The Iridophores of the Echinoid *Diadema antillarum*

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With one plate (fig. 1)

Summary

The blue pattern seen when the skin of *Diadema antillarum* is viewed by reflected light is due to iridophores. The quality of their blueness varies with the background provided by neighbouring chromatophores.

Microdissection reveals that they consist of gelatinous plates contained in a fibrous and cellular capsule. The study of fixed preparations shows that the plates are sheathed and that their structure is greatly altered by fixation which distorts, disrupts, or dissolves them.

The structure of the iridophores bears little resemblance to the description of the so-called 'eyes' of *D. setosum* given by Sarasin and Sarasin, with which they appear to correspond.

Examination of their optical properties indicates that Rayleigh scattering by the colloidal contents is the most likely cause of their colour.

Introduction

THE striking colour of the echinoid *Diadema antillarum* Philippi depends on three factors: ubiquitous black (provisionally identified as melanin) and red or purple (hydroxynaphthaquinone) pigments, interlaced with a brilliant blue pattern. The last is due to light reflected from structures in the skin, to which brief reference has already been made (Millott, 1953 *a*, *b*, *c*), though the ideas concerning the precise means whereby the colour is produced have needed revision.

Conspicuous blue spots appear also in the skin of other diadematids (Mortensen, 1940), but, apart from those of *D. setosum*, little has been written on their structure and on the means whereby their colour is produced. Sarasin and Sarasin (1887) have presented the most detailed existing description of the blue spots in a species from Ceylon, but it seems uncertain whether the species to which they refer is *D. setosum* (Leske), since before 1904 this species appears to have been confused with *D. savignyi* (Audouin) Michelin (see Mortensen, 1940, p. 259). Subsequent brief references to these structures in this and other diadematids by Cuénot (1891, 1948), Mortensen (1940), and Millott (1953 *a*, *b*) raise doubts as to the adequacy of the Sarasins' account.

Döderlein (1885) believed the blue areas to be luminous, while Sarasin and Sarasin believed they were eyes. The evidence is wholly inadequate, and in the case of *D. antillarum* the structures appear to be iridophores (Millott, 1953 *a*, *b*), a view which has been fully substantiated by subsequent

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study. They may perhaps function like a tapetum in the highly organized photoreceptive surface that covers the test (Millott, 1960).

Distribution of the Iridophores

When the living skin is examined microscopically by light reflected from a microscope point-light, an elaborate and exceedingly beautiful pattern of brilliant blue lines and spots appears against the red and black background of the skin. We have studied their distribution more closely in individuals that are about half-grown (roughly 3·5 cm across the ambitus).

Their distribution follows mainly that of the white pattern which is developed especially in young urchins, when the chromatophore pigment is concentrated (Millott, 1952) so that the iridophores are largely interambulacral and in greater number aboral to the ambitus.

They surround the periproct in a position corresponding to the inner border of the white ring that forms in this area, being arranged predominantly in a single row to form a discontinuous line that passes round the madreporite (fig. 1, A). Here and there the row is double.

In the middle line of each interambulacrum a band of iridophores extends toward the ambitus along each margin of a shallow depression or gutter in the test (fig. 1, A), which is conspicuously white when the chromatophore pigment is concentrated. The bands are irregular owing to the uneven spacing of the iridophores, which may be isolated or packed in a row (fig. 1, D). Aborally each band approaches the ring around the periproct, but it does not join it. As the bands pass to the ambitus they diverge sharply where the

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Fig. 1 (plate). A, the distribution of the iridophores aboral to the ambitus shown by a portion of the test from which most of the spines have been removed. The iridophores, seen by reflected light, appear as discontinuous white lines. The irregular white areas are the cut surfaces of the spines or test. The specimen is partly light-adapted so that some of the white pattern, formed when the chromatophore pigment is concentrated, is still visible between the rows of iridophores which run radially down the middle of each interambulacrum.

B, C, D, the effect of spreading skin pigment (p. 184) on the appearance of the iridophores which lie alongside the white lines developed in darkness in the gutter-like depressions of the interambulacra.

B, an early stage; the iridophores are barely distinguishable along the right margin, while those outside the white areas are well defined. The punctate or stellate black bodies in the white area are chromatophores.

C, the same area after longer exposure to light. Pigment has encroached on the white area (particularly along the right margin) in the processes of chromatophores underlying the iridophores, which are now more easily distinguished.

