacrum, centre experiment no.				ambulacrum, margins experiment no.				interambulacrum, outside white pattern experiment no.				interambulacrum, white (≡blue) area* experiment no.			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	.	.	.	.	.	.	.	.	.	×	.	.	.	.	.	.	.	.	.	.
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3	.	.	.	.	×	.	.	.	.	.	×	.	.	.	.	.	.	.	.	.
5	×	.	.	.	.	.	×	.	.	.	.	.	.	.	.	×	.	.	.	.
10	.	.	.	.	.	.	.	.	.	.	.	×	.	×	×	.	.	.	.	.
15	.	.	.	.	.	.	.	.	.	.	.	.	×	.	.	.	.	.	×	.
20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
30	.	.	.	.	.	×	.	.	.	.	.	.	.	.	.	.	.	.	.	×
60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	×	.	.
120	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
180	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

× indicates the time at which the spine response first appeared (see p. 204).
* See p. 204.

It is thus clear that the various regions of the surface differ considerably in sensitivity. In order of decreasing sensitiveness the regions are as follows:

ambulacral margins →ambulacral centre→dark areas of interambulacrum
→white areas of the interambulacrum.

The results confirm previous indications that the entire test surface is sensitive to changes in light intensity, and show further that the areas which are blue in strong light, far from being the localized seat of such sensitivity (as one would expect if they were eyes), are the least sensitive of all areas of the test. Thus, in so far as the spine responses to shading of *D. antillarum* are concerned, there is no experimental evidence to suggest that the blue areas act as photoreceptors, and therefore, if they have this function at all, they are not significantly involved in the animal's most striking and characteristic response to light. Their function is thus obscure, but I have already suggested that they may well be iridophores (Millott 1953*a*, *b*), though much further investigation is called for. Their function in *D. setosum* thus becomes even more in need of reinvestigation.

The results also show another relationship between colour and sensitivity, in that the parts of the surface showing least change in colour are the most sensitive and vice versa. It is most significant that the ambulacral margins which bear the tube feet are distinguished by the depth of their pigment as well as their relative sensitivity. In the dark-adapted phase of young forms they become very conspicuous because they alone on the test retain the blackness so characteristic of the light-adapted phase (see figure 12*B*, plate 16). Examination of histological preparations shows that the deposition of melanin

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is exceptionally heavy in the walls of the tube feet (both in those with suckers and those without, see figure 1). The distinction is not so well marked in older forms owing to the progressive and widespread deposition of pigment already noted (Millott 1952, 1953*a*). Whether the pigment is entirely melanin, or whether other pigments are involved, must remain for the moment uncertain, but it may be mentioned that considerable quantity of at least one reddish pigment occurs in *Diadema* (Millott & Jacobson 1951; Millott 1953*a*), but its properties and distribution are imperfectly known and are now being investigated.

The high degree of sensitivity of the tube feet agrees with the findings of workers such as Crozier (1914), Hess (1914) and Moore (1922), who described the tentacles of *Holothuria* and the podia of *Asterias* and *Astropecten* respectively, as especially sensitive to light. It is also noteworthy that in *Astropecten* their sensitivity increased after a sojourn in darkness.

In *Diadema*, the correlation between the degree of sensitivity and the depth of colour seems at first anomalous, for when the overall sensitivity of light-adapted and dark-adapted phases is considered, dark colour implies diminished, not increased, sensitivity. If, however, it be imagined that the most sensitive areas require the protection of a screen of absorptive pigment, then it is only to be expected that they would be darkest, and furthermore, show the least change in colour.

We have been assuming, however, that the change in sensitivity which occurs with change in colour is entirely due to differing degrees of dispersion of at least one light-absorbing substance in superficial melanophores, and we have taken no account of possible changes in the light-sensitive system already envisaged (p. 203). Also, it must be remarked that all the evidence of a diffuse sensitivity to localized changes in intensity has been obtained from dark-adapted forms; it still remains to be shown that such sensitivity exists in light-adapted forms, and that the relative degree of sensitivity of the various areas of the test is the same as in the dark-adapted phase.

Both of these points are covered by the series of experiments on forms maintained in a light-adapted condition, described below, in which any effect of superficially situated pigment is largely eliminated by stimulating the superficial tissues from the inside of the test. Under these circumstances, the test being translucent and the melanophores superficial, any light-sensitive elements beneath them can be stimulated without the interposition of large amounts of pigment. In order to ensure light adaptation, the method of producing the localized changes in intensity was altered and is shown in figure 13.

A beam from the lamp (*L*) was projected on to the inside of a fragment of test with both radial and interradial portions. By means of the stand and clamp with rack and pinion (*M*), a piece of wood (*P*) was lowered into the beam so as to cast a sharp shadow (*S*) approximately 1·0 cm in diameter on to the inner surface of the test. At 15 s intervals, the edge of the shadow was lowered 4·0 mm measured on the scale (*R*), and the resulting movements of one of the spines was recorded on a smoked drum. The piece of test was cut and mounted so that the ambulacral structures (*A*) formed a horizontal band across the middle, with the result that as the shadow advanced, it shaded successive areas measuring approximately 10×4 mm^2, at first in the upper interambulacrum, then in the intervening ambulacrum and finally in the lower ambulacrum. To ensure that the piece of test was fully light-adapted before beginning an experiment, it was placed for 1 h beneath a 40 W lamp. The results are shown in figures 14 and 15.

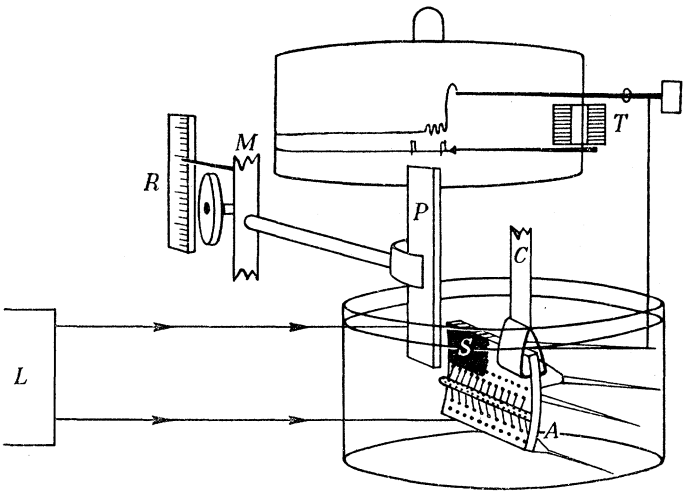

FIGURE 13. Diagram showing the method of recording spine movements produced by casting a shadow on to the *internal* surface of a light adapted fragment of test. *A*, ambulacrum of isolated fragment of test; *C*, clamp holding fragment of test; *L*, light source producing beam indicated by arrows; *M*, stand with rack and pinion for lowering *P*; *P*, piece of wood lowered into beam; *R*, scale indicating position of *P*; *S*, shadow cast on internal surface of test; *T*, signal marker indicating each successive advance of shadow.

