MESOGLOEAL ULTRASTRUCTURE AND REVERSIBLE OPACITY IN A TRANSPARENT SIPHONOPHORE

G.O. Mackie, G.V. Mackie

MESOGLOEAL ULTRASTRUCTURE AND REVERSIBLE OPACITY IN A TRANSPARENT SIPHONOPHORE

by G.O. MACKIE and G.V. MACKIE

Department of Zoology, University of Alberta, Edmonton, Canada

ABSTRACT

The reversible opaque response of *Hippopodius*, a transparent siphonophore, is caused by the appearance of light-scattering granules in the mesogloea. The transmission of the opaque response depends on the presence of intact covering epithelium. The mesogloea components (granules, collagen and elastin) have been studied by optical and electron microscopy, and the biological significance of blanching is discussed.

INTRODUCTION

Hippopodius hippopus Forskal is a calycophoran siphonophore common throughout the Mediterranean, and readily obtainable at the surface during winter and spring in the Bay of Naples and at Villefranche-sur-Mer (LELOUP, 1935). It has five to ten medusoid nectophores (swimming bells) tightly articulated together and enclosing a central space into which the stem and its appendages may be withdrawn (Fig 1). In a fresh colony which has been undisturbed for a sufficient period the nectophores are transparent, but if the colony is then touched a wave of opacity spreads across the nectophores and they become milky white. If left undisturbed, the organism becomes transparent again.

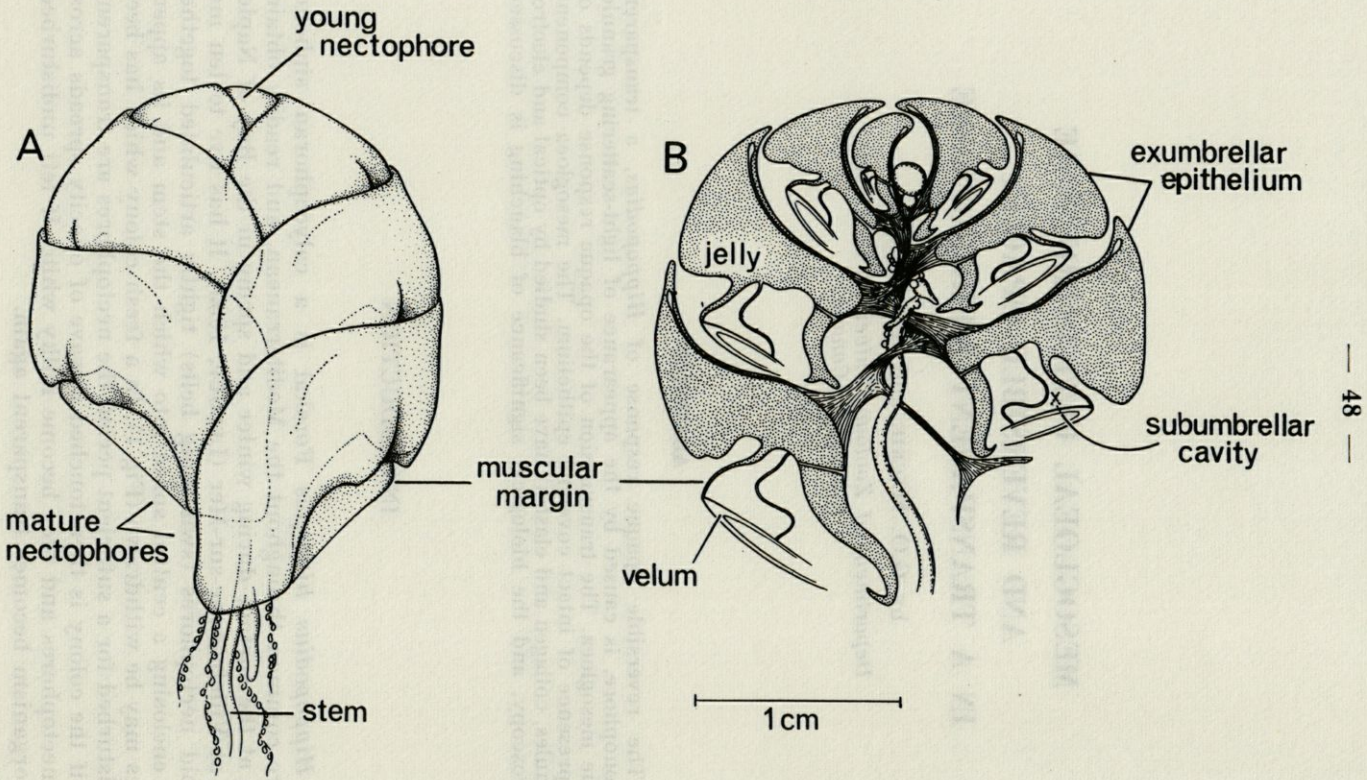


FIG. 1. A. Intact *Hippopodius* - B. Dissection, after Chun (from Mackie, 1965).

This reversible response was first clearly described by KOROTNEFF (1884) who attributed the opacity to Brownian movement of granules in the epidermis covering the nectophores. CHUN (1897), DUBOIS (1898) and IWANTZOFF (1928) also held that the opacity was produced in the epidermis, but several earlier workers commenting on the opacity of preserved *Hippopodius* believed that it was a property of the mesogloea (Fig. 1. " jelly ") and KÖLLIKER (1853) specifically attributed the opacity to the presence of granules in the outer layer of the jelly. The process of going opaque, known to German writers as 'Milchweisswerden', will here be called 'blanching'.

KOROTNEFF noted a second phenomenon associated with stimulation of *Hippopodius*. At night, the organism luminesces when agitated mechanically. KOROTNEFF believed that the blanching observed by day corresponded to the luminescence seen at night. DUBOIS (1898) associated both blanching and luminescence with granule formation in the epidermis, which he likened to the 'formation of crystals in a supersaturated solution'. NICOL (1958) provides spectral emission curves and gives estimates of radiant flux intensity for several luminescent siphonophores including *Hippopodius*. He was not able to localize the source of the luminescent emission, and he does not refer to blanching.

The majority of siphonophores do not blanch, and no other marine organisms known to us have a comparable response. Some animals possessing chromatophores (e.g. squids) include white in their repertoire of colours, but the mechanisms involved are completely different. The siphonophore *Ceratocymba* shows reversible blanching in its bracts, the opacity being caused by the appearance of granules in the mesogloea according to CHUN (1888). Species of *Heteropyramis* and *Chuniphyes* show opaque patches (TOTTON, 1954) but it is not known if opacity is reversible in these cases. *Bassia bassensis* shows permanent opacity at the edges of the nectophores and bracts (HUXLEY, 1859).