D, a later stage from an area aboral to that shown in B and C, where more chromatophore pigment has spread beneath the iridophores on both sides so that they are now clearly defined and appear blue.

E, a cluster of iridophores showing the dispersion of melanophore pigment in fine processes around them.

F, a horizontal section of an iridophore fixed in Carnoy (1½ h), stained by the PAS technique, and showing the thin plates separated by droplets which are strongly PAS-positive (see p. 190).

G, a vertical section through an iridophore of a young individual, fixed in Champy (2½ h), stained in Heidenhain's iron haematoxylin and light green. For description see p. 188.

H, a horizontal section through an iridophore fixed in Champy (2½ h), stained in Weigert's haematoxylin and light green. For description see p. 190.

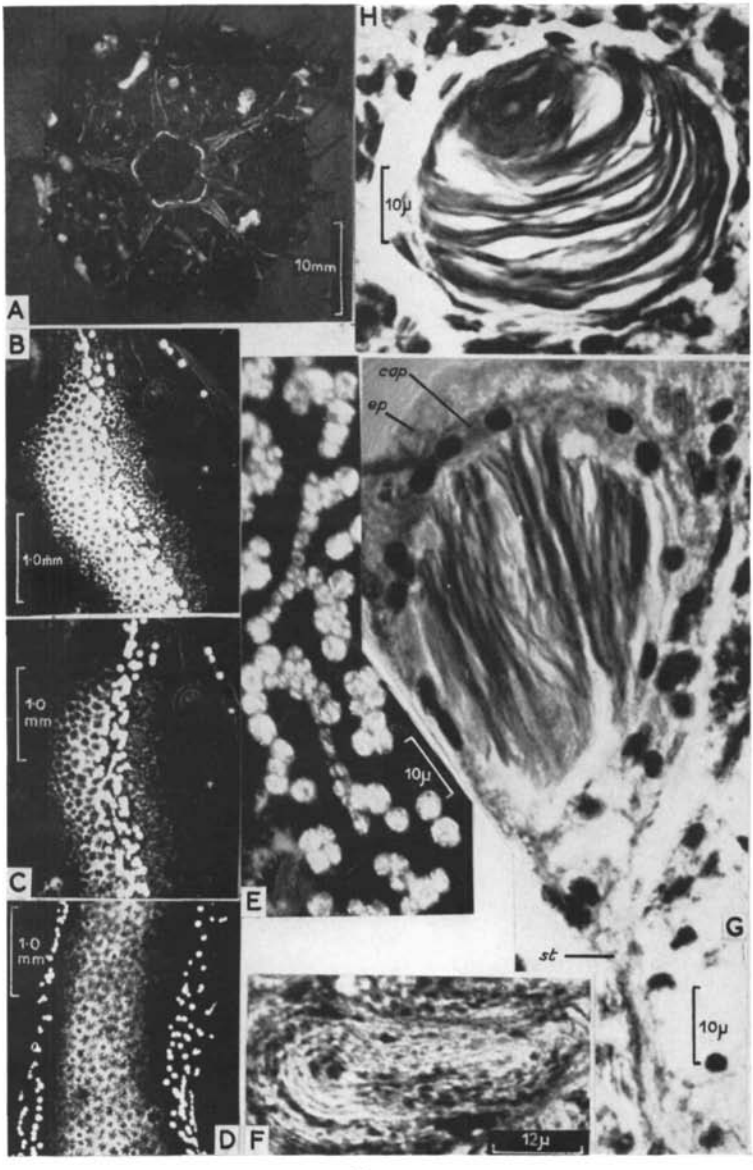


FIG. 1

gutter bifurcates to form a figure resembling the head of a lancet arch, which when devoid of pigment forms such a conspicuous and characteristic pattern in the interambulacrum. Each band of iridophores follows the outer margin of this figure, becoming thicker and irregularly branched, sometimes to such an extent that the ramifications form a lace-like network (fig. 1, E).

Here the iridophores are more densely packed into ridges or cushion-like masses, superficially resembling the faceted cornea of a compound eye, a likeness which, from the accounts given by the Sarasins, seems to have its counterpart in *D. setosum* and to have been partly responsible for their suggesting that these structures are eyes.