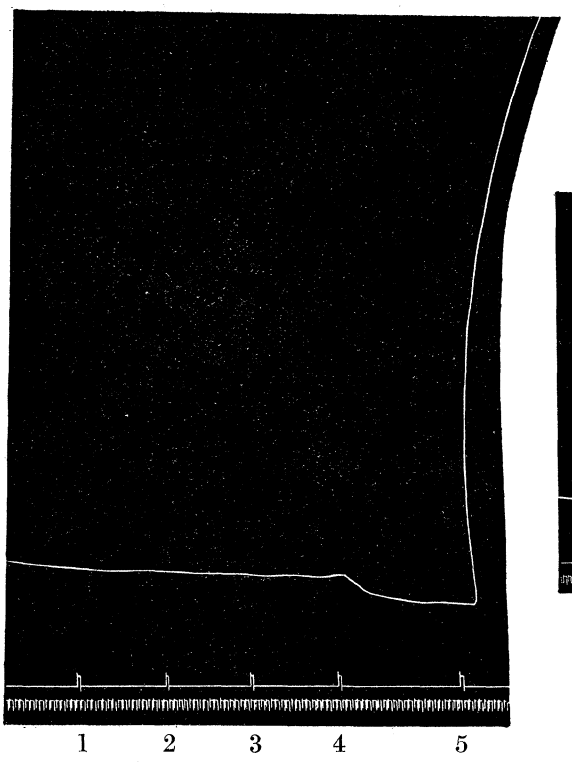

FIGURE 14

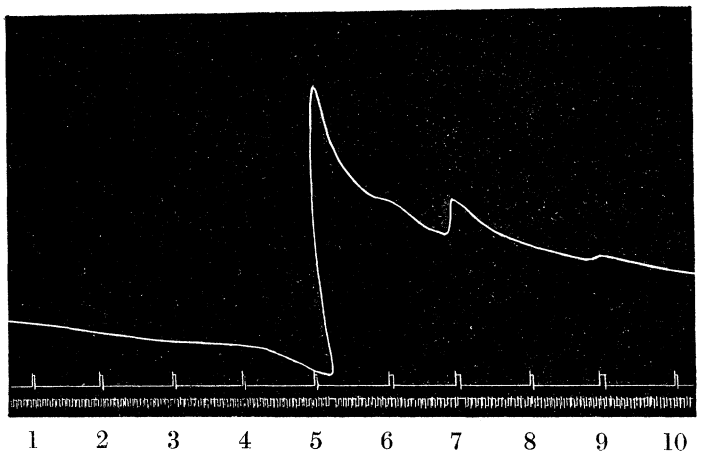

FIGURE 15

FIGURES 14, 15. Spine movements resulting from casting a shadow on the inside of a test fragment. In these, and all succeeding figures, the record should be read from left to right and the time scale is in seconds. The signals mark the successive advances of the lower edge of the shadow. Signals 1–3 mark the passing of the shadow across the upper inter-ambulacrum; at signal 4, the shadow reached the upper margin of the ambulacrum and at signal 5, the shadow reached the lower margin of the ambulacrum. In figure 15 signals 6–10 mark the passage of the shadow across the lower inter-ambulacrum.

So far as the interambulacra are concerned, the effect of the shading was variable, in some experiments (figure 14) no response resulted, but in others (figure 15) a response was obtained. Shading of the ambulacral margin always called forth a response, which was sometimes so extensive that the end of the recording lever was swept off the drum (figure 14). The overall picture must therefore be interpreted as showing a sensitivity in all parts of the interambulacrum and a much greater sensitivity at the margins of the ambulacrum. The experiments also demonstrate the directional character of the response, but unfortunately this does not appear strikingly on the records in view of the limitations of the recording apparatus. Thus in the experiments shown in figures 14 and 15, the spine attached to the recording apparatus was situated very near the upper margin of the ambulacrum. As the edge of the shadow reached this point, the spine moved upwards towards it, but the friction between the writing point of the recording apparatus and the smoked-drum paper was too great to allow the fall of the writing lever under its own weight to be recorded satisfactorily, since a very light lever had to be employed. When the shadow had advanced to the lower margin, the spines moved downwards, causing the writing point on the lever to rise against its own weight, giving a more satisfactory tracing.

These results show that the overall sensitivity of the test surface exists in the light-adapted as well as in the dark-adapted state, and further, that the relative insensitivity of the light-adapted state is due to the interfering effect of overlying pigment as suggested above rather than to any profound change in the photosensitive system, though it is still possible that such a change may occur. It cannot, however, be more than a minor factor in so far as the observed change in sensitivity is concerned.

It is now permissible to suggest that the concentration of melanin in the chromatophores, in rendering the animal more sensitive in dim light, will enable it to perceive fainter shadows, and by conferring an added awareness of the approach of potential enemies will increase its chances of survival in a dimly lighted environment. This must follow automatically, since the spines erected towards a shadow are poisonous.

Having eliminated the so-called 'eyes', in order to learn more concerning the photoreceptive surface, it is now appropriate to examine more closely the differential sensitivity reported on p. 205. The indications of this obtained by stimulation of the inside and the outside of the test are mutually confirmatory, but in view of the fact that, when stimulated from the outside, the margins of the ambulacra were found to be more sensitive than the centre (p. 205), we should determine whether this is also the case with internal stimulation.

For this purpose the simple method of employing an advancing shadow is clearly insufficiently accurate, and so it was necessary to resort to the glass-rod method. The apparatus employed was the same as that described on p. 201. The end of the glass rod was placed as closely as possible against the internal surface of a fragment of test which included the radial structures mounted horizontally as before. In view of the white internal test surface, it was necessary to take an added precaution against light spreading from the end of the glass rod and affecting other portions of the surface by reflexion. This consisted of enclosing the end in a tiny hood of insulating tape, projecting very slightly beyond the end of the tube, and which could be pressed firmly to the test. With such a device no noticeable spreading of light could be detected by the eye during experiments, even when they were conducted in total darkness.

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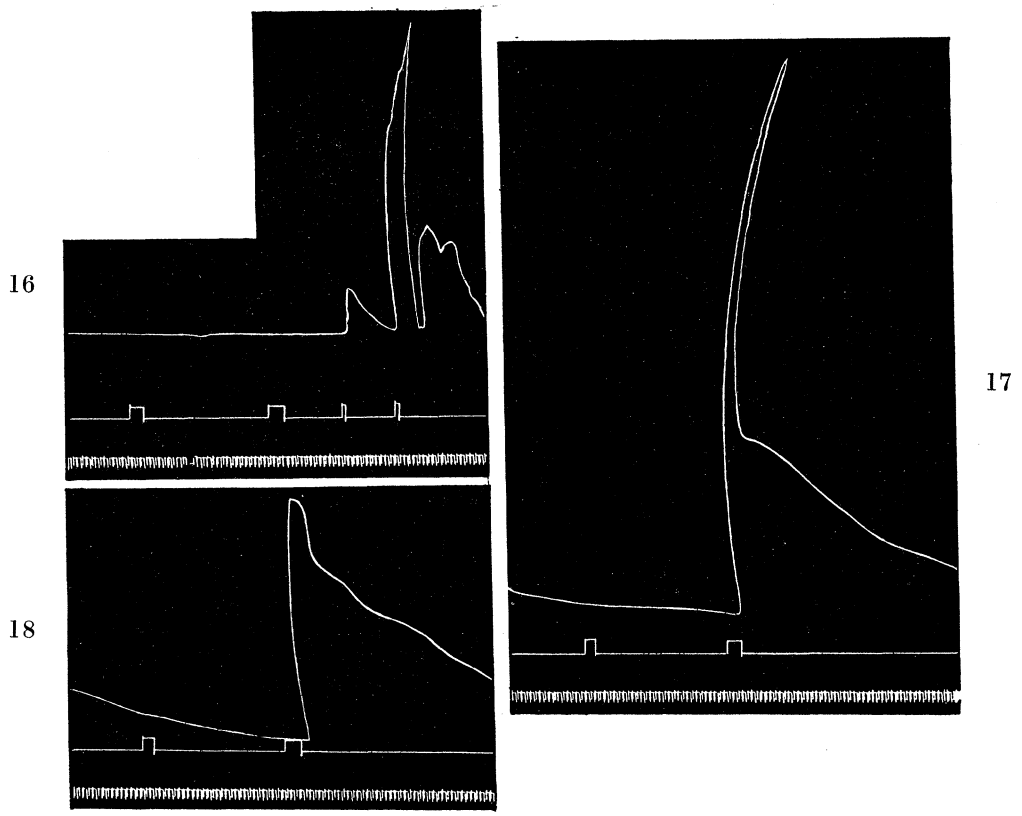
The results, embodied in figures 16, 17 and 18, show that if the relative size of the response to a stimulus that is the same in all cases be used as an index to the degree of sensitivity, then the ambulacral margins are more sensitive than either the ambulacral centre or the interambulacrum. Thus the distribution of sensitivity over test fragments is the same whether they be stimulated internally or externally (see p. 205). In view of this



FIGURES 16, 17, 18. Movement of a spine borne on an isolated fragment of the test in response to localized changes in light intensity occurring inside the test.

