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THE PHOTOSENSITIVITY OF ECHINOIDS

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I. INTRODUCTION

It has long been known that echinoderms respond to light. All classes have received attention but asteroids and particularly echinoids have attracted most of it. Photosensitivity has largely been inferred from behaviour since echinoderms have proved singularly intractable for electrophysiological studies. Larvae have received little attention, indeed almost all of it has been directed to adults.

Echinoids show their photosensitivity in a variety of responses: morphological and physiological colour change, in the responses of particular effectors such as spines and podia and possibly in reproductive activity. In life such responses may be integrated into complex activities such as those of covering behaviour or locomotion. In the quest for simplicity and understanding several investigators (and the writer is no exception) have sometimes dismembered such elaborate responses by studying the responses of effectors isolated singly or in small groups. It is as well to emphasize at the outset a fact which has not always been kept in mind, namely that in so doing mere fragments of behaviour as well as of the animal are being studied. Receptors no less than effectors can interact and the simplicity which may emerge can be misleading, for it is partly the creation of the investigator due to his methods. More will be said of this in relation to von Uexküll's famous and time-honoured aphorism of the *Reflexrepublik* as applied to echinoids.

In recent years these photic responses have been reviewed several times: by Millott (1966a) and in wider context by Millott (1957b), Steven (1963), Yoshida (1966) and Millott (1968). The successive reviews reflect the progress of the work, and since the last, though impetus has for the moment slowed down, there has been further work. This, together with the reflexions on earlier work in echinoids cast by later research on the photosensitivity of molluscs, means that some measure of reappraisal is possible.

For the purpose of this account the term " photosensitivity " will be used as implying sensitivity to ultra-violet as well as to visible radiation.

II. Movements of the Whole Animal

For almost a century echinoderms have been favourite targets for the study of so-called elementary patterns of behaviour. In general, analysis has been grossly inadequate, partly because it has been so incomplete, partly because it has been based on assumptions which are a legacy from the over-simplifications prevalent in the early part of the century and partly because in interpreting responses the animals have been relegated to a level of organization which is so lowly as to belie their true nature. The reactions of various echinoids to light have been described in scattered, brief accounts. It is generally agreed that the reactions of whole urchins are profoundly influenced by their physiological state, a complex and intangible factor that involves sensory adaptation (see below).

Diebschlag (1938) reported that *Psammechinus miliaris* (Gmelin) overturns when suddenly illuminated on the oral side. This was categorized as a dorsal light reaction despite the sheer inappropriateness of the term as applied to an animal which has undergone such radical changes in orientation in its development.

The accounts of phototaxis are disparate, workers using various species have recorded positive, negative and variable responses with respect to the light source (see Yoshida, 1966). *Centrostephanus longispinus* (Philippi) and *Diadema setosum* (Leske) are described by von Uexküll (1900a) as avoiding light. This is substantiated for the latter by Thornton (1956) and Magnus (1967) who describe migration at sunset of urchins which sought the shade of crevices during daylight, but they display a lesser tendency to seek shade after light adaptation. Such migrations recall those of clypeastrids reported by Mortensen (1948).

Some of the disparity could be the result of adaptation. Simple experiments on *Diadema antillarum* Philippi (Millott, 1954) showed that

the sign of the response depends on the light intensity to which urchins had been subjected before the experiment so that at first they appear to seek light intensities to which they had become accustomed, but the sign of the response does not persist. In all, the results suggested the existence of an optimum intensity which changes in correspondence with a photosensory system that undergoes adaptation. It is not clear, however, that this is the only factor involved. One additional complication stems from the colour change (see below); juveniles at least changed colour during the course of some experiments. This could influence their photosensitivity. This possibility will be considered again (p. 25). However, according to Sharp and Gray (1962) *Lytechinus variegatus* (Lamarck) shows no such adaptation, remaining positively phototaxic in artificial light for hours at a time, though it is negatively phototaxic to direct sunlight and to wavelengths shorter than 295 nm.

A general negative phototaxis is described in *Arbacia punctulata* (Lamarck) by Holmes (1912) and confirmed by Sharp and Gray (1962). Holmes claims that the animals quickly adapt and become irresponsive, but their responsiveness returns after chemical or mechanical stimulation. It also requires the oral nerve ring.

