The Fine Structure of the Mesenteries of the Sea-Anemone *Metridium senile*

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With six plates (figs. 2-7)

SUMMARY

Sections of the retractor and radial face of mesenteries of *Metridium senile* (L.) fixed with osmic, osmic-phosphotungstic, or formol-phosphotungstic fixatives were examined by electron microscopy. Each muscle-fibre forms the basal part of a musculo-epithelial cell and is in contact through the cell membrane with a differentiated surface layer of the underlying mesogloea. The muscle-fibres bear crests of cytoplasm containing mitochondria. Each crest is continuous with a cytoplasmic stem which passes through the intercellular space into the epithelial part of the musculo-epithelial cell. The muscle-fibre consists of densely packed longitudinal filaments 40–80 Å across. The muscle-fibres present a similar appearance over a great range of extension of the muscle. The filaments show no gross periodic structure like that of striated muscle; nor has any fine regular structure like that described in some other 'plain' muscle-fibres yet been detected. There is, however, an irregular beading on the filaments at about 240 Å. Though much smaller, the muscle-fibres of the very slowly contracting radial muscle sheet have the same fine structure as those of the very rapidly contracting retractor muscle. The epithelial part of the cell bears a flagellum arising from a cytoplasmic 'crater'. The flagellum contains vesicles as well as the typical fibrils. It passes continuously into a complex basal corpuscle which continues into a striated fibrous root. Near the epithelial surface there are conspicuous cross-connexions between adjacent cells. Mucus cells and amoebocytes are described. The mesogloeal lattice of Chapman, which permits free extension of the actinian body, is composed of fibres which seem to be built up of tubular fibrils of about 100 Å diameter. The fibrils show well-developed banding at 260 Å. Where fibrils adhere together their banding is congruent. The criteria of correspondence between the electron micrographs and the living structure are discussed, and the value of information from artifacts and varied methods of fixation is noted.

INTRODUCTION

COMPARED with the higher animals, the Actinozoa are very simply organized. The body is built up of sheets of tissue, on each surface of which there is an epithelium, resting upon a middle layer of fibrous mesogloea. This generally contains no cellular elements other than some scattered amoebocytes and, locally, genital cells.

The structure of the mesenteries of *Metridium senile* (L.) is typical of this organization. The fibrous mesogloea separates two epithelia, in this case both endodermal. At its surface of contact with the mesogloea each epithelium bears a sheet of muscle-fibres consisting of a single layer. This muscle-sheet may be folded to produce muscles of considerable size, but even when this is so it

remains essentially two-dimensional, and but one fibre thick. On one surface of the mesentery the fibres run vertically along the oral axis and form the retractor muscle, on the other surface the fibres run transversely (fig. 1, A).

Batham and Pantin (1950 *a* and *b*, 1951) have given an account of the structure and the mechanics of action of these muscle-sheets. Physiologically they are of particular interest because of their great contractility, to less than one-fifth of their extended length, the absence of any fixed resting length between
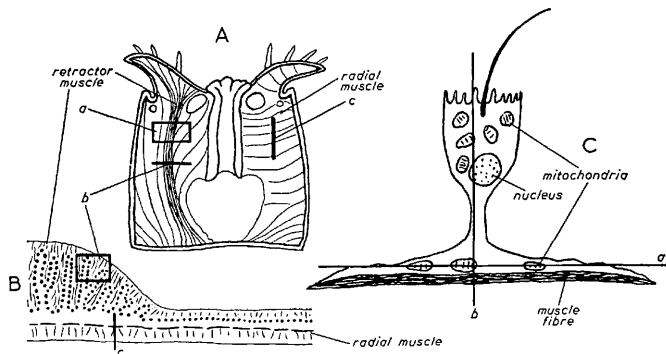

Fig. 1. Diagram to show plane of sections *a, b, c.* A, section through whole *Metridium* exposing two 'perfect' mesenteries. Left shows a retractor muscle surface, right shows a radial muscle surface of mesentery. B, transverse section of part of a 'perfect' mesentery showing retractor above and radial muscle beneath. C, stylized epithelio-muscular cell of mesentery showing basal muscle-fibre with cytoplasmic crest and mitochondria above; and stem connecting this with flagellate epithelial part of the cell.

these limits, and because of the great tension per unit area of cross-section they seem able to exert. All the muscle-sheets can contract slowly, taking a minute or more to develop tension. Under different conditions of excitation some of them can also contract rapidly, developing tension in a fraction of a second.