FIGURE 16. Effect of a change in intensity localized in the region of the interambulacrum bordering on the ambulacral margin. First signal, light beam passed through conducting rod; second signal, light cut off. As a control, to test the sensitivity of the piece as a whole, at third signal a 40 W lamp immediately overhead was switched on; at fourth signal, the lamp was switched off.

FIGURE 17. Effect of a change in intensity at the margin of the ambulacrum. At first signal, the beam was passed along the glass rod; at second signal, it was cut off.

FIGURE 18. Similar experiment with the same fragment, but with the change in intensity localized in the centre of the ambulacrum.

the question arises whether the light-sensitive elements are situated inside or outside the shell, especially since during the foregoing experiments it was observed that in dark-adapted forms a considerable measure of light could pass through the substance of the test, and there were indications that light-sensitive elements may exist below it (see p. 197). The question can be settled by eliminating the participation of any light-sensitive elements that might exist below the test, either by removing them or by rendering the test opaque.

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In the first type of experiment the technique just described was used, but, in addition, the inside surface of the interambulacral region of the fragment of test was thoroughly scraped until the inner layers had been removed. Casting a shadow on to the inside surface of the scraped region called forth a vigorous response of the spines, provided that the radial nerve and associated structures had not been damaged. It is clear, therefore, that the photoreceptive surface is, at least, not entirely inside the test.

The converse experiment, performed by casting a shadow on the outside surface of an area of test from which the spines and superficial skin had been removed, showed that a response of the spines in the adjoining intact areas was obtained only when the shadow fell upon the ambulacra. There must, therefore, be photoreceptive elements below the surface layers, but only in the ambulacral areas.

This conclusion is supported by the results of localized stimulation of the radial structures inside fragments of test. The technique used was the same as that described on p. 201, but the glass rod was adjusted to illuminate an area of the radial nerve and associated structures. To eliminate the possibility of light spreading through the test and affecting photosensitive structures on the outer surface, a fragment of test was used from which the spines and superficial skin had been scraped off above the radial nerve. Thus in the experiment recorded in figure 19, the area of the radial structures which was subjected to a sudden change in light intensity was about 2·0 mm in width, and in the second part of the experiment shown in record *B* it was situated within an area of denuded test, measuring about 100·0 mm^2. The spine attached to the recording apparatus was situated about 25·0 mm away from the stimulated region. A clear spine response followed a change in intensity, and tracings *A* and *B* show that although removal of the surface tissue leads to a somewhat diminished response, there is no doubt that the radial structures inside the test are photosensitive.

Thus, considered collectively, the foregoing experiments indicate that the photosensitive surface is superficial in the interambulacrum, but in the ambulacrum it is, in part at least, situated beneath the test. This at once raises the question as to whether the sensitivity of the ambulacra may not be attributable entirely to the radial nerve and associated structures within the test. Clearly this is possible, since the test is translucent, and changes in light intensity occurring at the outer surface might be sufficient to affect structures lying apposed to the inner, despite the extensive deposit of melanin in the ambulacra. Alternatively, both superficial and deeply seated photosensitive elements might be involved.

Both questions can be answered by testing the sensitivity of the inner and outer surfaces of test fragments such as those described on p. 208, with a glass-rod stimulator, after making the test opaque by artificial means. It was found that a most effective way of preventing the passage of light through the test was by inserting a piece of thin metal foil measuring about $1{\cdot}5 \times 2{\cdot}5$ cm between the radial nerve and the inner surface of the test as shown in figure 20.

This necessitated cutting through some branches of the radial nerve and radial water vascular canal. To avoid disturbing effects due to the reflexion of light from the surface of the metal foil, it was covered with a mat black enamel. It was also necessary to scrutinize the foil carefully by a lens for cracks, which might spoil the experiment.

The result of subjecting the radial structures separated from the test by the blackened

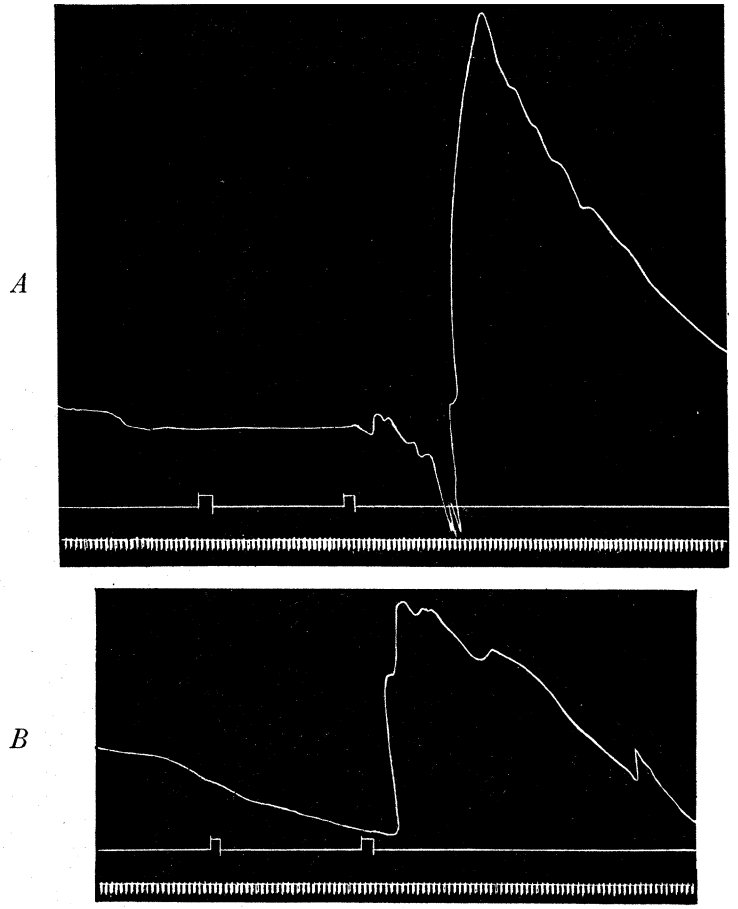

FIGURE 19. Experiment illustrating the movement of spines as a result of stimulating the radial nerve and associated structures by a localized change in light intensity. *A*, effect of stimulating the radial nerve on a spine 25·0 mm distant. First signal: light admitted; second signal: light cut off. *B*, repeat of the above, after the outer surface of the test for an area of 100 mm^2 around the end of the light conducting glass rod, had been thoroughly scraped of all tissue.

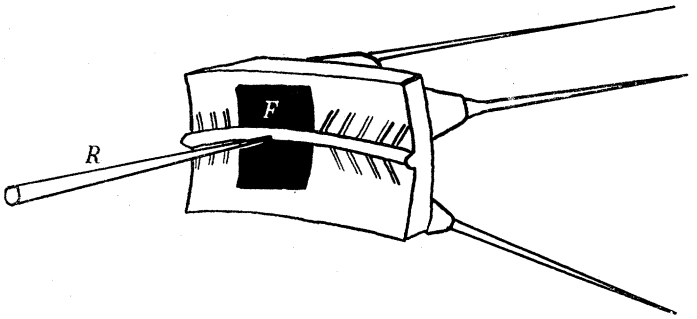

FIGURE 20. The technique used for rendering opaque a portion of the test below the radial nerve to show that the latter and its associated structures are light sensitive. *F*, piece of blackened metal foil inserted behind a section of the radial nerve; *R*, glass rod used to conduct light to the radial nerve, etc., above the metal foil.

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foil to a localized change in light intensity is shown in figure 21. Only about half of the experiments gave such unequivocal results, but this is perhaps not surprising, in view of what we must presume to be the severe disturbance occasioned by such an operation.