Yoshida and associates (see Yoshida, 1966) report that *Temnopleurus toreumaticus* (Leske) ceases to show positive phototaxis after a sojourn in darkness, but that this behaviour is progressively restored on illumination. They also showed that separation of a radial nerve from the nerve ring abolishes positive phototaxis in that sector. In animals from which most of the aboral hemisphere had been removed, leaving only one or two intact ambulacra, the latter took the lead in locomotion when they were illuminated. In darkness, however, it was the incomplete ambulacra which took the lead. Yoshida attempts to explain this by a formal scheme which invokes the existence of excitatory and inhibitory influences in each radial nerve of which the latter normally predominates unless the nerve is illuminated when inhibition is suppressed. Excitation then becomes the over-riding influence so that the illuminated ambulacrum takes the lead. Removal of the greater part of each remaining radial nerve means that much of their inhibitory influence is lost, so that in darkness most of the inhibition is on the side of the intact ambulacra and consequently movement is toward the opposite side.

Very little is known of the responses of larvae. Fox (1925) noted that plutei migrate downwards in light and upwards in darkness. Yoshida (1966) studied the behaviour of developmental stages of *Hemicentrotus pulcherrimus* Barnard from the early gastrula onwards, which aggregate at certain light intensities. He used microdensitometry

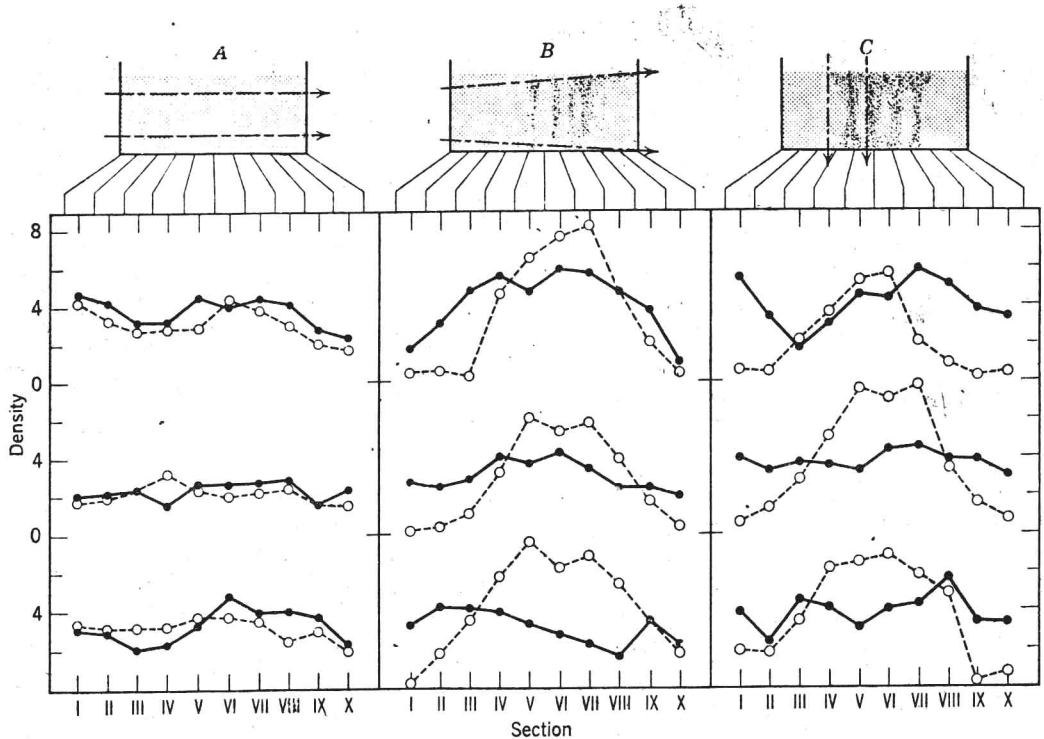

FIG. 1. Comparison of the pattern of distribution of larvae of *Hemicentrotus pulcherrimus*, in a square trough before (filled circles) and after (open circles) illumination. Illumination: A, horizontal parallel beam; B, horizontal beam diverged by 5 degrees; C, vertical parallel beam. The trough was divided, on photographic negatives, into three horizontal layers (upper, middle, and bottom) and ten vertical sections; the latter are shown in the abscissae. The optical density of each section of the photographic negatives, which reflects the degree of aggregation, was determined microdensitometrically and it is shown in the ordinates. In each graph the upper, middle, and bottom groups of curves correspond respectively with each of the three horizontal layers mentioned above. Reproduced with permission from Yoshida (1966).

and photographic methods to determine the degree of aggregation. Parallel horizontal beams produce no effect but larvae aggregate just outside vertical or diverging horizontal beams, which suggests they are doing so at a preferred intensity (Fig. 1). Light also affects their swimming speed, high intensities slowing down upward migration. The presence of an air–water interphase was also necessary for inducing aggregation. Yoshida therefore surmises that light eventually achieves the observed effects by a combined action on random movements at the surface and on the speed of vertical movements.