Existing accounts of blanching leave uncertain exactly what the opacity consists of, where it is produced, how it is propagated and how it is related to luminescence. CHUN (1897) and MACKIE (1964) conjectured that it was associated with waves of non-nervous excitation travelling in the epidermis. It was then assumed that the opacity was produced in the epidermis as proposed by KOROTNEFF and others. However, it is now clear that the opacity is due to the appearance of granules in the mesogloea, as described by CHUN (1888) for *Ceratocymba,* and although it is true that the epidermis is involved in propagation of the response, it is equally apparent that the mesogloea in which the granules form 'must in

a certain sense be capable of transmitting a stimulus' (CHUN, 1888) even if the stimulus is only a chemical one speading downward into the mesogloea from excited epidermal cells.

A description of the histology of the epidermis covering the nectophores was given earlier, along with an account of its capacity for propagated depolarizations (MACKIE, 1965). We shall here provide a general description of blanching, with details on the histology and ultrastructure of blanching mesogloea in *Hippopodius* and with some additional information on mesogloeal structure in gelatinous planctonic hydrozoans of other species.

MATERIAL AND METHODS

Hippopodius were dipped from the sea in plastic or glass vessels and quickly transferred to large tanks of slowly circulating water in the dark at 14 °C, where they stayed in good condition for several days.

Observations on living tissue were made using Zeiss darkfield and phase contrast equipment. A Zeiss 'Ukatron' flash attachment was used in making Figs. 9 A and B. Paraffin sections of fixed material were examined. Material was also fixed in 2 % OsO_4 and embedded in Araldite or Epon according to standard procedures. 1 µ sections were stained with toluidine blue for light microscopy. Ultrathin sections were examined with a Philips EM 100 after staining with uranium, lead or tungsten salts.

RESULTS

General Description of Blanching and Allied Responses

The two photographs, Figs. 2A and B, show a specimen of *Hippopodius* before and after stimulation. In A the colony is floating the right way up, near the surface, with transparent nectophores. The stem and its appendages are partially relaxed and hang below the nectophores, where they are seen as an opaque mass. In B, after a blow from a glass rod, the animal has contracted its stem, pulling it up into the central space enclosed by the nectophores. This has altered the centre of gravity and the specimen is now

upside down (QUOY and GAIMARD, 1827; JACOBS, 1937). The stimulation has caused opacity in the majority of the nectophores. Not shown in the photograph is the curling inward of the margins of the nectophores which also takes place following exumbrellar stimulation (MACKIE, 1965). In addition, if the specimen were observed in the dark, a brief flash of luminescence would be seen to take place on stimulation.

It was shown previously (MACKIE, 1965) that the muscular response in the margin can be obtained by stimulating any part of the exumbrellar epithelium of a nectophore. Tactile or electrical stimulation may be used. In the latter case, the shock can be given by electrodes that are not quite touching the epithelium. Following such a stimulus, an electrical event is propagated over the epithelium, travelling at a velocity of 30-40 cm/sec. This potential change can be recorded directly by an electrode in the vicinity of the epithelium. The epithelium is in part syncytial, in part cellular, and it varies in thickness and in granularity over different areas of the nectophore, but the cytoplasm is without myofibrils and there are no nerve cells in any part of the exumbrella. Non-nervous conduction in exumbrellar epithelia has been found in several siphonophores and it also occurs in hydromedusae (MACKIE, PASSANO and PAVANS DE CECCATTY, 1967).

Conduction within a single nectophore appears to be all-or-none following a single shock. This can be demonstrated by direct recording from the epithelium or by recording of the muscle potentials evoked at the margin. Each exumbrellar shock evokes a muscle potential or a sequence of potentials, even when the margin is curled up in a state of tonus as a result of previous stimulation. The opacity produced by applying a single shock to the exumbrella of a transparent specimen does not, however, increase in density with subsequent stimulation, although resumption of transparency can be delayed indefinitely by repeating the stimulus. In a whole colony, a single tactile stimulus may cause a muscle response and blanching in one or a few nectophores, several stimuli being needed to cause the animal to blanch all over. However, examples were seen where a single tactile stimulus appeared to get through to all nectophores and it is possible that through-conduction can occur between adjacent nectophores as well as within one nectophore. It is not known whether a tactile stimulus elicits a single electrical potential or a series in such cases, and we cannot therefore say if through-conduction or facilitation is involved. Repetitive firing to a single shock has been found in the epithelia of *Sarsia* (MACKIE, PASSANO and PAVANS DE CECCATTY, 1967).

Experiments were carried out which showed that an intact covering epithelium is needed both for the propagation of blanching

and for the subsequent restoration of transparency. Such an experiment is illustrated in Fig. 4. An island of intact epithelium is isolated by a circular abrasion. The nectophore blanches all over as a result of the tactile stimulation caused by the operation (A), but after several minutes the regions covered with epithelium become transparent. The ring of denuded mesogloea remains white (B). If a stimulus is now given at point S, the peripheral region re-blanches, but the central island remains transparent (C). Nectophores show permanent blanching wherever the epithelium is damaged or removed. The epithelium can regenerate and cover such areas, and transparency may then be restored.

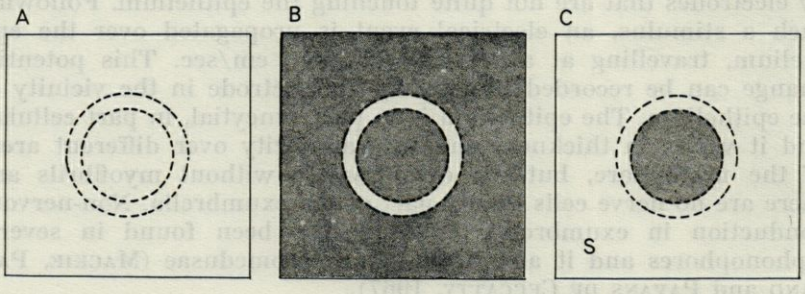


FIG. 4. (See text). Experiment demonstrating the role of the covering epithelium in the propagation of blanching and in subsequent resumption of transparency.

Blanching is not immediate following the passage of an electrical event, but takes 1-2 seconds to build up to full density. Deblanching usually takes 15-30 minutes, and may take much longer. Some regions remain opaque longer than others. Specimens that are not completely fresh may take abnormally long to deblanch and their subsequent blanchability may be less than that of fresh specimens, but exact figures were not obtained on these points. A form of non-propagated blanching is frequently seen, which will be called ' spot-blanching '. A light touch with a probe will produce a small white spot, without causing overall blanching. An animal floating in a tank frequently spot-blanches in areas where it has touched the walls of the tank. These areas usually become transparent again when the animal is freed from contact with the tank. It is possible that in some cases spot-blanching is the result of damage to the epithelium and exposure of the mesogloea directly to seawater, which we have already seen to cause irreversible blanching. However, the possibility should be borne in mind that the epithelium may respond to sub-threshold stimuli by local, non-propagating potential changes which evoke blanching in the immediate locality.