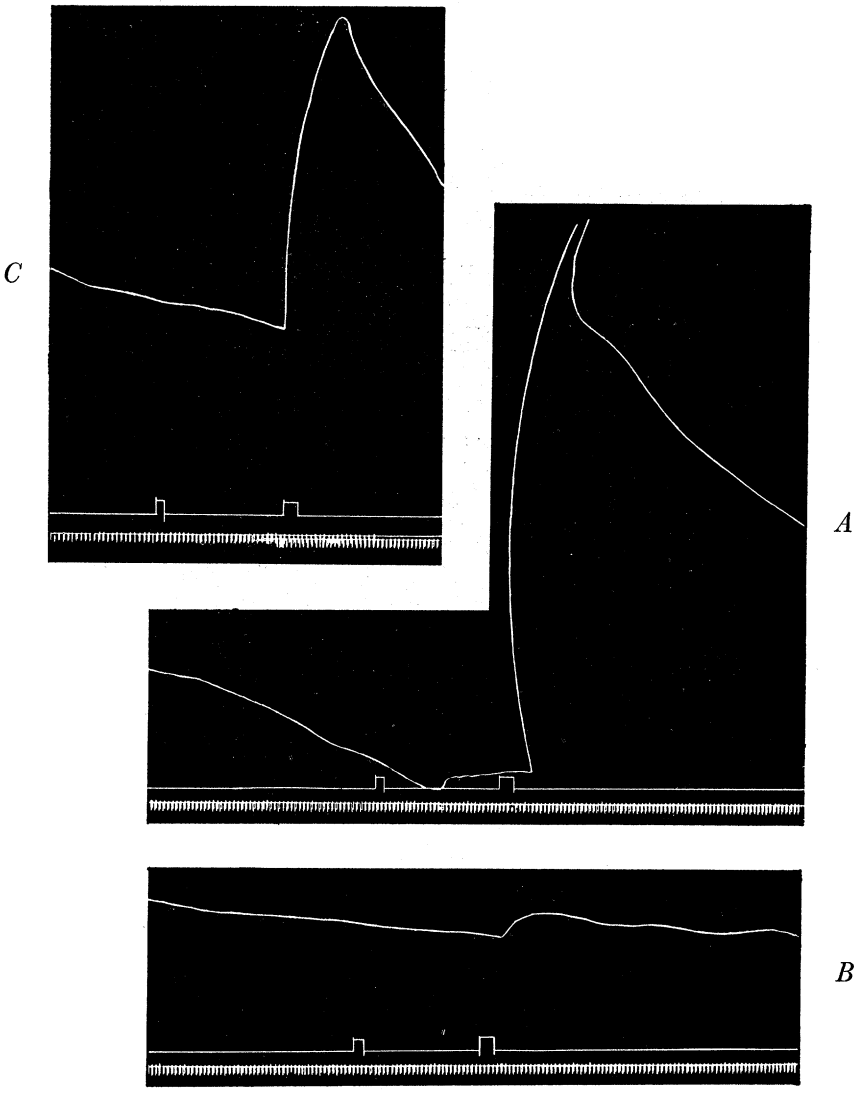

FIGURE 21. Experiments showing that the radial nerve and its associated structures are sensitive to changes in light intensity. In each case a minute area (about 1·5 mm in diameter) of the radial nerve of an isolated piece of test, was stimulated by cutting off a light beam directed on to it by a solid glass rod (figure 20, *R*) as described on p. 210. Movements were recorded in a spine situated 10·0 mm away from the region of the radial structures stimulated. At first signal, the light beam was directed on to the radial nerve; at the second, it was cut off. *A* shows the results using a normal test fragment. *B* shows the results obtained by repeating the same experiment on the same fragment, immediately after a piece of blackened metal foil (figure 20, *F*) had been inserted behind the radial nerve. *C* shows the result of repeating the experiment on the same test fragment one hour after inserting the foil.

Allowing the preparation to rest for an hour or so after the operation sometimes improved the result, as can be seen by comparing figure 21 *B* and *C*. The positive results are significant, however, and confirm previous indications of the existence of radially disposed light-sensitive elements below the test.

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The result of testing the sensitivity of the outer surface of such test fragments is shown in figure 22. Stimulating the skin by cutting off the light from an area about 2·0 mm in diameter, situated opposite the inserted metal foil, elicited a clear reflex in a spine 10·0 mm away. We may therefore conclude that photosensitive elements also exist on the outer surface of the ambulacrum.

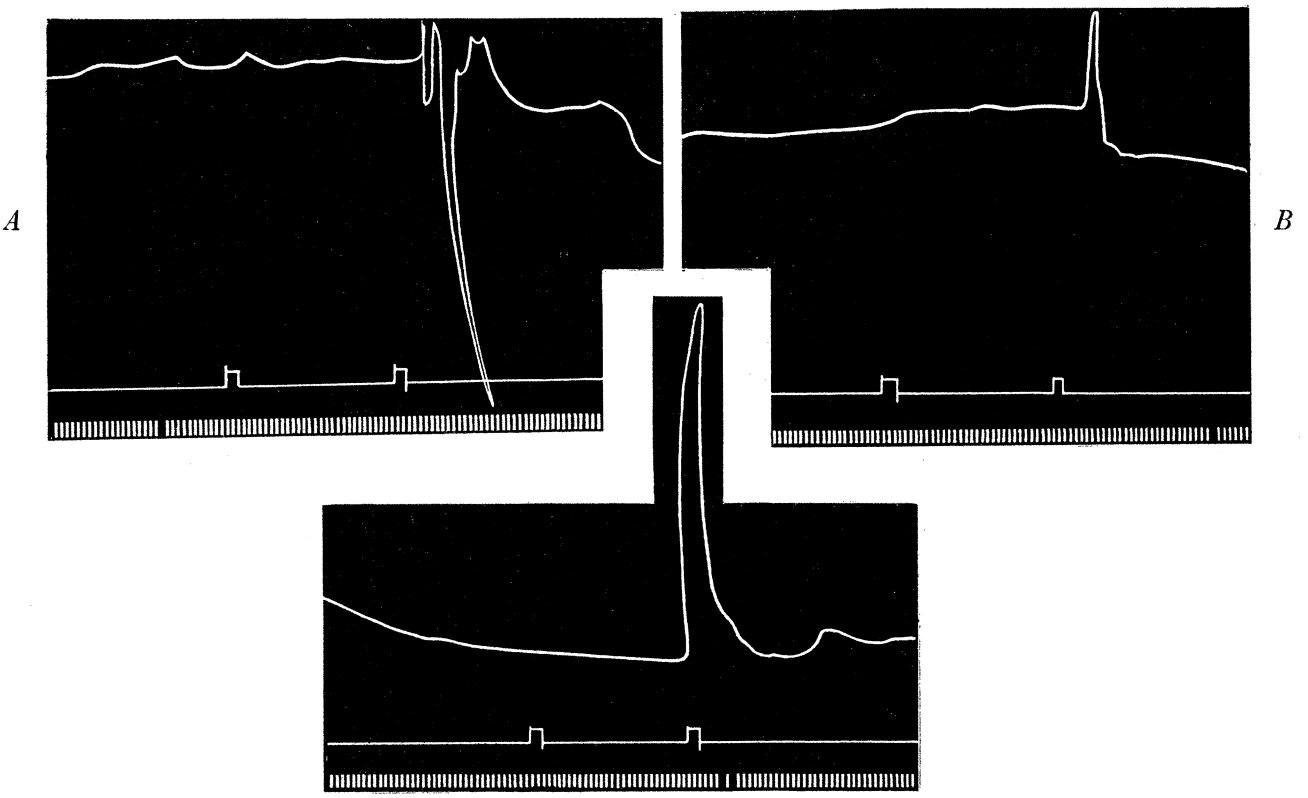

FIGURE 22. Experiments showing that light sensitive elements exist both inside and outside the test. Stimulation of the radial nerve and associated structures and the outer surface of an isolated fragment of test, was accomplished by cutting off a light beam carried by a glass rod, the end of which, adjacent to the piece of test, was 1·5 mm in diameter. The spine attached to the recording apparatus was about 10·0 mm from the stimulated area. The experiments were performed in daylight. first signal: light on; second signal: light off. *A*, Stimulation of the radial nerve. Note waving of the spine which followed. *B*, same preparation, stimulated a few minutes later by switching an overhead 40 W lamp on and off. *C*, effect of stimulating the outer surface of the same preparation, opposite a piece of metal foil inserted below the radial nerve as shown in figure 20.