Robson (1957) has shown that each muscle-fibre of these sheets is the basal part of a musculo-epithelial cell. The superficial parts of these cells form a flagellate pavement epithelium; each epithelial part being connected with its muscle-fibre by a strand of cytoplasm which fans out as a crest along the fibre. Between these strands is a sub-epithelial fluid of considerable mechanical importance.

The mechanical properties of the muscle-fibres must be related to their fine structure. Since their diameter is of the order 0.5 to 1.0μ it is not possible to see this with the light microscope, though it is well suited to the electron microscope. Likewise, the fine structure of the epithelial parts of the cells and of their connecting strands can be investigated only by this means.

Of the structure of the mesogloea to which the muscle-sheets are attached

a good deal is now known. Chapman (1953) working with the light microscope showed that the mesogloea is organized in actinozoans as a three-dimensional lattice which permits great reversible extensibility. The lattice consists of collagen fibres in a fluid medium. The fibres tend to run more or less at $45°$ to the main axes of the animal, and to be arranged in alternating layers running obliquely right and obliquely left. The layers of the lattice are not wholly separate, and individual fibres may be seen to pass from one layer to the other.

Chapman raises the important question of whether this lattice structure could arise by mechanical stresses set up in a matrix of fibrous material, by the movements of the animal. He produces interesting arguments by analogy in support of that. Here again interpretation of the organization must depend upon knowledge of fine structure of the collagen fibres of the mesogloea; and these, seeming to be between $0·1$ and $1·0\mu$, are near the limits of resolution of the light microscope.

Though the actinozoan mesogloea is extensible, it must provide a support for muscle-fibres of the muscle-sheets. Batham and Pantin (1951) showed that the shortening caused by contraction of one muscle-sheet was accommodated by buckling of the opposing muscle-sheet on the other surface of the mesogloea. This sheet was thus passively thrown into folds at right angles to the direction of contraction. They argued from this that the part of the mesogloea in immediate contact with the muscle-layer must have a different and less extensible character from the mass of the mesogloea. There is some histological evidence for the existence of such a differentiated layer. But here again the structure cannot be fully analysed by the light microscope.

The objects of the present paper are thus to throw light on the fine structure of the muscle-fibres and the cells of which they are a part, and to see how far the fine structure of the fibrous mesogloea will account for its special properties.

METHODS

Moderately large *M. senile* were obtained from the Marine Biological Laboratory, Plymouth. The typical procedure was as follows. The *Metridium* was anaesthetized for about 3 to 5 h in sea-water with an equal part of isotonic $MgCl_2$ ($0·36$ M). These animals can recover perfectly after exposure to this mixture for as long as 24 h. The anemone was cut open and a small wax plate was slipped under one of the 'perfect' mesenteries with the retractor face upwards. To avoid distortion the mesentery was pinned round its margin to the wax plate. The preparation on the plate was then cut free from the animal and dropped into fixative.

Three methods of fixation were used:

(1) Some specimens were fixed in ice-cold 1% OsO_4 in sea-water for 30 min. They were then washed in ice-cold sea-water for 15 min. At this stage, small pieces, about $0·5$ mm square or less, of the fixed mesentery were cut out with razor blades under a binocular microscope.

(2) Some specimens were fixed in 1% OsO_4 and washed in sea-water as described above, and then small pieces were transferred to ice-cold 1% phosphotungstic acid in sea-water for about 30 h. They were then washed in ice-cold sea-water for 3 min (see Hanson, 1957).

(3) Some specimens were fixed for about 30 h in ice-cold neutral formalin, made up with sea-water to about 5% CH_2O. Pieces were cut out, washed in sea-water, passed to 1% phosphotungstic acid for 24 h, and washed as before.

Material prepared by each of these methods was dehydrated by passing through ice-cold 50%, 70%, 85%, and 95% ethanol, allowed to warm up to room temperature in the last of these, and then transferred to absolute ethanol at room temperature. Specimens were then passed through several changes of a mixture of butyl and methyl methacrylates (9 : 1 or 3 : 1) and finally into pre-polymerized methacrylate containing 1% benzoyl peroxide. Polymerization was at about 60° C. Flat embedding (Borysko, 1956) was commonly used. Sections were cut on a microtome of the type described by Hodge, Huxley, and Spiro (1954), floated on 25% acetone, and picked up on collodion-coated grids. They were examined without removing the embedding medium in a Siemens electron microscope (Elmiskop I). Orientation was sometimes additionally checked by observation of sections under phase contrast by the light microscope.