Some of the branches from the bands are more extensive, passing between the bases of the adjoining primary spines, around which they extend to form discontinuous rings composed mostly of single rows, thickened irregularly to two or three deep. From the rings small centrifugal branches pass between the bases of the surrounding spines of lower orders.

In addition to the above, there are a few iridophores scattered singly or in small groups over the interambulacrum, aboral to the area which bears the primary spines.

Below the ambitus the bands of iridophores follow a wavy course, becoming thinner and more compact, losing many of their branches. As they approach the peristome the bands break up into progressively smaller sections, separated by increasing gaps, finally disappearing before the peristome is reached. Because skin pigment is less dense here, many of the bands of iridophores can be seen to overlie the sutures between the test plates.

There are few iridophores in the ambulacra, where they are scattered irregularly between the bases of the spines.

The Colour Produced by Iridophores and its Relation to Skin Pigment

When examined in their natural position by light reflected from a tungsten lamp, the iridophores appear predominantly blue, lilac, or turquoise. Each iridophore may be uniformly coloured or show patterns of the three colours in which blue and lilac predominate. The intensity of light reflected is not uniform, but areas of greater brilliance appear as fine lines which may be isolated or grouped in parallel or concentric array, and disposed with respect to the surface in many different ways. When the direction of the incident light is altered, the pattern of both the coloured areas and the lines of high intensity may change completely or disappear, but the range of colour does not alter.

The coloured light thus reflected produces a bluish glow in the surrounding areas of the skin, which is particularly evident in the grooves between the spine bases and alongside the white pattern developed in the interambulacra and around the periproct.

The quality of the colour is profoundly affected by the background provided by the skin pigment. This changes markedly in the vicinity of many

iridophores, owing to the activity of the surrounding chromatophores (both black and red), which are affected by the prevailing light intensity. When the pigment is fully concentrated the iridophores overlie a white background due to the test, appearing almost colourless and difficult to see (fig. 1, B). In bright light the chromatophore pigment disperses so as to obliterate the white pattern. As the tongues of pigment encroach on the white areas, the outlines of the iridophores and their blueness become increasingly evident (fig. 1, C, D). At first they are predominantly lilac, but as more black melanophore pigment comes to lie beneath them they change to blue (fig. 1, D). This accounts for the lilac and blue pattern mentioned above. As the black pigment continues to disperse, the iridophores come to lie on a background that is completely black and they then reflect only blue.

The importance of the background effect is shown directly by lifting the blue iridophores away from the underlying melanin by microdissection needles and deflecting them on to a paler part of the skin, when their colour reverts to lilac, perhaps mixed with bluish green. When excised, placed over matt black paper, and viewed by reflected light, their blueness again increases although not to the same degree as when observed in their natural position; considerable admixture with lilac and blue-green persists.

It will now be evident that the iridophores play a significant role in the complex play of colours that accompanies the dispersion of each pigment in the skin, and the effect is partly due to their changing background. This change was described as seen in young individuals, in which it is most extensive (Millott, 1952). As urchins age, the progressive accumulation of pigment, most of which is static in the sense that it is not subject to rapid dispersion and concentration, lessens and in some cases abolishes the change. Other factors, too, such as changes in the chromatophores, may be involved. However, it is most significant that the capacity for pigment movement is retained longest in the melanophores which border the gutter-like depressions of the interambulacra. It is these which lie below the greatest concentrations of iridophores. But even here there is differentiation, for where the iridophores lie along one side of the gutter, the melanophores beneath them are more numerous and more active than those on the other side (fig. 1, B, C). Further, their pigment tends to remain dispersed for a longer time and few become punctate.

The Structure of Living Iridophores

When excised and examined in sea-water, the iridophores appear as more or less globular transparent bodies. They are usually lifted off with their underlying cushion of melanin and naphthaquinone, some of which is present in the amoebocytes which swarm around them. Varying numbers may be associated in short strands (fig. 1, E).

The iridophores may lie flush with the epidermal surface as in young individuals, or they may stand out, forming a prominent ridge.

Each iridophore is surrounded by a tough capsule (fig. 2). Its contents, which appear faintly yellow by transmitted light, show a striated appearance, suggesting a laminated structure. Sometimes the striations can be seen to form definite patterns or systems, which are complicated and variable. Some extend across the capsule as groups of parallel and more or less straight lines. Very commonly they are folded in a simple way, so that the parallel array resembles a nest of letters 'U' of graded size fitting closely into one another.