Thus in addition to the entire skin covering the test, internal radial structures are sensitive to changes in light intensity. On the basis of experimental evidence obtained here we are not entitled to be more specific than this, but bearing in mind what is already known of the distribution of nerve elements (p. 188), it is highly suggestive that their presence is invariably accompanied by sensitivity to light, and yet no morphologically differentiated receptors have been discovered.

That nerve elements themselves are sensitive to light changes, though by no means an

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inescapable conclusion on the evidence obtained so far, is at least strongly indicated. Such a tentative conclusion would not only fit in with the observed distribution of sensitivity, but it would also account for its variation in the light- and dark-adapted phases and its relation to the change in colour, for, as will be clear from the photomicrograph in figure 4, plate 15, the elements of the superficial nerve layer, as well as those of the radial nerve, lie below the melanophores, and therefore the amount of light they receive must be affected considerably by the degree of dispersion of the melanin.

Discussion

The reactions to light and changes in its intensity of *D. antillarum*, described in the preceding pages, have their counterpart in other echinoids as well as in other echinoderms. Observations on any of these forms are, as yet, very incomplete, and an attempt at a detailed comparison would thus be both misleading and unprofitable. This is especially so in the case of the responses to steady directional illumination described on p. 189, and no attempt has been made to analyze them in relation to the classical hypotheses of Ray-Verworn, Leob, Jennings, etc. (see Mast 1938), or to classify them in terms of the schemes proposed by Kuhn, and those more recently formulated by Fraenkel & Gunn (1940).

However, in view of their special interest, the observations of Dahlgren (1916) may be specifically noted, for he mentioned the spine response described in the preceding sections, in a *Centrechinus* found in Tortugas. We must presume this to be *D. antillarum*. Von Uexküll (1900) also described essentially similar responses in two allied species from Dar-es-Salaam, and presented some evidence concerning the pathways of the nerve impulses involved. He claimed that the evidence showed the existence of different pathways for impulses resulting from a decrease in light intensity and those resulting from an increase.

With reference to the reflex responses of the spines to shading, in contrast with what has been found here, von Uexküll stated: 'Hier ist keine Rede davon, dass dem Reflex Nicht die nöthige Zeit zur Entwickelung gegönnt wird, denn der Reflex trit immer im ersten Moment der Beschattung auf oder gar nicht.' In *D. antillarum*, shading may sometimes call forth a prolonged response. A response to increased illumination is also described in the following terms: 'Auf plötzlich einfallendes Sonnenlicht antworten denn auch die Seeigel mit einer ganz allgemeinen Stachelbewegung, an die sich erst später die Fluchtbewegung anschliesst. Diese allgemeine Stachelbewegung, die zum Theil ein wirkliches Rotiren werden kann, ist auch am immobilisirten Thier und schliesslich an jedem Schälenstuck sichtbar.' My observations on *D. antillarum* do not agree with those of von Uexküll in so far as the delayed movement out of a light beam is concerned. Whilst this reaction may sometimes be delayed and follow a general spine movement, this sequence is by no means invariable; indeed, very often the movement out of the light beam begins immediately, especially in dark-adapted forms, a retreat into the shade being accompanied by a general waving of the spines.

The results of the preceding investigation have a special interest which is two-fold: first, they show that a diffuse photoreceptive surface exists in echinoids; secondly, there are indications that the nervous system itself may be photosensitive.

Indications of a diffuse photosensitivity of the body surface are not new, but a review of the literature shows that, in general, the methods employed to show it have not been

beyond reproach, in so far as the stimuli were not strictly localized. There often remains the uncertainty as to whether the light did not affect an area greater than the one assumed to be affected, especially where experiments were performed on animals immersed in water. Under such circumstances it was not always possible to be sure that the dispersed light rays did not affect some specialized photoreceptor situated at a distance. The use of a technique such as that described on p. 201 ensures that changes in light intensity are strictly localized, and the results of scanning the surface of the urchins indicate that a truly diffuse photosensitivity exists. Some of the photosensitive elements have been shown to exist in the tissues covering the test, and we may therefore refer to such sensitivity to light as 'dermal' in the sense previously used by many workers.

In higher animals at least, the perception of light is commonly assumed to be the function exclusively of photoreceptors. Usually they are aggregated to form morphologically differentiated organs which vary from simple 'eye-spots' to complex eyes. A survey of the literature, however, reveals the somewhat surprising fact that even in animals with relatively high levels of organization such differentiation is not always apparent, and the photoreceptive areas may not be defined, even histologically. Coupled with this there is evidence, which will be referred to again, that many of the so-called 'eye-spots', to which the photosensitivity of an organism has often been attributed, are not specially sensitive to light. It appears, therefore, that in such cases a diffuse photosensitivity exists, which is perhaps comparable with that of the simpler Protozoa, devoid of optic organelles, and attributable to a fundamental photosensitivity of protoplasm (see Mast 1941; Mast & Stabler 1937).

However, it is essential to bear in mind that the lack of histological differentiation may be apparent rather than real, due to inadequate histological technique, and that much further investigation is required, both in *Diadema* and many of the other instances where such sensitivity has been described, before stronger assertions may be made.

With this reservation we may note that members of almost all phyla have been described as showing a general dermal sensitivity to light in the reviews given by such authors as Willem (1891), Dubois (1892) and Nagel (1896).

It is among echinoderms, however, that the phenomenon has been most widely observed, and Cuénot (1891) regards the dermal light sense as general in these forms. Despite the large number of observations, the idea of a dermal photosensitivity has not been universally accepted. Thus, for example, Romanes & Ewart (1881) attribute the photic reactions of asteroids and echinoids to the stimulation of discrete 'eye-spots'.

Some investigators have described the co-existence of specialized photoreceptors and general dermal photosensitivity. In echinoderms this has been claimed for *Asterias* and *Solaster* by Plessner (1913), who suggested that the dermal light sense may serve for the perception of intensity changes, while the ocelli serve as receptors for phototactic responses.

It remains to be discovered whether a special photosensitive pigment is involved in the dermal light sense. Crozier (1915*a*) has claimed that this is so in *Holothuria*. Such knowledge would have great importance, not only in leading to a more complete understanding of photochemical processes in living matter, but also in providing sadly needed indications of the course of evolution of photoreceptors. It is possible that the photochemical processes of echinoderms may have a special significance in this connexion, because of the affinities

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between these forms and the lower chordates. Wald (1945) has already suggested that information concerning photochemical processes at this level may have implications parallel to those forthcoming from the well-known researches of Meyerhof and his school in the field of muscle metabolism (see Meyerhof 1930). The discovery of a remarkably light-sensitive echinoid such as *D. antillarum* clearly opens up new possibilities in this direction.

The remaining point of special interest, namely, indications of a direct effect of visible light on nerve elements, unlike the first, has been reported but few times. Prosser (1934) and Welsh (1934) have described photosensitive regions of the central nervous system in *Cambarus* and *Panulirus*, while von Fritsch (1911) and Scharrer (1928), working on *Phoxinus*, and Young (1935*a*, *b*) working on lampreys, have described effects that appear to depend on photochemical processes occurring in nerve. The recent suggestions made by Parker, Hendricks, Borthwick & Jenner (1952), in connexion with photoperiodism and the results of earlier investigators such as Klüver (1944) and Benoit & Ott (1946), are also noteworthy. They suggest that the central nervous system in various birds and mammals may be affected by visible radiation, and reference is made to Klüver's discovery of a porphyrin showing a strong fluorescence at 6250 Å in avian and mammalian central nervous systems. It is also worth noting at this juncture that the common association of highly absorptive pigments such as melanin with nerve tracts, around which it may sometimes appear in quantity, would receive an added significance if the nerve itself were light sensitive (see Young 1935*a*, *b*).