III. The Pigmentary System and Colour Change

Much has been learned concerning the pigments of sea urchins and although a great deal of attention has been devoted to biochemistry, on which a review would be misplaced here, the findings in *Diadema antillarum* have some relevance to photosensitivity.

This striking urchin shows both morphological and physiological colour changes, and both are influenced by light. The former is complex and the intimate mechanism of the latter appears in part at least to be of an unusual type. Accounts of both have been given by Millott and collaborators (for a summary see Millott, 1964). Several kinds of pigment are involved, hydroxynaphthaquinone (echinochrome), melanin, chromolipoid and an iron-containing pigment of nuclear origin. The pigment pervades the skin and viscera, that of the skin is contained in large intercellular spaces which form a network of channels disposed mainly parallel with the body surface (Fig. 2). In young urchins the

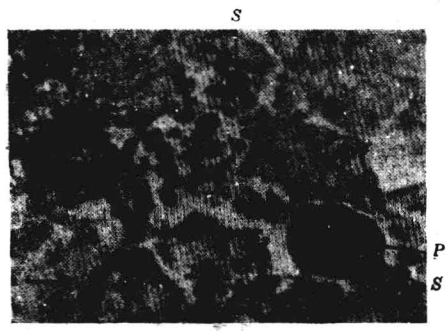

Fig. 2. *Diadema antillarum*. Portion of the intercellular network of the channels containing pigment forming a chromatoglyph, as seen in a tangential section of the skin. Note the compacted residual cytoplasm of a chromatocyte which forms the primary pigment (P.), surrounded by secondary pigment (S.). The pigment is stained selectively for melanin by Lillie's ferrous iron method. Scale, 8·0 μm. Reproduced with permission from Millott (1964).

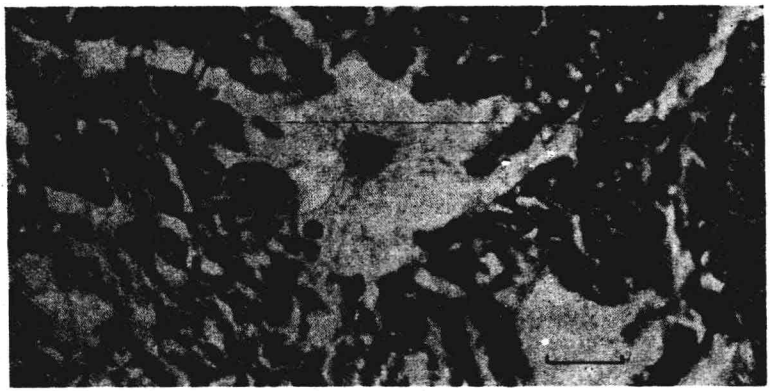

FIG. 3. *Diadema antillarum*. Portion of the intercellular network of pigment channels as seen in a section of the skin cut tangential to the surface, showing the system of fibrils left behind in the space formerly occupied by a chromatocyte (p. 9). Scale, 12 μm. Reproduced with permission from Millott (1966a).

pigment (primary pigment) is contained largely in cellular chromatophores (chromatocytes) but, as the urchins age, this is supplemented and eventually replaced to varying degrees by secondary extracellular pigment deposited in the channels. The melanin in the chromatocytes is finely granular (Fig. 4) so that they resemble the melanophores familiar in a wide variety of animals. The melanin deposited later appears in the form of much larger spheroids which accumulate together with echinochrome and the other pigments. This activity is largely due to amoebocytes which wander into the channels and degenerate leaving behind their contained pigment. To this is added that left behind in the channels as the pigment cells degenerate (Fig. 2). This morphological change is our concern insofar as the melanin is formed by a photosensitive process in amoebocytes which contain the requisite phenolases and tyrosine (the presumed substrate) (Jacobson and Millott, 1953; Millott and Vevers, 1968). The photosensitive nature of the process is supported by the observation (Kristensen, 1964) that pigmentation increases more rapidly in urchins kept in normal light intensities than in those kept in darkness. This has far-reaching implications for the accompanying process of physiological colour change (see below). In this context we may note in passing, the suggestion from Kennedy and Vevers (1972) in connexion with their discovery of chlorin e_6 and coproporphyrin I in the test of *Arbacia lixula* (Linn.), that potentially photosensitizing pigments may be sequestered in the test.