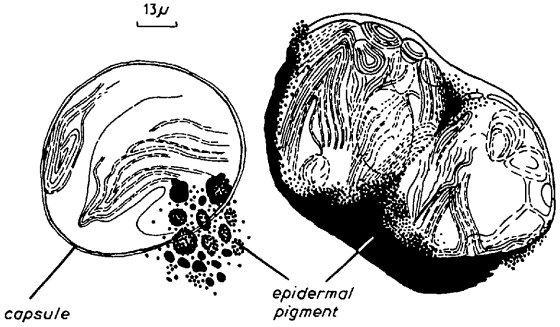


FIG. 2. Excised living iridophores, redrawn from preparations in sea-water (see p. 185).

Others form concentric systems, scrolls, or formations too varied to describe.

Where they are clearly visible, the arrangement of the striations suggests that they are paired, each member of the pair being separated by about 1 μ, with a distance of about 2 μ between the pairs. Though they are much more numerous, their disposition recalls that of the bright lines observed by reflected light (p. 183).

Microdissection reveals more of the contents. If an iridophore is cut open, the contents protrude as a laminated, gelatinous mass, one end of which remains firmly anchored to the inside of the capsule.

When the capsule is punctured, some of the contained jelly escapes, leaving well-defined channels in the remainder. Squeezing such iridophores expresses the contained gel as separate masses showing a laminated structure, or as hyaline globules which pass along defined channels to escape by the puncture, leaving behind the walls of the channels as an array of parallel striae arranged along the lines of flow by which the jelly escaped. In form and arrangement the striae recall some of those just described in the intact iridophore.

The expressed gel shows remarkable properties. When compressed, it does not disperse into sea-water but spreads out to form a stratified mass of regular laminae, which appears to change or 'set' (fig. 3). The laminae gradually separate, forming rods of jelly that eventually break up into pieces of varying length and about 1 μ thick.

A most significant property is their bright blue appearance when viewed by reflected light over a background of black paper, which was evident whether the contents escaped in a single mass or as separate globules. In the former

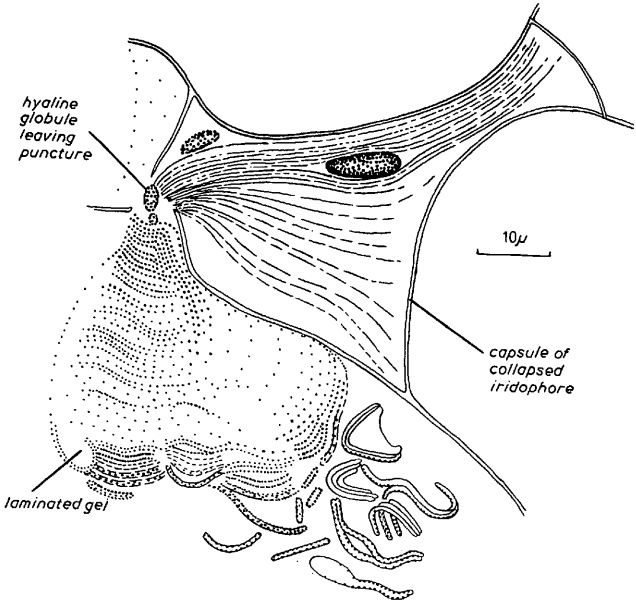


FIG. 3. Expulsion of the contents of a living iridophore into sea-water (see p. 185). Redrawn from a preparation subjected to pressure.

case, the gel showed the brilliant striations characteristic of the intact iridophore, and the degree of colour achieved was at least as great as when the contents were in the natural position.

There is thus no evidence whatever for the existence of the solid plates or crystals that are usually associated with iridophores. On the contrary, the foregoing evidence from manipulation of living iridophores consistently indicates that a large measure of their contents is formed into regularly arranged gelatinous laminae, the disposition of which in some degree resembles the systems of lines visible in the intact iridophore. The peculiar layering properties of the gel might be related to its laminated natural disposition, and it is worth recalling that the layers tend to separate with about the same thickness as the distance separating the paired lines which divide up the contents of intact iridophores. This property also hints at an orientated

ultrastructure, and the blue colour of the gel suggests the occurrence of light-scattering by colloids with a fine dispersed phase.