Observations involving ultra-violet rays have also been reported by workers such as Booth, von Muralt & Stämpfli (1950), who showed that irradiation of myelinated nerve brought about changes in rheobase. They interpreted some of their results as due to a selective photochemical action on substances associated with excitable membranes. Earlier observations on the effect of visible light on membrane permeability are also suggestive, since excitatory phenomena may well depend ultimately on changes in permeability. These observations have been reviewed by Heilbrunn & Mazia (1936).

In the echinoderms generally, the superficial position of much of the nervous system implies that such parts may well be readily accessible to light, and bearing in mind the fact that, in *Diadema*, the distribution of photosensitivity, both inside and outside the test, has been shown to correspond almost exactly with the distribution of nerve elements in the superficial nerve layer, radial nerves and their branches, the implications are obvious. Further, if direct excitation by light were possible, since so much of the echinoderm nervous system is superficial, it would account for the prevalent dermal photosensitivity in these forms.

However, such a conception is somewhat unorthodox, for it is usually assumed that light acts upon morphologically specialized photoreceptors, and where photosensitivity has been shown to exist, investigators generally have been at pains to ascribe it to morphologically differentiated receptors of some kind, if not in the form of eyes, at least in the form of eye-spots or ocelli. This has often led to inferences being made concerning the function of such organs, without adequate evidence, as already noted (see p. 199), and the blue spots of *Diadema* have been no exception.

It thus appears that we must be alive to the idea of a photoreceptor differentiated biochemically, but not necessarily histologically. This is essentially a succinct restatement

of the conclusions attained many years ago by Nagel (1896) and Crozier & Arey (1919), in relation to the light-sensitive elements in the skin of molluscs, and later by Welsh (1934) concerning the similarly sensitive elements in the central nervous system of crayfish. Bearing in mind the unique opportunities for research into the fundamental processes of vision presented by such instances, it is indeed surprising that these observations should have attracted so little attention.

In passing, it may be noted that Dubois (1892), working on *Pholas*, proposed a novel interpretation of the effect of light on the nervous system, which he believed to be indirect and due to mechanical stimulation of the nerve elements, following contraction of superficial cells brought about by the direct action of light on them. Such an idea would appear, at first, to offer a possible explanation of the spread of spine responses in *Diadema*, in view of the work of Kinosita (1941), who showed that the spreading of impulses in the superficial nervous system of echinoids involved mechanical stimuli set up by the stretching of the tissues on one side of the spine base as a result of spine movement. If, therefore, a localized stimulation of spine muscles by the technique described on p. 201 be imagined to result in a local contraction, then the mechanical stimulus which follows could excite contraction in a neighbouring spine, and so spread excitation in the manner described by Kinosita. However, experiments such as those described on p. 210, in which the internal radial structures were stimulated in an area denuded of spines, makes it clear that excitation cannot spread entirely in this way.

In the preceding investigation, it is sensitivity to decreases in intensity of illumination that has been studied in detail, and in the present discussion we have so far made no distinction between this and other forms of photosensitivity. It is obvious, however, that it constitutes a special case of differential sensitivity. It has been recorded widely in invertebrates, for example, in annelids (Nicol 1950) and molluscs (Dubois 1892; Nagel 1896; Crozier 1918). Many instances have been recorded in echinoderms, notably among echinoids (von Uexküll 1900; Mangold 1909; Holmes 1912; Hess 1914), asteroids (Cowles 1911; van Weel 1935) and holothurians (Pearse 1908; Crozier 1914, 1915*a*, *b*, 1917; Hilton 1923). Among these instances it is significant that some react to both increase and decrease, some only to an increase, but most only to a decrease. Even when there is a reaction to both, that given to shading is often the more vigorous, as in *Diadema*.

Thus, in reviewing this behaviour, one is struck by the general resemblance in the relations between stimulus and response to those shown by certain optic nerves and retinas. Hartline (1938) has shown that in the various types of nerve fibres composing the optic nerves of the frog, bursts of activity (as judged by trains of spike potentials) may follow either cessation, or cessation and onset, of illumination of the eye. Prosser (1934) also has described 'on' and 'off' bursts of activity in the optic nerve discharge pattern of crayfish. The general similarity between these bursts of activity and the spine responses of *Diadema* to rapid changes of light intensity, reported on p. 195, is noteworthy.

The similarity between the general organization of the dermal photoreceptor system of *Diadema* and that of a complex vertebrate or arthropod eye, extends further than this, for in the latter other changes, such as pupil reactions and photomechanical movements of retinal pigment, occur when the light intensity is altered. The pigment movements are of particular interest, since by them melanin is dispersed and concentrated so as to affect the

amount, or the pathways, of light falling on the photoreceptors (Parker 1932). Such pigment movements are reminiscent of the changes occurring in the melanophores of *Diadema*. Again, as in *Diadema* (Millott 1952), the movements of retinal pigment may show a diurnal rhythm as reported by Kiesel (1894), Demoll (1911), Welsh (1930, 1936) and Brown (1951). The resemblance extends still further, for many complex photoreceptors contain a reflecting pigment, forming a tapetum, for the purpose of reflecting and diffusing light of low intensity over the receptive surface (Parker 1932). This too has its counterpart in *Diadema*, in the iridophores, for, as already suggested (Millott 1953*a*, *b*), these structures, by virtue of their reflecting properties, may diffuse light over the photoreceptive surface. This factor may materially increase the capacity of the urchin to respond to the necessarily small intensity changes that could occur when the environment is dimly lighted.

Thus in *Diadema*, what appear to be adaptive changes occur, allowing activity of the photoreceptive surface to be spread over much of the 24 h. However, though the migration of retinal pigment is usually explained as a part of a process of adaptation, aiding vision under varying conditions of lighting intensity, caution has been urged in accepting this view unreservedly, notably by Arey (1915, 1916) and Detwiler (1943). Nevertheless, the pigment movements in the skin of *Diadema*, with its extensive superficial, and seemingly otherwise unprotected photoreceptive surface, exposed to full tropical sunlight by day and yet remaining sensitive to shadows by night, undeniably receive their most satisfying explanation on this basis.

These points of comparison between specialized photoreceptors and general skin sensitivity provide even more justification for a reversion to the study of the dermal light sense as an avenue to an eventual understanding of the fundamental processes of photosensitivity.

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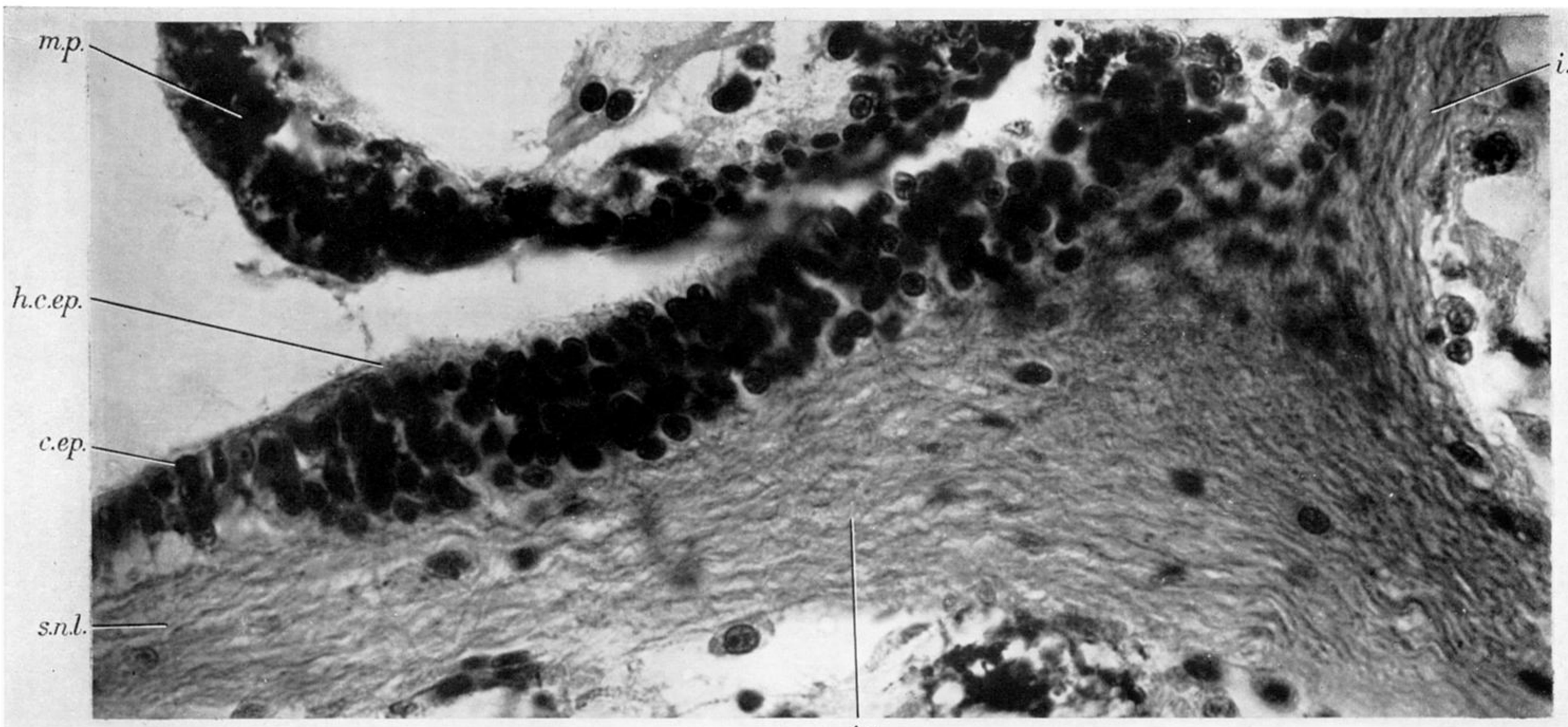