Like propagated blanching, luminescence is only exhibited in regions where the epithelium is intact. A single flash lasts about 1 sec., but with continued agitation, such as stirring the specimen in its bowl, almost continuous emission can be obtained. Specimens can luminesce immediately following transfer from light to darkness, which is not the case in luminescent ctenophores (MOORE, 1926). The colour of the light is best described as 'ice-blue'. It is quite unlike the greenish luminescence shown in the marginal light-organs of hydromedusae, but closely resembles the generalized subumbrellar luminescence of the jellyfish *Euphysa flammea*, which is also produced over a wide area of epithelium (MACKIE and MACKIE, 1963).

Luminescence, blanching, the responses of the musculature and of the radial muscle fibers of the nectophore margins are clearly dependent on epithelial conduction in the exumbrella. All three can be elicited by stimulation of specimens kept for 1 hour in 1 : 1 isotonic $MgCl_2$ - sea water. We have given figures elsewhere (MACKIE and MACKIE, 1963) on the sensitivities of various responses in hydromedusae to Mg^{++} anaesthesia and have shown that some effector systems are blocked almost immediately, while others are blocked only slowly if at all. Studies on sea anemones (ROSS and PANTIN, 1940), scyphomedusae (BULLOCK, 1943) siphonophores (MACKIE, 1964) and hydroids (JOSEPHSON, 1965) suggest that high susceptibility to excess Mg^{++} is an indication that nerves are involved. Thus low sensitivity of the *Hippopodius* responses accords with the general picture of non-nervous conduction. Swimming, however, in which the circular muscle system of the nectophores is involved is blocked rapidly by anaesthesia and is presumably controlled by the marginal nerve rings at the base of the velum, which are well-developed in the nectophores of all the siphonophores we have examined, including *Hippopodius*.

We were able to examine a eudoxid bract of *Bassia*, and to compare it with *Hippopodius*. The specimen examined was obtained from a plankton haul and showed some damage. The epithelium was intact over most regions however. Opacity was shown along the ridges (Fig. 6), as described by HUXLEY (1859). Removal of epithelium from transparent areas did not produce spot-blanching or overall blanching. The opaque areas remained opaque after several hours in which it was kept in still water. Thus, opacity appears to be permanent in the ridges and other regions lack blanchability. MOSER (1925) suggested that the blanched regions might be luminescent in *Bassia*, but this was not the case in the specimen we examined.

Morphological Basis of Blanching and Mesogloeal Ultrastructure

There are various reasons for stating that blanching occurs in the mesogloea where it is associated with granulation as Köl-liker (1853) suggested and that it is not produced in the epithelial cells as Korotneff (1884), Chun (1897), Dubois (1898) and Iwantzoff (1928) maintained :

1. Removal of the epithelium does not eliminate opacity, but renders it permanent.

2. Observation of living preparations by darkfield and phase contrast microscopy shows that granules appear in the mesogloea during blanching and disappear when transparency is restored (Fig. 3A and B). The epidermis, though it contains secretion bodies, shows no comparable changes, and is never opaque.

3. The mesogloeal granules appear in a surface layer whose depth varies in different parts, these differences being correlated with local differences in blanching intensity. A thick, strongly blanching layer is found on the superior and lateral abaxial sides; a thinner, more feebly blanching layer occurs over the axial side and subumbrella. In one region which does not blanch, granules are not observed. This is the region adjacent to a plexus of canals formed by dichotomy of the ventral, radial, endoderm canal in the subumbrella, known as the '*rete mirabile*' (Totton, 1965).

4. Siphonophores which do not blanch do not show granules in the mesogloea. *Ceratocymba,* which blanches reversibly, has granules (Chun, 1888). *Bassia* which has permanently opaque edges to its gelatinous members has been examined and granules have been found in the mesogloea of the opaque regions (*vide supra*).

Blanching then is caused by reversible granulation of an outer layer of the mesogloea in *Hippopodius*. Transparency depends upon the optical homogeneity of the transmitting substance, and the introduction of dense granules will thus have a light-scattering effect and will lead to opacity whatever the colour reflected by the granules. However, the granules themselves look both opaque and white when studied individually under incident light. The term 'milk-white' favoured by writers using the German language is peculiarly appropriate since it suggests the faint bluish overtones actually seen.

Blanched areas viewed under the microscope sometimes show an uneven distribution of granules. At the edge of a spot-blanch there is often a thin line of dense granulation. Where a spot-blanch

has been inundated by a wave of general blanching this dense line may still be seen and the area just beyond it may be less dense than the general background (Fig. 3A and B). Specimens observed during the deblanching process fade by gradual disappearance of granules, the more heavily granulated regions fading last. No local movement or general migration of granules has been observed. The granules merely cease to be visible. It is not clear if they do so by becoming smaller or by losing opacity. Under the 100X phase objective no evidence was seen for small, permanent ' nuclei ' which might serve as deposition sites in the ' development ' of the granules. At the fringe area where transparent mesogloea covered with epithelium lies beside a denunded (and hence opaque) patch, the granules are seen to extend a short distance underneath the epithelium. The ions or molecules which cause onset of granulation must therefore be assumed to be capable of limited diffusion horizontally beneath the epithelium as well as vertically downward into the blanching layer.

The granular layer varies from about 5 μ to about 55 μ in thickness in various parts of the nectophores. In the inner, transparent substance of the nectophores granules are rarely seen. The granules observed under the light microscope in sections and in whole mounts range in size from barely visible particles to a typical, larger particle size of about 5 μ. A few granules of up to 1.5 μ have been seen in a young nectophore. The granular layer is clearly demarcated from the other regions of the mesogloea, and these differences are well seen in paraffin sections of material fixed in 10 % formalin followed by postchromation. This treatment also preserves the epithelium in its correct position, preventing it from separating from the mesogloeal surface. Mallory staining shows an intensely blue basement membrane immediately beneath the epithelium, below this a less intense but still strongly blue layer about 3.0 - 5.0 μ thick and lacking granules, and below this the granular layer with a pale blue matrix and red granules. Below the granular layer lies a pale blue, agranular central substance, the typical ' jelly ' of hydromedusae and siphonophores. These zones are also shown well in 1 μ sections of araldite-embedded material stained in toluidine blue (Fig. 5).