The phenomenon of physiological colour change has excited more interest than the morphological. It was recorded for *Centrostephanus*

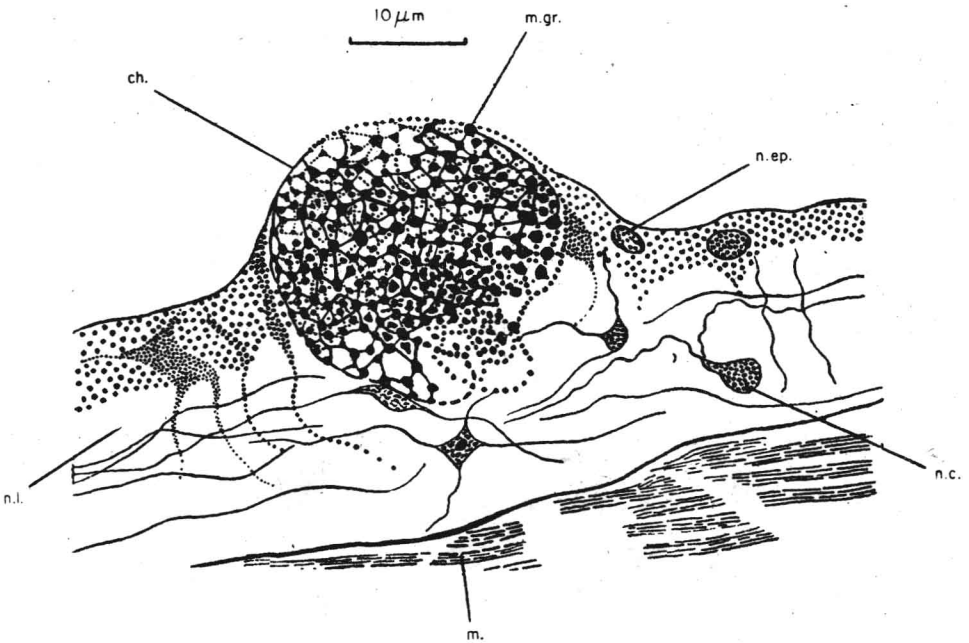

FIG. 4. Chromatocyte of a juvenile *Diadema antillarum* as seen in a transverse section of the skin. Note the finely granular pigment which should be contrasted with that of a chromatoglyph (Fig. 2). ch. chromatocyte, m. muscle, m.gr. melanin granule, n.c. neuron, n.ep. nucleus of epidermal cell, n.l. superficial nerve layer. Reproduced with permission from Millott and Jacobson (1952). *Journal of Investigative Dermatology*, **18**, 91–95.

and *Arbacia* by von Uexküll (1897a), and reinvestigated by Parker (1931), who failed to confirm its existence and again by Kleinholtz (1938) who confirmed von Uexküll's findings. Millott (1952) gave a brief description and analysis of the phenomenon in *Diadema antillarum*, which was followed by a fuller analysis in *Diadema setosum* by Yoshida (1956, 1957a, 1960). More recently Dambach and collaborators (Dambach, 1969; Weber and Dambach, 1972) have redirected attention to *Centrostephanus*.*

Responses of the chromatophores to light were evident in all these cases, but it became clear from the earlier studies that the responses were independent of the radial nerves and could be localized by the use of narrow light beams (Millott, 1952). This was confirmed by Yoshida (1956) who in an eminently elegant fashion, used light spots 3 μm in diameter to induce pigment dispersion in individual chromatophores.

* Defined by the authors as *Centrostephanus longispinus* Peters.

He was also able to show that the responses were obtained over a broad spectral band (450–500 nm) with a maximum at 470. Millott (1952) also revealed that the chromatophores manifest a diurnal rhythm of pigment concentration and dispersion that is independent of the immediate effects of environmental lighting.