The osmic-phosphotungstic preparations gave by far the clearest detail. The fixation with OsO_4 alone was good, but the contrast was weak, particularly in the mesogloea. The formalin-phosphotungstic preparations showed excellent contrast but manifestly imperfect fixation of some fine structures. Useful information was, however, gained by comparison of sections prepared by the three methods. The criteria of good fixation will be discussed later.

RESULTS

Retractor muscle-fibres and cytoplasm

The muscle-fibre is the evidently contractile part of the musculo-epithelial cell. It consists of a single strand attached to the mesogloea. With the light microscope the retractor muscle can be seen to bear a crest of cytoplasm. The crest passes into a narrow stem running to the epithelial part of the cell, which bears a flagellum (fig. 1, c). Between the stems is the intercellular fluid; the 'subepithelial fluid' of Robson. All the cytoplasm contains mitochondria; and these can be stained with Janus green B (Robson, 1957).

Under the light microscope, no essential difference can be seen in the structure of the muscle-fibres from different parts of the actinozoan body, except in size (Batham and Pantin, 1951). In the mesentery, the rapidly acting retractor muscle-fibres are about 1 mm long, whilst the slow radial muscle-fibres are only about 100μ long.

Fig. 2, A shows a transverse section (osmic-phosphotungstic fixation) of part of the highly folded muscle-sheet which constitutes the retractor muscle.

mesogloea · retractor muscle fibre · cell membrane

⊢————⊣ 1μ

cytoplasmic stem · mitochondrion · radial muscle fibre · mesogloea
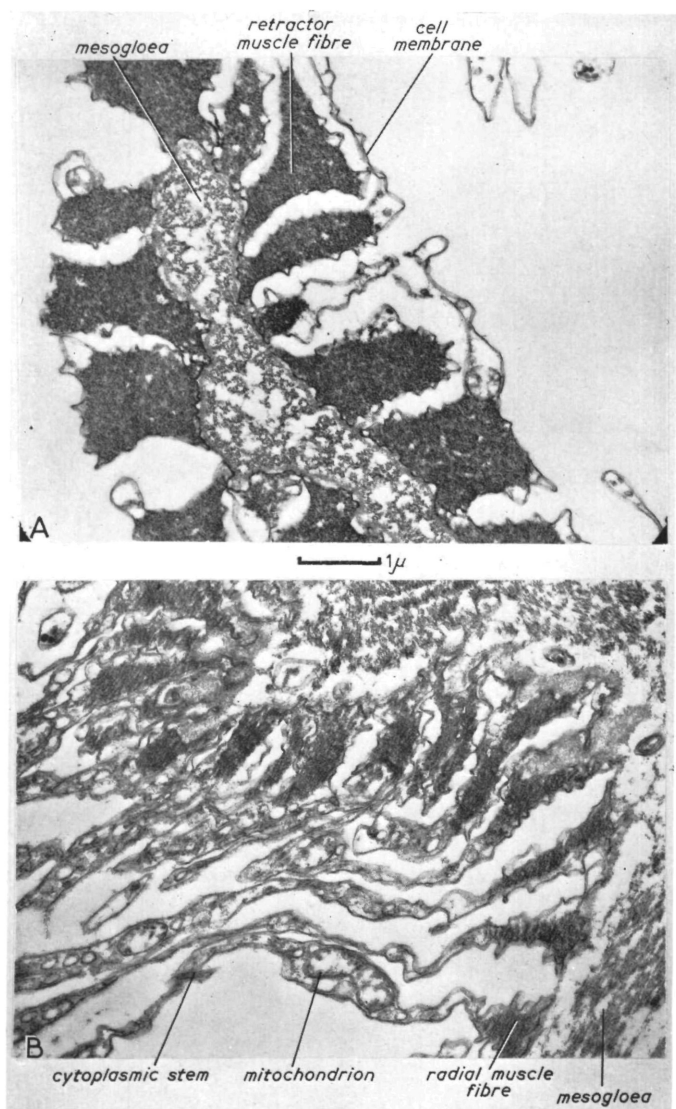
FIG. 2

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The muscle-fibres are well defined and form an almost continuous layer over the mesogloeal surface. Indeed, occasional lateral contacts of the cell membranes between the muscle-fibres suggest that there is some shrinkage in fixation and that in this muscle contiguous fibres form a complete covering to the mesogloeal surface.