The umbrellar mesogloea of these nectophores is a wholly extracellular material, and unlike that of some hydromedusae (e.g. *Proboscidactyla*), it contains no free or bridging cells, nor does it contain cytoplasmic extensions from the cells of the bordering epithelia such as are found in the mesolamella of hydroids. It does, however contain conspicuous fibres which run in an orderly way across the mesogloea, branching at their tips where they approach the mesogloeal surface (Figs. 7, 8). GEGENBAUR (1959) shows por-

tions of these fibres in a drawing of *Hippopodius* jelly and similar fibres occur in many other siphonopores and hydromedusae. KÖLLIKER (1865) provides an excellent drawing of them in *Aequorea* in his review and classification of mesogloeal types. Very similar fibres occur in Scyphomedusae, sometimes reaching diameters up to 5 μ (HERTWIG and HERTWIG, 1878). A succession of workers from KÖLLIKER onward have agreed in describing these as elastic fibres. When the mesogloea is damaged the fibres assume a convoluted or cork-screw form, where previously they ran in sweeping curves or straight lines. It has always been supposed that they have an architectural function, serving to provide the mesogloea with its spingy and resilient consistency. In more recent times CHAPMAN (1953) has suggested that the fibres are collagenous, a view he maintains (CHAPMAN, 1959), in spite of new evidence presented to the contrary (BOUILLON and VANDERMEERSSCHE, 1956). Our findings may be summarized as follows :

Collagen is certainly present in the mesogloea of *Hippopodius* as shown by X-ray diffraction studies (RUDALL, 1956). Under the electron microscope, a fine background fibrillar mesh is seen (Fig. 13) which is assumed to be collagen although periodic banding has not been seen in these fine, isolated fibrils. The fibrils are 50-80 Å in diameter, and lack regular orientation in most parts. Near the mesogloeal surface however, they are denser, and may tend to run parallel to the surface. They show no tendency to form bundles of dimensions which would permit their observation under the light microscope, and they are quite independent of the elastic fibres. It is of interest to note in this connection some observations we have made on *Euphysa flammea* (O. Anthomedusae). In this medusa, the background collagen mesh closely resembles that of *Hippopodius* but in this case the fibrils aggregate in certain regions to form sizeable fibres up to 1 μ in diameter which can be seen with the light microscope. Under the electron microscope, the fibres can be seen to be cross striated (Fig. 12) although the striations have not been detected in the dispersed fibrils with which they are connected. The fact that *Euphysa* possesses optically visible collagen fibres might appear to support Chapman's contention that the large fibres of other jellyfish are collagenous. However, the reverse is true. The collagen fibres in *Euphysa* differ from the fibres of other forms in showing no tendency to assume convoluted forms, in being periodically striated, in staining strongly with basic dyes and in showing strong electron density after osmium fixation without supplementary impregnation with other metallic stains. So far, *Euphysa* is the only form known in which the fibres are clearly collagenous. In other hydromedusae, the collagen fibrils are dispersed and no striations are seen (CHAPMAN, PANTIN and ROBSON, 1962;

KAWAGUTI and HAMAKOSHI, 1963), as in *Hippopodius* and the other siphonophores we have studied. Collagen fibres in coelenterates vary widely in their banding periodicity : 200 Å in *Metridium sp.* and *Physalia* (PIEZ and GROSS, 1959), 220 - 250 Å in *Metridium senile* (GRIMSTONE, HORNE, PANTIN and ROBSON, 1958), 420 - 460 Å in * *M. canum* (BATHAM, 1960), 640 Å in the medusae studied by BOUILLON and VANDERMEERSSCHE (1957), and 640 Å in *Pelagia* (CHAPMAN, 1959). The periodicity observed in our preparations of *Euphysa* is 260 Å. Much of this variation is probably due to differences in the preparation techniques employed or in the age of fibres.

The elastic fibres of siphonophores are shown in Figs. 7, 8 and 11, all in the contracted condition. They branch at their tips, the branches penetrating the granular layer in *Hippopodius*. They run across the mesogloea from one side to the other, and are concentrated in regions adjacent to the subumbrellar muscle layers. In 1956, samples of mesogloea from these regions where the fibres are densely aggregated were sent to Dr. K. M. RUDALL for X-ray diffraction study along with samples from non-fibrous regions. In the latter, collagen was abundant, but in the former only traces were found and other material, which could not be defined, was 'obviously present' (RUDALL, 1956). This non-collagenous component was presumably the elastic fibres.

The fibres are chemically inert, judged by their failure to stain with histological dyes, and by the difficulty with which they are seen under the electron microscope, even after lead and uranium staining. In whole mount preparations treated by a reduced silver method (Fig. 7) the silver precipitates on the fibres, but this is not necessarily an indication of chemical affinity. Chemical inertness is a characteristic of mature elastin in vertebrates, and this, coupled with the obvious mechanical elasticity of these fibres and their lack of periodic banding reinforces the evidence put forward by BOUILLON and VANDERMEERSSCHE that they are a form of elastin.

Mention should be made of the agranular zone adjacent to the *rete mirabile* (see above, p. 54). The mesogloea here stains a deeper blue in Mallory slides than elsewhere, suggesting proximity to a region of mesogloeal synthesis. The nuclei of the *rete* are exceptionally large and are often lobulated or fragmented (CHUN, 1890; MÜNTER, 1912). They are strongly Feulgen positive, with conspicuous chromonemata. Cells of this type are not uncommonly found in tissues showing high synthetic or metabolic activity. The *rete* is large and covers most of the subumbrella roof in young necto-

(*) This sea-anemone has been renamed *Mimetridium cryptum* (HAND (C.), 1961. Two new acontiate New Zealand sea anemones. *Trans. roy. Soc. New Zealand*, 1, 75-89).

phores but in those which have reached their definitive size the *rete* is vestigial, which implies that functional activity is greatest in the growing stage. Growth of nectophores is primarily a matter of expansion of the mesogloea. The covering cellular layers merely stretch out and become thinner. There will certainly be secretion of mesogloeal materials during growth, and it is likely that the *rete* carries it out. BOUILLON and VANDERMEERSSCHE (1956) also implicate the endoderm in mesogloeal synthesis in medusae.

The granules responsible for blanching, seen under the electron microscope, appear as electron-dense objects without any detectable sub-structure. They range in size from about 100 to 500 mμ, and are usually more or less spherical in form. They lie scattered in the collagen mesh, the fibrils appearing to adhere to them. Comparison of superficial mesogloea in *Hippopodius* and *Chelophyes* (which does not blanch) by electron microscopy reveals two major differences : the absence of granules in *Chelophyes* and the absence of a distinct basement membrane layer. In *Chelophyes*, the region immediately beneath the epidermal layer is occupied by a layer of regularly arrayed collagen fibres, and there is nothing comparable to the thick, electron-dense basement membrane (MACKIE, 1965). Whether this layer in *Hippopodius* is significant in terms of the blanching reaction has yet to be determined.