The Effect of Fixation on Colour

Elucidation of more detailed structure necessitates the study of fixed material. If any inference is to be made from such studies concerning the origin of colour in the iridophores, the effect of fixation on their colour should be known. Mortensen (1910) reported that the blue colour was not preserved by alcohol or formalin, and this has been found to be true for most of the fixatives we have used. However, we cannot assume that this is entirely due to the effect of fixation on the iridophores themselves, for some fixatives burst the chromatophores (Millott, 1953*a*), causing them to discharge their contents over the surface, and this might make the iridophores invisible without destroying their intrinsic colour-producing mechanism.

Pieces of test-bearing skin were fixed in formaldehyde-saline, Bouin, a modified Bouin (Atkins, 1937), Duboscq–Brasil, Carnoy, Heidenhain's 'Susa', Flemming's strong fluid, and Champy. Only the last preserved any of the blueness, which survived the subsequent washing in water, dehydration in ethanol, and clearing in methyl benzoate.

To discover whether fixation had transient effects on the production of colour, which might be significant, the action of several fixatives was followed under a microscope by delivering a few drops on to a cushion of iridophores viewed by reflected light.

Champy destroyed the lilac colour, so that the iridophores reflected a paler but more uniform blue, though the parts immediately overlying the melanophores still appeared more blue than the rest. No discharge of skin pigment was observed, and the capsule became noticeably whitish and less transparent.

Flemming's fluid rapidly destroyed the intense blueness. Most iridophores became opaque white, while a few continued to reflect a very pale blue which disappeared on subsequent washing in tap-water. A unique and noteworthy effect was to cause a small number to reflect intense red.

Bouin, Susa, and Carnoy completely destroyed all blueness, the iridophores rapidly becoming white and opaque. The action of Carnoy was instantaneous, and that of Susa required 5 to 10 sec. Very shortly afterwards they liberated floods of pigment from the surrounding red chromatophores, which rapidly turned brown to form a sooty deposit over the surface obscuring the iridophores. Thus the destruction of colour occurs first, and is independent of pigment discharge.

It may be mentioned here that osmotic changes, produced by immersing living iridophores in mixtures of sea-water and M/4 KCl or in distilled water, affect the colour. The former abolished it, but not permanently, for it returned on re-immersion in sea-water. Immersion in distilled water destroyed the blue colour, which partly returned in sea-water, but to what extent the effect

on the iridophores is reversible remains unknown because much pigment is discharged.

The Structure of Fixed Iridophores

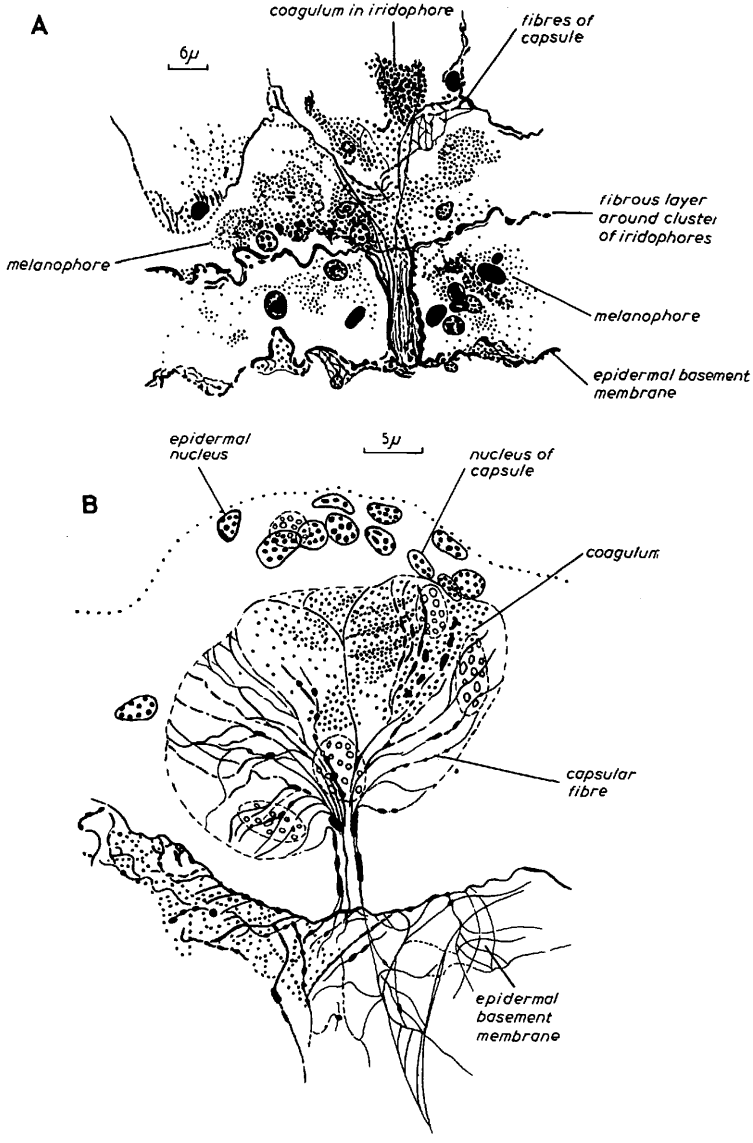
Paraffin sections 4 to 6 μ thick were prepared of skin and test. Owing to the kindness of Professor D. M. Steven of the University College of the West Indies, we were able to extend this part of our study to include iridophores of young animals measuring about 0·6 cm across the ambitus. Many staining techniques were used, including Delafield's haematoxylin and eosin, Masson's trichrome, Heidenhain's iron haematoxylin (alone and with light green), Weigert's haematoxylin (with light green or ponceau fuchsin). In addition, a number of special methods were employed, such as silver staining for reticulin (Wilder, 1935), alcian blue for acid mucopolysaccharide (Steedman, 1950), and the periodic acid / Schiff and Feulgen techniques.