In material fixed with osmic alone, adjacent muscle-fibres are often in close contact. And though formolphosphotungstic fixes imperfectly, with this fixation also the cell membranes of adjacent muscle-fibres are commonly in close contact. This apparent close packing of the moderately extended muscle-field contrasts with the open network of fibres seen in whole mounts of fully stretched actinozoan muscle-fields; fig. 2, A may be contrasted with plate 2 of Batham and Pantin (1951). Robson (1957) noted that it might be possible for small cytoplasmic processes from the base of the muscle-cells to provide some attachment between adjacent fibres. Batham and Pantin's evidence that the muscle-field was not a syncytium is reinforced by electron microscope preparations which give many examples of contact but not of syncytial connexion.

The sarcoplasmic crests rising from muscle-fibres are seen in transverse section in fig. 2, A. The crests lie freely in the intercellular space and are bounded by the well-defined cell membrane. Under high magnification the entire cell is seen to be bounded by such a cell membrane about 80 Å thick which, when viewed in strictly transverse section, is clearly double (figs. 4, B; 5, A; 7, D). A variety of methods used on a variety of other cells indicate that the cell membrane is double, about 75 Å thick (Rothschild, 1958). The cytoplasmic crests are often somewhat empty of inclusions, except mitochondria (figs. 2, A; 5 F). The appearance of the cytoplasm and mitochondria resembles that illustrated by Hess and others (1957) in the musculo-epithelial cells of *Hydra*. Occasionally mitochondria seem to be embedded in the substance of the muscle-fibre.

The mitochondria are spherical or somewhat elongated and, as in fig. 5, F, they are commonly about 0·3 to 0·5 μ in diameter. The walls are about 160 Å thick and seem double. In cross-section there are some 5 or 10 cristae, which in favourable sections seem to arise from the inner lamina of the wall and themselves to consist of two laminae separated by 120 Å. No mitochondria densely packed with cristae or with internal tubular structures were seen. The cristae are fewer in *Metridium* than in mitochondria in the higher animals (cf. Sjöstrand and Hanzon, 1954 *a*).

In contrast with the general cytoplasm of the epithelial part of the cell, the muscle-fibre consists of a dense mass of longitudinal filaments. This clearly

FIG. 2 (plate). Osmic-phosphotungstic fixation, same scale for A and B.

A, transverse section of fold of moderately extended retractor (as in fig. 1, A (*b*) and B (*b*)). Note: cell membranes, mitochondria, filaments of muscle-fibres and of mesogloea in cross-section, boundary layer of mesogloea.

B, transverse section of extended radial muscle-sheet of mesentery (as in (*c*) of fig. 1, A, B). Note smaller muscle-fibres, but with similar filamentous structure to that of A, and cytoplasmic stems leading to epithelial part of cell.

corresponds to the densely staining substance of the muscle-fibre described by Batham and Pantin (1951). Using the light microscope, they noted that the fibre sometimes gives the impression of consisting of two halves or fibrils separated by a core of less dense material. No evidence of this was obtained in our present observations and the appearance is doubtless an optical artifact.

In transverse section, the muscle-fibre seems clearly composed of filaments (figs. 4, C, D, E). They are shown by all methods of fixation, though their diameter varies with the fixative. In an extended muscle fixed with osmic-phosphotungstic acid they seem between 40 and 80 Å across. Despite the great contractility of these muscles the filaments of shortened fibres do not seem much thicker than extended ones.

In longitudinal section the filaments of extended fibres seem even thinner (30–40 Å) than in cross-section. Hanson (1957) gives 50 Å for the thickness of the filaments of earthworm muscle. The filaments in *Metridium* show no gross periodic structure comparable to that of striated muscle or indeed of some kinds of smooth muscle. The absence of this is evident in fig. 4, B. But near the limit of resolution available to us, the extended filaments in thin osmic-phosphotungstic sections such as this do show a rather irregularly beaded appearance with an interval of about 240 Å. There seems to be a tendency for the fibres to adhere laterally at these beads. Several fibres connected in this way may give some slight appearance of a transverse banding locally.