The mesogloea of *Hippopodius* thus contains three microscopically distinct components : 1. Granules responsible for blanching, 2. fine, dispersed fibrils, presumed to be collagen and 3. thick, branching fibre bundles, presumed to be a form of elastin. BOUILLON and VANDERMERSSCHE (1956) demonstrated the presence of polysaccharides in the mesogloea of medusae, and on general grounds it may be assumed to be present as a fourth component, perhaps constituting a major component of the basement membrane in the case of *Hippopodius*.

EFFECTS OF REAGENTS

The supply of *Hippopodius* failed suddenly in late May 1964 before a clear picture of the chemistry of blanching could be obtained, but it is doubtful if in any event, with the rather simple methods adopted, much progress would have been made towards explaining the reactions involved. Blanching is apparently something quite unique, and needs serious biochemical study.

If a pinch of versene (E.D.T.A.) is added to sea water containing a strip of the granular layer, freed of its covering epithelium, the

strip deblanches within a few minutes. If the strip is then washed in clean sea water, blanching is restorted. It was found that acidification of the water has the same effect, and it therefore seemed possible that versene was causing deblanching because of its acidity. However, .02 % versene buffered to pH 7.9 with 0.1 M boric acid/sodium borate buffer also causes deblanching, so the mechanism is distinct from that given by acidification, and is perhaps due to chelation of Ca^{++}. Controls maintained in borax-buffered sea water did not deblanch.

Tests with natural sea water adjusted to various pH levels by addition of small quantities of HCl or NaOH indicated that maintenance of full blanching requires a pH over 7.0. Between pH 5.0 and 6.5 deblanching is rapid and complete. Below about pH 3.5 deblanching does not occur. Degrees of deblanching and the times taken to deblanch vary with pH, but accurate estimation of these variables was not obtained.

Deblanching produced by acids is reversible. Material can be washed in sea water and the granules reappear, and this can be repeated several times with some fall-off in the intensity of the response. Restoration of blanching following deblanching is not obviously dependent on a particular balance of ions, because isotonic NaCl adjusted to pH 7.9 with NaOH is effective in bringing it about. The nature of the acid or base used does not seem to be critical. Oxalic, citric and acetic acids all caused deblanching and sodium borate, sodium hydroxide and sodium bicarbonate all restored blanching.

The tonicity of the sea water does not seem to be very critical. Sea water concentrated by evaporation to 63 $^{0}/_{00}$ was buffered to pH 7.9 using one part of 0.1 M boric acid-borax buffer to ten parts of water. Mesogloeal strips transferred to this solution remained blanched, and when deblanched with versene and returned to the solution, recovered their blanched condition. Sea water diluted to 12 $^{0}/_{00}$ was buffered at pH 7.9 and strips placed in it retained their blanched condition. However, deblanched pieces seem slow to recover their opacity when washed in this solution.

Some observations were made on tissue strips using small crystals placed directly on the mesogloeal surface. Crystals of versene, citric acid and oxalic acid placed on the mesogloea produce similar effects (Fig. 9A and B). As the crystal begins to dissolve, a halo of deblanching appears around it, and gradually spreads out, but the area immediately under and around the crystal remains blanched until the crystal has dissolved completely. The two pictures were taken 2-1/2 minutes apart by electronic flash arranged to give a darkfield effect. It will be seen how the de-

blanching wave spreads out and invades surrounding areas. The retention of blanching in the area of highest solute concentration was noted in all cases. It is tempting to align this observation with the finding noted above that sea water below pH 3.5 fails to produce deblanching. An effect not shown in the photographs was seen in tests with citric acid crystals : the advancing halo of deblanching which spreads out around the crystal may show a dense white outer fringe at first, as if there was a transitional intensification of blanching which precedes deblanching. Mixtures of acid and basic crystals sprinkled on the surface of the mesogloea compete locally, producing interesting patterns.

All the foregoing tests were carried out on mesogloea from which the covering epithelium had been removed. If the epithelium is left intact it protects the granular layer to same extent and will delay the effects of versene and acids.

Some whole nectophores were fixed in alcohol or formalin and were then treated with acids and versene in concentrations which could cause deblanching of fresh material. No deblanching occurred, and the granules in this fixed material appeared insoluble. This suggests that they are not calcareous particles.

DISCUSSION

Coelenterate mesogloea can be regarded as a material equivalent to the acellular component of the connective tissue of higher animals and like the latter it varies widely from one species to the next and often in different regions of the same animal. KÖLLIKER (1865) clearly recognized this in his review and classification of mesogloeal types. Cells may be present in the mesogloea, but, in the large number of cases where they are not, it must be assumed that the covering epithelia in whole or in part are specialized as the source of mesogloeal materials. The consistency of the mesogloea varies from a flabby gel to a tough membrane to a rigid, calcified endoskeleton. By analogy with vertebrate connective tissues, these variations would appear to be due to differences in proportions of the fibrous proteins, proteinpolysaccharides, acid mucopolysaccharides, water and mineral salts present. The material is usually elastic and resilient but this is a variable factor. Scyphozoan jelly is more elastic than the mesogloea of sea anemones (ALEXANDER, 1965). *Hydra* mesogloea is tough, fibrous, elastic and resists penetration (SHOSTAK, PATEL and BURNETT, 1965). Elasticity may

be associated with the presence of specialized elastic fibres or it may be due to the way in which relatively inelastic collagen fibres are woven together into a yielding fabric. A whole spectrum of physical consistencies and textures is in evidence. If jellyfish mesogloea is incised, fluid collects quickly in the cut, showing that mesogloeal fluid in this case is free to move within the substance of the mesogloea. This fluid is low in SO_4^{--} ion (Robertson, 1949), a deficiency which probably contributes to buoyancy (Denton and Shaw, 1961). Lenhoff (1964) has shown that extracts of *Aequorea* mesogloea contain a substance which inhibits swimming in *Stomotoca*. Now, we have a specialized mesogloeal blanching system in *Hippopodius* and *Ceratocymba* as yet another example of the remarkable versatility of this material.

We have shown in this paper that the phenomenon of blanching is due to the formation of light-scattering granules in the mesogloea. The covering epithelium is clearly involved in the propagation of the response and in the subsequent restoration of transparency. These is, however, little to go on in attempting to explain the actual mechanisms involved in the formation and disappearance of the granules. It is unlikely that activation involves a diffusible, chemical substance secreted by the epithelium, since blanching can be turned on and off repeatedly by pH adjustments in mesogloea deprived of its epithelial covering. Possibly pH changes in the blanching layer are induced by the passage of the electrical change in the epithelium. According to the so-called 'Wien effect' alteration in the strength of an electrical field penetrating a medium containing acids in solution can cause an alteration in the pH of the medium. On the other hand, the experiments with versene suggest that calcium ions may be admitted into the mesogloea during the epithelial event, triggering granule formation in the blanching layer. Deblanching might thus represent pumping out of calcium ions by the epithelium. It would evidently be a slow process, much longer than the refractory period of the epithelium where impulse transmission was concerned.