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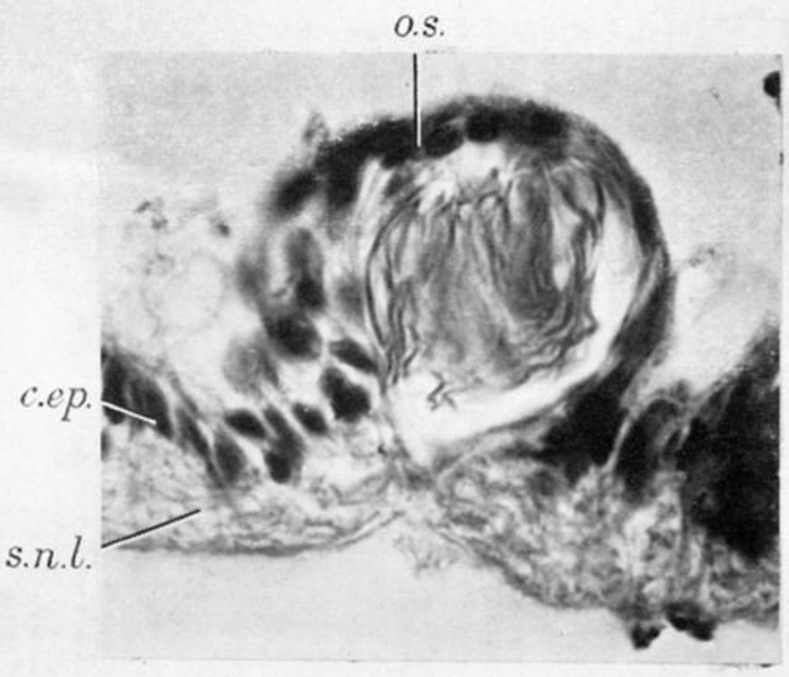

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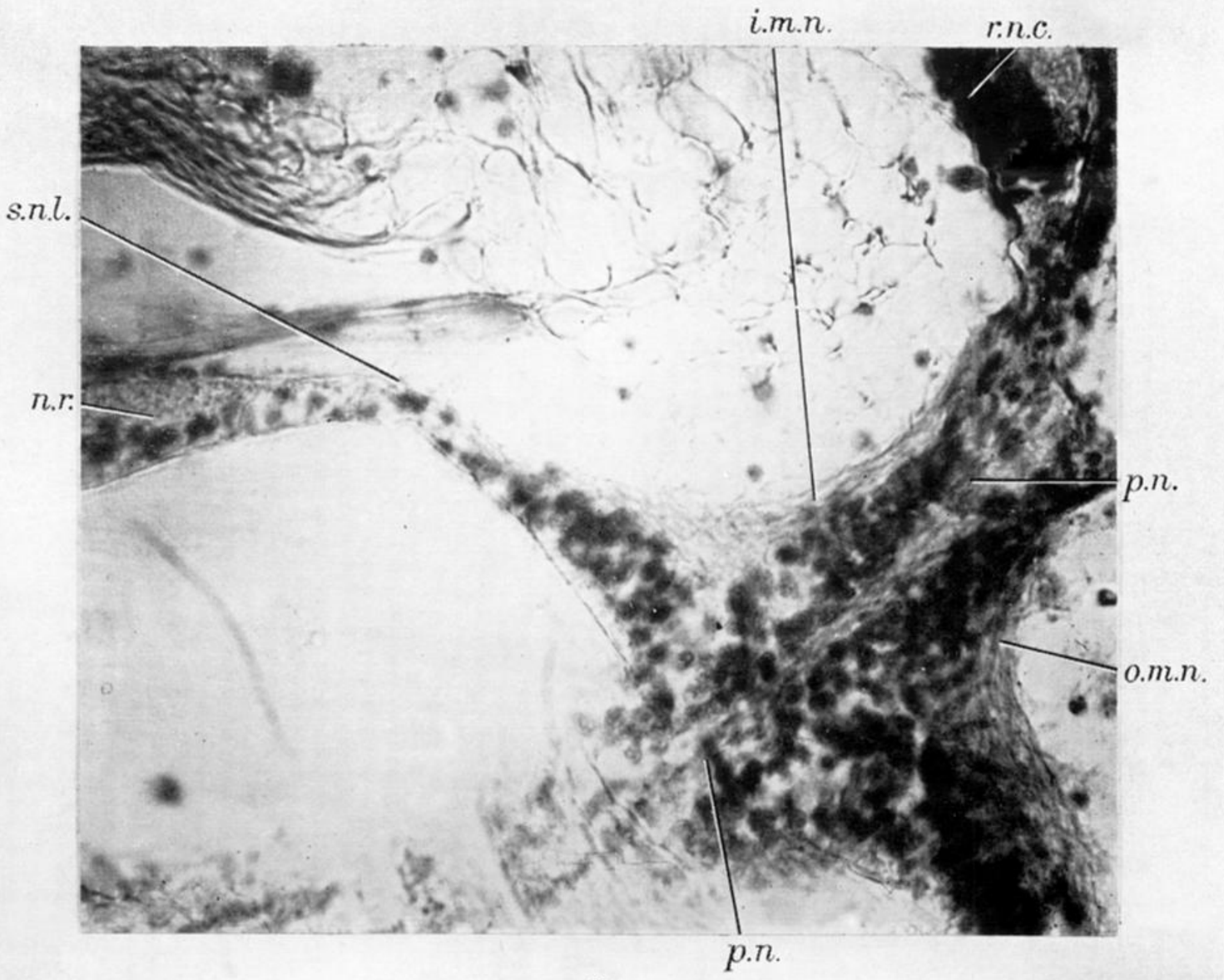