However, longitudinal sections are not always easy to interpret. The appearance naturally depends upon the precise angle of the section as well as on the fixation. It also depends upon whether the muscle is in a contracted or relaxed state at any length between the wide limits of the fully extended and the fully shortened muscle.

At all lengths, when in a state of contraction, the muscle seems composed of parallel filaments. But if the muscle is not in a state of tension, as shown by its buckled appearance in the whole section, it has the appearance of a network. Fig. 3 shows both well-extended muscle and fully shortened muscle (one-fifth extended length), in each case in the tensile state and in the relaxed. Examination of longitudinal sections of muscle under tension, particularly at full extension, suggest that this appearance of a network is due to adhesion of relatively long filaments, and is not due to a truly anastomosing meshwork. This is borne out by examination of formalin-fixed sections; even though the fixation in this case seems defective compared with osmium fixation. In transverse section the filaments clump together, whilst in longitudinal section the filaments adhere into an irregular number of bundles $1–2\,\mu$ long. But though the whole appearance and comparison with osmic and osmic-phosphotungstic

FIG. 3 (plate). Longitudinal sections of retractor muscle. All osmic-phosphotungstic fixation. All same magnification.

A, extended and under tension. Note fine filamentous structure.

B, extended but relaxed. Note network appearance.

C, shortened to about one-fifth extended length, under tension. Note filamentous structure. Filaments apparently thicker than in A.

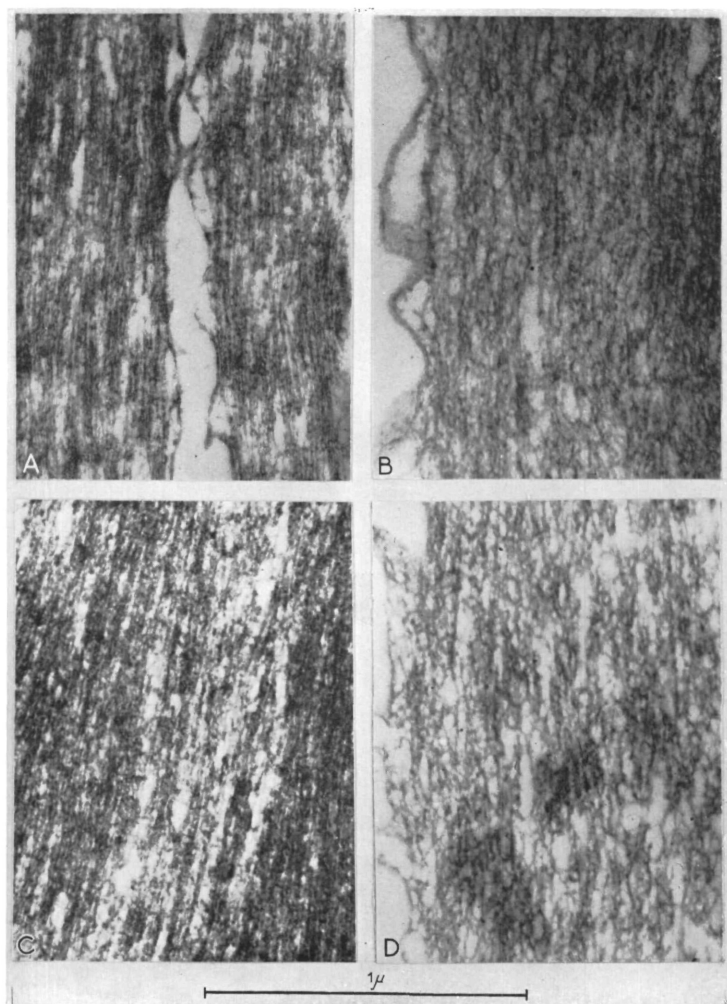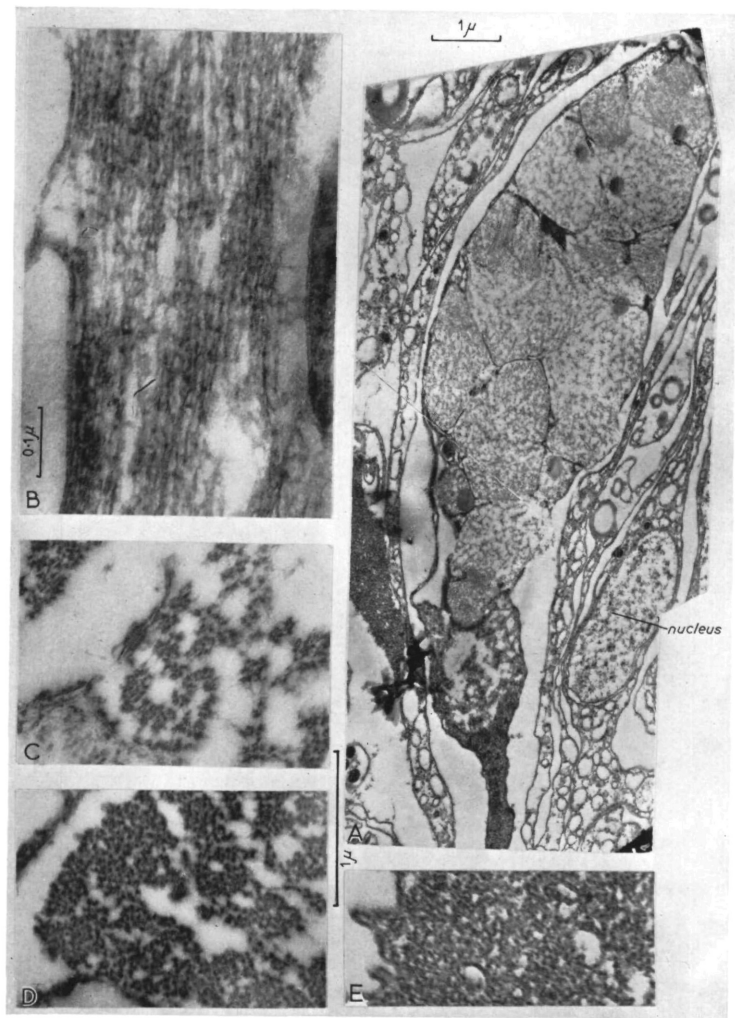D, shortened but relaxed. Note network appearance.