Assuming that the granules are proteinaceous, the protein can be assumed to exist either in a dispersed form or, when aggregated, as visible granules. Such a change could be evoked by slight ionic changes in the mesogloeal milieu, causing an alteration of the charge on the dispersed molecules and making them attract instead of repel one another. In attempting to elaborate some such hypothesis we are handicapped by a lack of precise knowledge on the composition of the mesogloeal ground substance and the rapidity with which diffusion of materials can occur within it. It is perhaps unfortunate that workers on mesogloea have devoted more attention to the mechanical properties of the material, in

particular to its fibrous proteins, and less to questions of permeability and ion content.

A second discussion point concerns the role of blanching in the life of *Hippopodius*. Spectacular adaptations of planktonic animals such as transparency, luminescence, colour change, etc. are generally assumed to have evolved because they confer definite advantages on their possessors. It is not axiomatic that this must be so, but from what is known of the rigor with which natural selection operates in highly competitive situations of this sort it is unlikely that these phenomena have arisen and persisted meaninglessly. In the case of blanching we are dealing with one component of what is clearly a generalized protective response. The muscle responses are both protective. The curling in of the margin of the nectophores protects the most vulnerable region of these members. The drawing in of the stem protects it and the attached feeding and reproductive members from damage. Thus it may be assumed that the blanching and luminescent flash which accompany the muscle responses are also defensive in some way.

Hippopodius feeds on microscopic plankton and has no obvious means of defence against larger animals. Its one asset is its large size. It is well known that many birds, fishes and mammals show threatening or defensive displays involving erection of feathers, fins or hair which give the animal a larger and more formidable aspect. *Hippopodius* is normally almost invisible, but in the blanched or luminescent condition it looms up suddenly as a very large object, and would be avoided by schools of small fishes, euphausids, etc. which otherwise might collide with it and damage it severely. The nectophores are very vulnerable to damage, having a covering epithelium only a few microns thick and lacking defensive spines or nematocysts. (*Vogtia,* a close relative, does not blanch, but has conspicuous spiny projections). Prevention of damage to the epithelium is probably important in more than one way. The epithelium is part of the stimulus-transmitting system (Mackie, 1965). Also, it may be involved in adjusting the contained mesogloea from the point of view of buoyancy. Jacobs (1937) has shown that *Hippopodius* can make itself more or less dense, and since there is no adjustable gas-filled float such as some physonectid spihonophores possess, it must be assumed that the buoyant material, which is here the mesogloea, is susceptible to density adjustment. The findings of Denton and Shaw (1961) suggest that active transport of lighter and heavier ions across the covering epithelium may here be responsible for density changes. Damage to the epithelium would presumably lead to an increase in density and descent into deeper and inhospitable water layers. Damaged

and moribund *Hippopodius* sink, while fresh ones can float without muscular exertion.

The question arises, if blanching is advantageous in making the animal visible, why is the blanched condition not retained permanently ? It can only be supposed that the organism passes much of its time in situations where it is not threatened by collision and that invisibility here offers important advantages, perhaps in the business of food capture.

The bulk of this study was carried out at the Station Zoologique, Université de Paris, at Villefranche-sur-Mer during the spring of 1964 and we are indebted to Prof. Paul BOUGIS, Sous-Directeur, and to his Assistants and personnel for providing for our research needs and general well-being. M^lle^ Mari-Luz HERNANDEZ and Mrs. Leah MITCHELL helped with the electron microscopy. Prof. Max PAVANS DE CECCATTY was instrumental in obtaining a photostat of the relevant passages from the rare work by Raphaël DUBOIS (1898). Mr. A. K. TOTTON and Dr. L. M. PASSANO collaborated in certain phases of the study, and their comments and suggestions were of great value. M^me^ Sabine BOUSANI drew Fig. 1, which is here reproduced from the *American Zoologist* by permission of Dr. Sears CROWELL. The strip to Villefranche was made possible by the award to G. O. MACKIE of a Post-Doctoral Overseas Research Fellowship by the National Research Council of Canada. The Council also made the award of an Operating Grant which provided for various research expenses, particularly use of the " Maurice Bedot " for collecting material in the environs of Villefranche and St. Jean - Cap Ferrat.

SUMMARY

Hippopodius, a transparent siphonophore, becomes opaque following tactile or electrical stimulation. This opacity is caused by the appearance of light-scattering granules in mesogloea of the nectophores. The response is a reversible one. Although the granules are produced extracellularly, transmission of the opaque response (' blanching ') depends on the presence of intact covering epithelium, which is a non-nervous conducting tissue also capable of luminescence. Disappearance of the granules and resumption of transparency (' deblanching ') also depends on the covering epithelium. The mesogloea contains two sorts of fibrous components, regarded as collagen and elastin, as well as the granules responsible for blanching. All three have been studied by optical

and electron microscopy. Experiments on the granules in living mesogloea show that their appearance and diasappearance is influenced by pH changes, and by E.D.T.A. They are regarded as proteinaceous bodies. The biological significance of blanching is discussed.

RÉSUMÉ

Hippopodius est un Siphonophore transparent qui devient opaque à la suite d'une stimulation tactile ou électrique. L'opacité est due à l'apparition, dans la mésoglée des nectophores, de granules qui diffusent la lumière. Cette réponse de « blanchiment » est réversible. Quoique les granules soient extracellulaires, la transmission de la réponse d'opacité dépend de l'intégrité de l'épithélium de recouvrement qui est un tissu conducteur aneural, aussi capable de luminescence. La disparition des granules et le rétablissement de la transparence (le déblanchiment) dépendent aussi de cet épithélium. La mésoglée contient deux sortes de composants fibreux, considérés comme du collagène et de l'élastine qui, comme les granules de diffusion, ont été étudiés en microscopies photonique et électronique. L'expérimentation *in vivo* sur les granules de la mésoglée montre que leur apparition et leur disparition sont influencées par les changements de pH, et par l'E.D.T.A. Ils sont considérés comme des éléments protéiques. La signification biologique du blanchiment est enfin envisagée.