Although Yoshida assumed that the chromatophores were cellular entities and comparable with those of other animals, he revealed in them some singular properties which are difficult to interpret on this basis. Thus his minute light spots did not exert an effect on a whole chromatophore, but only on the part that was illuminated so that the illuminated branches remained deeply pigmented for so long as the light was projected on to them, while the rest of the unit changed to the punctate form (Fig. 5A). Again chromatophores could be displaced

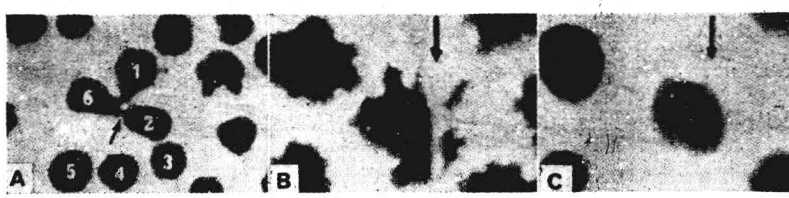

FIG. 5. Behaviour of the chromatophores of *Diadema setosum*. A. Effect of projecting a minute light spot in the position indicated by arrow between the chromatophores labelled 1, 2 and 6. Note the localized dispersion of pigment induced. B. A chromatophore is cut across by a glass needle at the position arrowed, while pigment is dispersed. C. The same chromatophore after 30 min. in darkness. Note the apparent re-union of the two portions. A. reproduced with permission from Yoshida (1956). B. and C. reproduced with permission from Yoshida (1960).

bodily by centrifuging as well as by illuminating the adjacent areas of skin. Most remarkable of all was his demonstration that when the pigment was dispersed they could be cut in two by a fine glass needle (Fig. 5B) whereupon the two halves appeared to re-unite into a functional unit when they assumed the punctate condition (Fig. 5C).

These somewhat puzzling findings were complemented and highlighted by Millott (1964, 1966a) in *Diadema antillarum*. Thus when the channels pervading the living skin are punctured by a micro-manipulator, pigment escapes freely and it is forcefully discharged in minute jets on fixation, forming a sooty deposit over the skin. In coverslip preparations of living skin, pigment masses are seen to undergo continual movement dividing and re-joining so that under the influence of light and shade, when pigment is dispersed and subsequently concentrated, it may become re-distributed among the interlacing channels. Again the masses could be cut across and the separated portions continued to disperse and concentrate under appropriate lighting. As in the case of Yoshida's work these findings were difficult to explain on

current concepts of the working of pigmentary effector systems and the precise cause of movement remained unsolved. In this context it should be noted that the walls of the intercellular channels are pervaded by a well developed system of fibrils that in living preparations appear to be elastic.

The histology of the system exhibits some other significant features. In juveniles the black pigment is contained in what are clearly cells (chromatocytes, Fig. 4) situated in well defined cavities that form the nodes of the intercellular channels, the walls of which embody spindle-like cells attenuated into fibrils. As the animals age the chromatocytes degenerate and their remains, supplemented by secondary pigment of all varieties (Fig. 2), form aggregations of pigment cast in the mould of their cellular precursors. Millott (1964) distinguished these structures as *chromatoglyphs* (γλυφις). As the accumulation of pigment continues, the reversible colour change becomes less evident, disappearing completely in many individuals, but in some it persists in limited areas, so that when the pigment concentrates, a characteristic pattern of white lines develops in the periproct and interambulacra (Fig. 6). Nevertheless sections of these areas of mobile pigment show that much of it is contained in chromatoglyphs.

At this stage a most striking feature of the histology is the elaborate web of fibrils that spans the chromatoglyphs and radiates from a nucleus, presumably the relict of a degenerated chromatocyte. This is suggested by the deeply pigmented pycnotic condition of many of these nuclei, but others, such as that shown in Fig. 3, appear normal and could therefore be the remains of cells which have suffered rupture and dissolution on fixation and discharged their pigment (p. 8). Their regular occurrence and disposition suggests that, together with the fibrils in the channel walls, they could be concerned with pigment movement.

These features could explain at least some of the peculiar behaviour of the chromatophores of *Diadema antillarum* and it is tempting to suggest that they could also account for some of the behaviour reported by Yoshida in the allied species. Lacking knowledge of the histology of the latter no stronger assertion is warranted. Thus the strictly localized effect of minute light spots on chromatophores could be the reflexion of their effect on restricted areas of the channel walls. The apparent reunion of separated portions of bisected chromatophores, the bodily displacement of such structures by centrifuging and the redistribution of pigment under the influence of light (p. 9), are easier to explain in the case of chromatoglyphs than in the case of chromatocytes of the usual type forming a tissue constituent.

Dambach and collaborators, as a result of their more recent work on *Centrostephanus*, offer a different explanation. Curiously, Weber and