In size and form, fixed iridophores approximate to the living, but it is possible to see that there is great variation in size among them even within one cushion, where the smallest are about 20 μ in diameter and the largest over 5 times this width. Iridophores of young animals are much smaller, being commonly about 20 to 30 μ across.

It is now possible to see that despite their superficial position they lie below the epidermis, which is reflected over them, changing its character to become a shallow layer of more or less cuboid cells (compare fig. 1, G), recalling the so-called 'cornea' of the Sarasins' description. Nothing corresponding to the cuticle they described in *Diadema setosum* exists here, though the epidermis is usually covered by a thin, even layer of apparently mucoid secretion, which stains brightly with alcian blue.

The capsule (fig. 1, G) lies immediately beneath the epidermis and is largely an elaborate network of branched fibres apparently continuous with those of the epidermal basement membrane, which they resemble in form, arrangement, and staining properties. Sometimes the capsule may be drawn out into a stalk of varying length (*st*, figs. 1, H; 4), anchored to the deeper layers of the test by a bunch of prominent fibres recalling those figured by the Sarasins. The fibres appear brown with silver and stain sharply with the periodic acid / Schiff technique; some respond to alcian blue. Interspersed among the fibres are small cells which form a more or less regular layer (*cap*, fig. 1, G) over the top of the iridophore beneath the shallow epidermis. Round the sides and beneath, the cells become sparse and attenuated so that, apart from their compressed nuclei, it is difficult to discern their presence. Where

FIG. 4. Details of the capsule from preparations stained to show the fibres. A, basal portion of two iridophores, showing the relation between the stalk (of the iridophore on the right), the common fibrous layer extending around the cluster of iridophores, and the epidermal basement membrane. Fixed in formaldehyde-saline; silvered for reticulin. B, portion of the capsule of a single iridophore of a very young individual. The stalk and some of the capsule lie along the plane of section; the main body of the iridophore, being obliquely disposed, is below this. Some of the nuclei of the capsule, with some of its contents in the form of a coagulum, appear in deep focus. Fixed in Bouin: silvered for reticulin.


A
coagulum in iridophore
fibres of capsule
6μ
fibrous layer around cluster of iridophores
melanophore
melanophore
epidermal basement membrane
B
epidermal nucleus
5μ
nucleus of capsule
coagulum
capsular fibre
epidermal basement membrane

iridophores are compacted into groups, the whole is bound together by an additional fibrous layer (fig. 4, A).

The contents appear in the same bewildering variety as in the living structures, but it is now possible to see that they are a variable mixture of plates, coagulum, and what appear to be fibrillae.