ZUSAMMENFASSUNG

Hippopodius, eine transparente Siphonophore, wird undurchsichtig wenn man sie durch Berührung oder elektrisch reizt. Die Opazität wird hervorgerufen durch das Auftreten von lichtstreuenden Körnchen in der Mesogloea der Schwimmglocken. Die Reaktion ist reversibel. Obwohl die Körnchen ausserhalb der Zellen entstehen, hängt die Übertragung der Opazität-Reaktion (« Milchweisswerden », « blanching ») von der Anwesenheit eines unversehrten Deckepithels ab, welches ein nicht-nervöses Leitgewebe darstellt, das auch Lumineszenz zeigt. Auch das Verschwinden der Körnchen und Wiederscheinen der Transparenz (« deblanching ») hängt vom Deckepithel ab. Die Mesogloea enthält zwei Arten von Faserkomponenten, die als Kollagen und Elastin angesehen wurden, und die

Körnchen die für das Milchweisswerden verantwortlich sind. Alle drei wurden mit Mikroskop und Elektronenmikroskop erforscht. Versuche an den Körnchen in der lebenden Mesogloea zeigen, dass ihr Auftreten und Verschwinden von Veränderungen im pH und von E.D.T.A. beeinflusst werden. Es wird angenommen, dass die Körnchen eiweissartig sind. Die biologische Bedeutung des Milchweisswerdens wird besprochen.

REFERENCES

ALEXANDER, R. McN., 1963. Visco-elastic properties of the mesogloea of jellyfish. *J. exp. Biol.*, **XLI**, 363-369.

BATHAM, E.J., 1960. The fine structure of epithelium and mesogloea in a sea anemone. *Quart. J. micr. Sci.*, **CI**, 481-485.

BOUILLON, J. and G. VANDERMEERSSCHE, 1956. Structure et nature de la mésoglée des Hydro- et Scyphomeduses. *Ann. Soc. roy. zool. Belg.*, **LXXXVII**, 9-25.

BULLOCK, T.H., 1943. Neuromuscular facilitation in Scyphomedusae. *J. cell. comp. Physiol.*, **XXII**, 251-272.

CHAPMAN, D.M., C.F.A. PANTIN and E.A. ROBSON, 1962. Muscle in coelenterates. *Rev. Canad. Biol.*, **XXI**, 267-278.

CHAPMAN, G., 1953. Studies of the mesogloea of coelenterates 1. Histology and chemical properties. *Quart. J. micr. Sci.*, **XCIV**, 155-176.

CHAPMAN, G., 1959. The mesogloea of *Pelagia noctiluca*. *Quart. J. micr. Sci.*, **C**, 599-610.

CHUN, C., 1888. Bericht über eine nach den Canarischen Inseln im Winter 1887-1888 ausgeführte Reise. *S.B. preuss. Akad. Wiss.*, **XLIV**, 1141-1173.

CHUN, C., 1890. Die Bedeutung der direkten Kernteilung. *Schrift. Physik-ökon.* Ges. Königsberg, Jahrg. 31, Sitz.-Ber. April 30, 16-18.

CHUN, C., 1897. Histologie der Hydromedusen *in* Klassen und Ordnungen des Thier-Reichs, Bd. **II**, Abt. 2 (ed. H.G. Bronn).

DENTON, E.J. and T.I. SHAW, 1961. The buoyancy of gelatinous marine animals. *J. Physiol.*, **CLXI**, 14-15.

DUBOIS, R., 1898. Leçons de Physiologie générale et comparée. Paris (Carré and Naud).

GEGENBAUR, K., 1859. Neue Beiträge zur näheren Kenntnis der Siphonophoren. *Nova Acta Leop. Carol.*, **XXVII**. 333-424.

GRIMSTONE, A.V., R.W. HORNE, C.F.A. PANTIN and E.A. ROBSON, 1958. The fine structure of the mesenteries of the sea-anemone *Metridium senile*. *Quart. J. micr. Sci.*, **XCIX**, 523-540.

HERTWIG, H. and R. HERTWIG, 1879. Der Organismus der Medusen. Jena (Fischer).

HUXLEY, T.H., 1859. The Oceanic Hydrozoa. London (Ray Soc.).

IWANTZOFF, N.A., 1928. Beiträge zur Kenntnis der Histologie der Siphonophoren. *Bull. Soc. Nat. Moscou*, **XXXVII**, 1-36.

JACOBS, W., 1937. Beobachtungen über das Schweben der Siphonophoren. *Zeit. vergl. Physiol.*, **XXIV**, 583-601.

JOSEPHSON, R.K., 1965. Three parallel conducting systems in the stalk of a hydroid. *J. exp. Biol.*, **XLII**, 139-152.

KAWAGUTI S. and T. HAMAKOSHI, 1963. Electron microscopic studies on the striated and smooth muscles of an anthomedusa, *Spirocodon saltatrix*. *Biol. J. Okayama Univ.*, **IX**, 127-139.

KÖLLIKER, A., 1853. Die Schwimmpolypen oder Siphonophoren von Messina. Leipzig (Engelmann).

KÖLLIKER, A., 1865. Icones Histologicae oder Atlas der vergleichenden Gewebelehre. Leipzig (Engelmann).

KOROTNEFF, A., 1884. Zur Histologie der Siphonophoren. *Mitt. Zool. Sta. Neapel*, **V**, 229-288.

LELOUP, E., 1935. Les siphonophores de la Rade de Villefranche-sur-Mer (Alpes-Maritimes, France). *Bull. Mus. Hist. Nat. Belg.*, **XI**, 1-12.

LENHOFF, H.M., 1964. Reversible inhibition of swimming in *Stomotoca atra* by mesogloeal extracts of some other medusae. *Biol. Bull.*, **CXXVI**, 115-120.

MACKIE G.O., 1964. Analysis of locomotion in a siphonophore colony. *Proc. roy. Soc. B.*, **CLIX**, 366-391.

MACKIE G.O., 1965. Conduction in the nerve-free epithelia of siphonophores. *Amer. Zool.*, **V**, 439-453.

MACKIE G.O. and G.V. MACKIE, 1963. Systematic and Biological notes on living Hydromedusae from Puget Sound. *Contr. Zool. Nat. Mus. Canada*, Bull. **CXCIX**, 63-84.

MACKIE G.O., L.M. PASSANO et PAVANS DE CECCATTY, 1967. Physiologie du comportement de l'Hydroméduse « *Sarsia tubulosa* » Sars. Les systèmes à conduction aneurale. *C.R. Acad. Sc. Paris*, **CCLXIV**, 466-469.

MOORE, A.R., 1926. Inhibizione della luminescenza nei ctenofori. *Arch. Sci. Biol. Napoli*, **VIII**, 112-123.

MOSER, F., 1925. Die Siphonophoren der Deutschen Südpolar Expedition, 1901-03. *Dtsch. Südpol. Exp.*, **XVIII**, Zool. 9, 1-541.

MÜNTER, H., 1912. Inaugural-Dissertation. Zoologisches Institut, Westfälischen Wilhelms-Universität, Münster.

NICOL, J.A.C., 1958. Observations on luminescence in pelagic animals. *J. Mar. Biol. Ass. U.K.*, **XXXVII**, 705-752.