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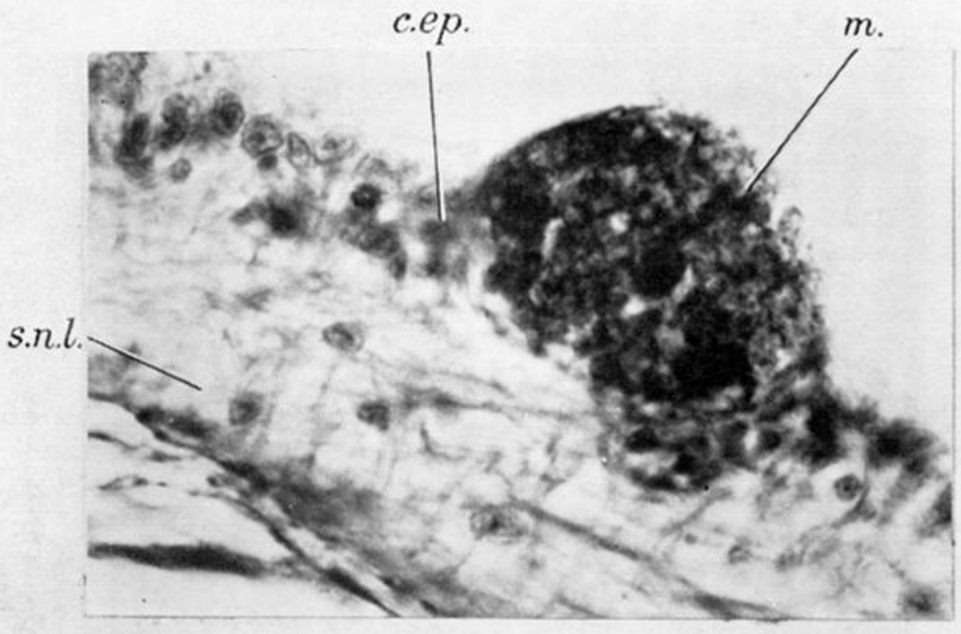


4

Description of plate 15

Figure 2. Photomicrograph of portion of a transverse section through a radius of *Diadema antillarum*, showing the integumentary nerve, (*i.n.*) emerging at the surface to join the superficial nerve layer (*s.n.l.*) (see p. 188). Note that at the point where the integumentary nerve joins the superficial layer (=ambulacral margin), the covering epithelium is thickened (*h.c.ep.*). The extensive deposition of melanin (*m.p.*) in the walls of the tube foot is also evident. Prepared from a young individual, measuring about 1·3 cm across the ambitus. Fixed in Bouin's fluid; stained by Masson's method for the argentaffine reaction; counterstained in Mallory's triple stain $10\mu \times 660$.

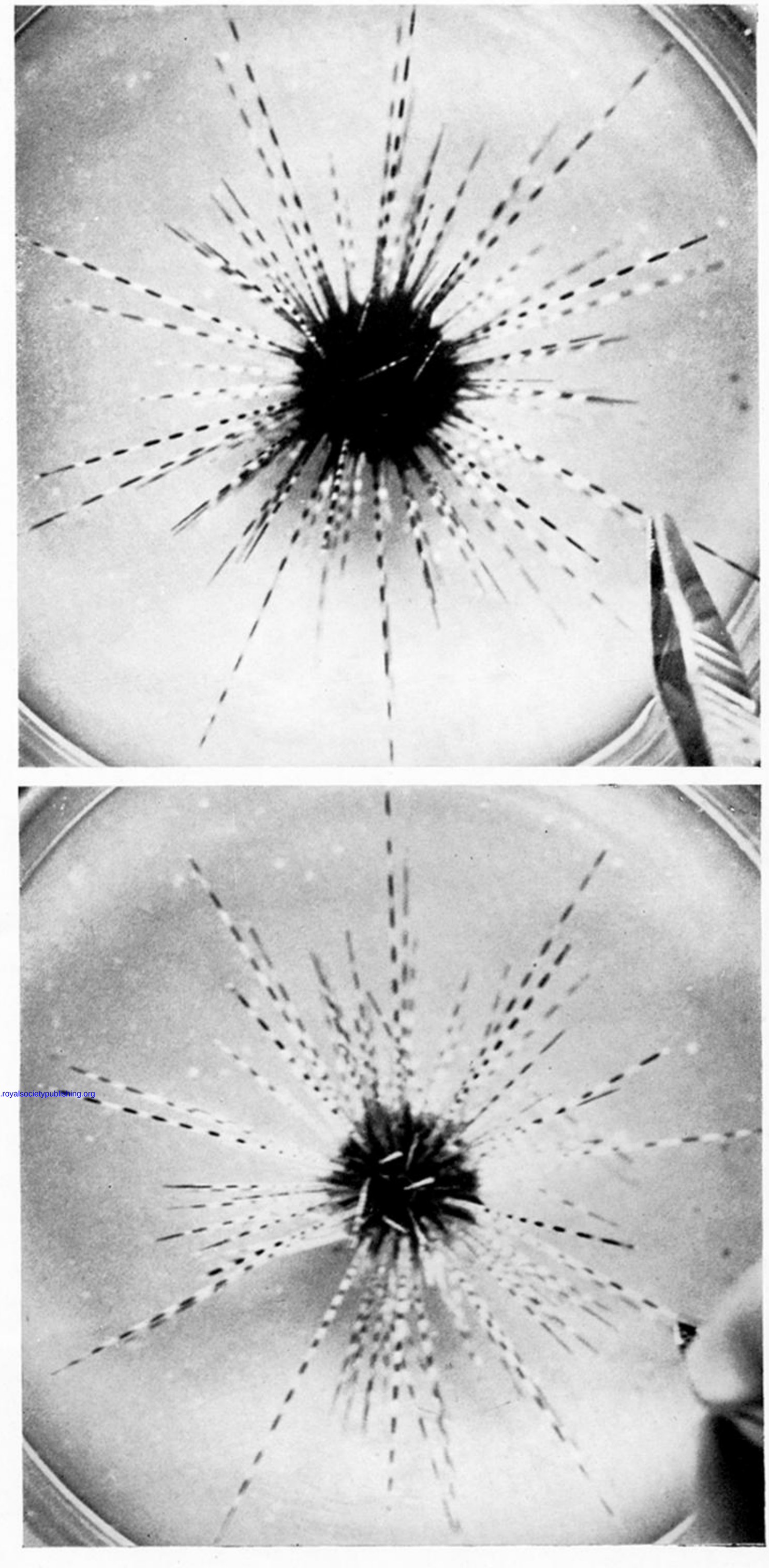
Figure 3. Photomicrograph of a portion of a transverse section through a radius of *Diadema antillarum* showing the origin of the podial (*p.n.*) and integumentary nerves from the radial nerve (*r.n.c.*). The integumentary nerve whose radial and interradial margins are shown respectively by the letters *i.m.n.* and *o.m.n.*, joins the superficial nerve layer (*s.n.l.*) (see p. 188), which is thickened to form a ring (*n.r.*) around each spine base. Prepared from a very young individual measuring 2·5 mm across the ambitus. Fixed in Bouin's fluid; stained in Delafield's haematoxylin and eosin. $5\mu \times 500$.

Figure 4. Photomicrograph of portion of a section through the base of a spine, showing the relations between the superficial nerve layer (*s.n.l.*) and the melanophores (*m.*) in the covering epithelium (*c.ep.*) (see p. 204). From a young individual measuring about 1·0 cm across the ambitus. Fixed in Bouin's fluid; stained in Mallory's triple stain. $10\mu \times 660$.

Figure 5. Photomicrograph of a section through an iridophore (=so-called 'eye') of *Diadema antillarum* (see p. 200), cut in a plane normal to its outside surface (*o.s.*). From a young individual measuring about 1·3 cm across the ambitus. Fixed Bouin's fluid; stained by Masson's method for the argentaffine reaction, counterstained in Mallory's triple stain. $10\mu \times 660$.

Lettering

c.ep. epithelium covering surface of test.
o.s. outside surface of iridophore.
Other letters as in figure 1.



A

B

FIGURE 12. Photographs of the aboral aspect of the young individual used in experiment E12 A (see p. 203) showing the light adapted (*A*) and dark adapted (*B*) phases. Approx. natural size. The urchin was photographed in each case in identical artificial lighting, on the same type of plate, using identical exposures and settings of the same camera. The plates were developed simultaneously in the same tank, and printed under exactly the same conditions. In the dark-adapted phase, note the white pattern developed on the test. The intervening regions of the test are also conspicuously paler than in the light-adapted phase, with the exception of the ambulacral margins (see p. 205) which stand out prominently by virtue of their persistent blackness.