After fixation in Champy (which also preserved the colour) the capsule contents took the form most closely resembling that seen in life, appearing as regularly arranged lines about 0·3 μ thick, grouped in pairs (fig. 1, G, H), each pair being separated by spaces about 1 μ wide and folded to varying degrees so as to form the same variety of patterns. Correlation of sections in different planes confirms what was already suspected (p. 186), that the patterns result from systems of variously folded partitions inserted by their edges on to the capsule (fig. 1, H). Those folded like a letter 'U' are most commonly inserted by their edges on to the region of the capsule which lies immediately below the epidermis.

The double lines form the thickened margins of plates (fig. 1, H). Each plate consists of a hyaline central core about 0·3 μ across, staining feebly with the periodic acid / Schiff stain and not at all with alcian blue, invested by a sheath of similar thickness which is coloured brown by osmium tetroxide, red by periodic acid / Schiff; it takes up light green but not alcian blue. The sheaths, which form the patterns of lines, are continuous with the capsule. No substance is visible between such plates. But not all the plates are like this even in the same iridophore. Some may appear swollen and distorted, with their sheaths separated from the core. Others may appear incomplete, failing to join the capsule, with gaps in the core and sheaths, either one or the other of which may be partly or completely lacking. The spaces between such distorted plates is usually occupied by a coagulum.

Other methods yield different appearances. After Carnoy or Susa fixation, particularly in conjunction with the periodic acid / Schiff technique (fig. 1, F), the plates are thin, often lacking sheaths and showing signs of dissolution, but they retain their regular arrangement so that the contents look rather like a thumb-print. Strongly PAS-positive droplets appear between the plates. Elsewhere the plates may be bundled together and often their identity is lost, the capsule being filled with coagulum which may be more or less homogeneous or pervaded by PAS-positive droplets or by what appears to be a tangled mass of fibrils. Shrinkage and distortion of the plates is particularly marked after fixation in Bouin.

Sections in different planes show that the fibrils are fragments of the sheaths. Further, there is a roughly reciprocal relationship between the number, size, or integrity of the plates and the amount of coagulum or the number of PAS-positive droplets. This suggests that the varied appearances represent stages in the progressive distortion and eventual breakdown of the sheathed plates under the action of fixatives, the material derived from disintegration accumulating between the remains of the plates as coagulum or droplets. The idea is confirmed by appearances after the PAS technique

when the sheaths of the plates stain progressively less as the amount of red-staining material between them increases.

It is clear that fixed material has strictly limited usefulness and can only be interpreted in conjunction with the living structure.

The notion of sheathed gelatinous plates would fit in with the behaviour of living iridophores under compression already described (p. 185). Thus if the hyaline core alone were expressed (and the sheaths are now known to be anchored to the inside of the capsule), not only would it tend to escape along definite channels walled-in by the sheaths, but the empty sheaths left behind would appear as the parallel striae observed. Moreover, their arrangement would be expected to resemble that of the striations seen in living intact iridophores (p. 185).

The Production of Colour

The means whereby the iridophores produce their blue colour presents a difficult problem. The blueness of the iridophore contents (p. 186) shows that it is not due in significant measure to the superjacent tissue as previously conjectured (Millott, 1953 *a*, *b*, *c*).

The fact that the blue is obvious when the iridophores are seen by reflected light, yet is lacking when they are viewed by transmitted light, indicates that the colour is produced by structural means rather than by pigmentation. Possible participation of pigment is not ruled out by this, however, because there might be minute amounts of blue pigment in the iridophores, insufficient to be evident when light is passed directly through them but sufficient to produce obvious colour when disposed in a thick layer. The equivalent of such a layer might be produced if light were passed by internal reflexion along the laminae, acting as light guides in such a way that the incident light passed down one limb of the 'U' to be reflected back along the other. The yellowish tinge observed when iridophores are viewed by transmitted light might be attributed to the naphthaquinone which often surrounds their bases.

However, the production of blue by pigment means that significant absorption of spectral yellow must occur, so that the possible participation of pigment can be tested by examining iridophores by reflected yellow light. If such absorption takes place, the iridophores should appear dark. They do not. When viewed by light passed through a Wratten No. 12 filter (which transmits maximally between 550 mμ and 700 mμ and cuts off all visible light of wavelength shorter than 500 mμ), they appear yellow. There is thus no significant absorption of yellow light, and therefore no evidence of blue pigment.

The use of an Ilford filter No. 805, to remove any ultra-violet in the light beam by which iridophores are examined, makes no appreciable difference to their blue colour. Again, iridophores are invisible in ultra-violet light, so that fluorescence plays no significant role.