PIEZ, K.A. and J. GROSS, 1959. The amino acid composition and morphology of some invertebrate and vertebrate collagens. *Biochim. Biophys. Acta*, **XXXV**, 24-39.

QUOY, J.R.C. and J.P. GAIMARD, 1827. Observations zoologiques faites à bord de l'Astrolabe en mai 1826 dans le détroit de Gibraltar. *Ann. Sc. nat.*, **X**, 1-56.

ROBERTSON, J.D., 1949. Ionic regulation in some marine invertebrates. *J. exp. Biol.*, **XXVI**, 182-200.

ROSS, D.M. and C.F.A. PANTIN, 1940. Factors influencing facilitation in Actinozoa. The action of certain ions. *J. exp. Biol.*, **XVII**, 61-73.

RUDALL, K.M., 1956. Unpublished studies on material provided by the

authors, cited in part by L.E.R. Picken, 1960. The organization of cells and other organisms. Oxford (Clarendon Press).

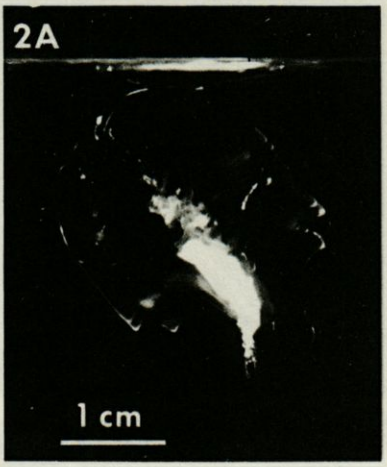
SHOSTAK, S., N.G. PATEL and A.L. BURNETT. The role of mesogloea in mass cell movement in *Hydra. Devel. Biol.*, **XII**, 434-450.

TOTTON, A.K., 1965. A synopsis of the Siphonophora. London (British Museum).

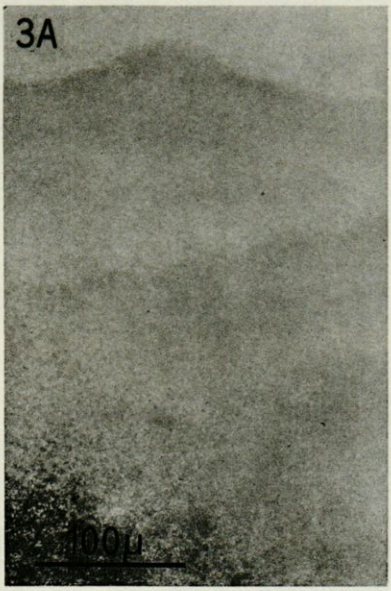
Reçu le 14 octobre 1966.

FIG. 2. A. Unblanched *Hippopodius*. - B. The same specimen blanched.

FIG. 3. A. Blanched mesogloea, showing overlapping waves of opacity. - B. The same, after 5 minutes, showing some loss of opacity.


2A
1 cm


2B


3A
100μ

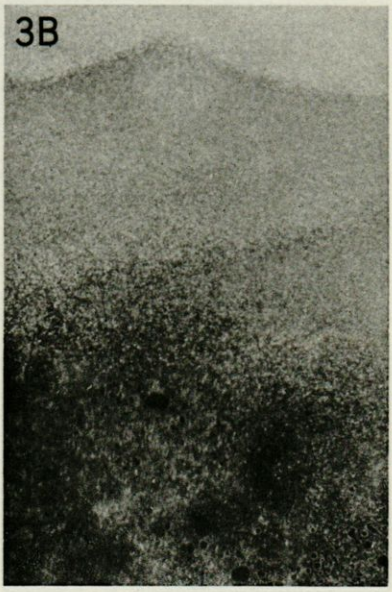
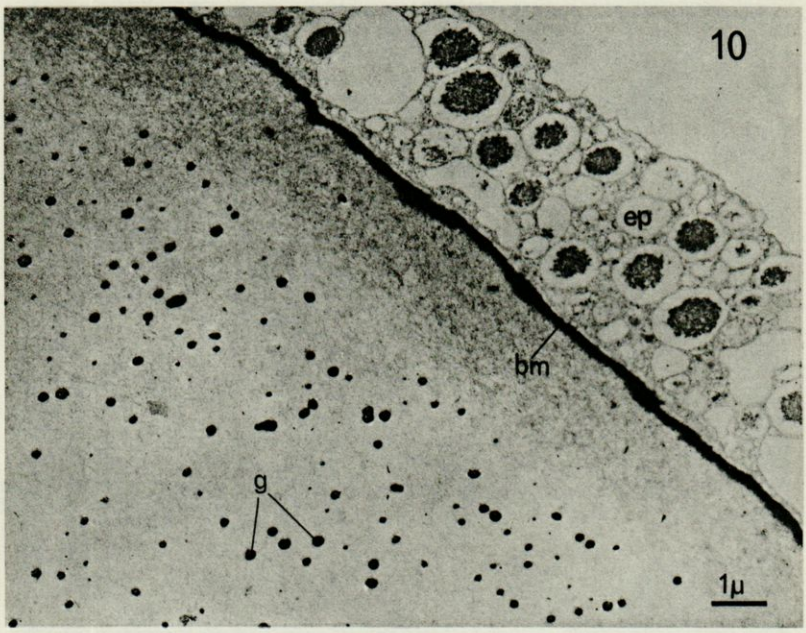

3B

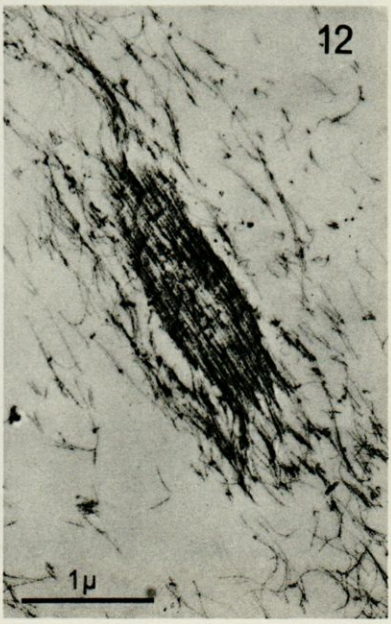
FIG. 10. *Hippopodius*. Low power electron micrograph of area similar to that shown in Fig. 5 (for lettering see Fig. 5) Os_4O fixation. Lead citrate stain.

FIG. 11. *Nanomia cara* (O. Physonectae). Elastic fibre. OsO_4 fixation. Uranyl-acetate and lead citrate stain.

FIG. 12. *Euphysa flammea* (O. Anthomedusae). Collagen bundle. OsO_4 fixation.


10
ep
bm
g
1µ


11
1µ


12
1µ