The evidence thus supports the previous indication that the colour is

structural in origin. It may result from refraction, interference, diffraction, or scattering.

The first is eliminated, because when the iridophores are viewed in the natural position against a black background the only colour visible is blue, and there is no change when the angle or direction of the incident beam is altered.

Interference and diffraction are more difficult to eliminate, and it must be admitted that the structure of the iridophores with their regularly arranged plates hints at such an origin for their colour. The spacing of the plates is necessarily a critical factor in production of colour by these means, but we have not been able to obtain measurements that are sufficiently reliable to test the idea. On the other hand, stretching and compressing iridophores by microdissection would be expected to alter the spacing of the plates, in view of the gelatinous nature of the iridophores, but it does not alter the colour of the light reflected from them. Again, the intense blueness evident in drops of gel expressed from iridophores suggests that critical spacing of the plates is not a major factor, although it is impossible to be certain that such droplets did not contain intact fragments of the laminated structure. Further, there is no directional effect, blue being reflected in all directions, and the colour does not vary appreciably when the incident beam is rotated through an angle of 35° (the maximum possible with the optical means available). Though this evidence is not conclusive, because the systems of the plates within one iridophore may be disposed at a variety of angles with respect to the surface, movement through such an angle would be expected to make an appreciable difference to the colour; but this was not observed.

Scattering seems a more likely source of the colour. The obvious preponderance of blue in the emergent beam, as well as its intensity, would be expected with fourth-power scattering. The slightly yellowish colour shown by iridophores when light is passed through them and the purification of the blue colour which occurs when iridophores are viewed against a black background agree with this idea. Again, when iridophores are examined by polaroid filters the beam reflected from them is seen to be polarized. In the balance, therefore, such facts favour scattering as the means of producing colour.

The intensity of blueness observed in structures so small as the iridophores would suggest Rayleigh scattering by an optically heterogeneous system with particles less than 0·1 μ in diameter, and the delicate colloidal plates of the iridophores could embody such a system.

Discussion

To what degree the iridophores of *D. antillarum* correspond with the blue spots in other diadematids is uncertain. Nevertheless, in their position in the skin, as well as in the general features of their distribution and structure, the iridophores resemble the blue spots described by the Sarasins in a species from Ceylon which they called *D. setosum* Gray (see p. 181). In each case the

structures are seated among pigment cells and rest on the superficial nerve-layer, being bounded externally by the epithelium covering the test, which is here greatly thinned out.

Caution is necessary in attempting detailed comparisons, because existing descriptions of the structures in *D. setosum* are based on fixed material alone. Again, doubt has been cast on the accuracy of the Sarasins' account (Cuénot, 1891; Mortensen, 1940), parts of which the authors admit can be accepted only with reservation because it applies to structures that they found difficult to interpret and which changed considerably on fixation. Neither this, nor lack of experimental evidence, appears to have influenced their conviction that the blue spots were eyes!

Nevertheless, certain features they described can be matched with those of the iridophores of *D. antillarum*. Thus their 'cornea' corresponds with the thin epithelium overlying the iridophore, while their 'nuclear cap' seems to correspond with the nuclei of the capsule cells lying beneath the epithelium. It is difficult to match any structures with the so-called 'retinula'.

The contents of the capsule seem to have proved something of an enigma. The Sarasins interpreted them as lenses formed of more or less regularly arranged, vacuolated cells, while Cuénot (1948) seemed inclined to regard them as mucoid. Mortensen (1940, p. 248) is curiously inconsistent. Accepting the view that the whole structure is an eye, he disagrees with the Sarasins' figures and describes the contents as a 'dense mass of fibrillae wound up like a ball', yet he figures them as what might be a uniform coagulum (fig. 12, plate LXXIII).

Examination of the very few specimens of *D. setosum* to which we have had access reveals that the contents not only show signs of a laminated structure but also a variety of appearances which parallel those seen in the iridophores of *D. antillarum* after fixation. This not only strengthens the previous conjecture (Millott, 1953*a*) that the blue spots in the two species are produced by the same kind of structure, but also suggests that changes which follow fixation of the so-called 'eyes' of *D. setosum* may have been partly responsible for the different and confusing descriptions just mentioned. Re-investigation of these structures is long overdue.

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