Fig. 4

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fixation shows that these long bundles are artifacts, their formation suggests that the filaments whatever their length are only connected firmly with each other at distances of several μ.

Radial muscle-fibres and cytoplasm

Fig. 2, B shows a transverse section of the muscle-field of the radial face of a perfect mesentery (fig. 1, A (*c*)). It may be compared with that of the retractor face as shown in fig. 2, A. When there is incomplete longitudinal extension of the mesentery the transverse muscle-field is thrown into folds at intervals of about 20μ—the 'buckling' of Batham and Pantin (1951). The curve of the muscle-field in fig. 2, B is due to such a fold.

Functionally the radial muscle differs from the retractor in that it is much weaker and that it only contracts slowly. Fig. 2, A, B shows, however, that the general structure is essentially the same in both muscles except for the much smaller development of the muscle-fibre in the radial muscle-cells. The filaments are fewer in number than in the retractor, but their diameter (about 60 Å) and their packing are similar in the fibres of both muscles.

In both radial and retractor muscle the contractile filamentous material is wholly or almost wholly confined to the muscle-fibre. Robson has pointed out that the stems between the fibres and the epithelium are elastic and possibly contractile and follow changes of cell shape on extension of the muscle-sheet. Our preparations show the cytoplasm of the stems as well as of the crests to be rather empty of contents apart from mitochondria and vacuoles. The cell membrane, which is clearly defined, does sometimes show indications of fibrils in contact with it, and occasionally small amounts of fibrillar material somewhat resembling muscle-filaments can be traced into the epithelial stems. But such fibrillar material is always small in amount. The shortening of the surface of the epithelial stems which necessarily takes place when the muscle-fibres extend would seem operated by contractility or elasticity in the cell membrane itself.

The epithelium

As Robson (1957) showed, each stem arising from the cytoplasmic crest of the endodermal muscle-fibre passes up to a nucleated epithelial portion of the cell, bearing a flagellum. These same features are seen in detail in electron micrographs. The stems on the long thin epithelial parts of the cells carry nuclei (fig. 4, A). Some sections show a prominent nucleolus, but perhaps

FIG. 4 (plate). Figs. C, D, and E are on the same scale.

A, osmic-phosphotungstic fixation. Transverse section of epithelium over retractor muscle-sheet (plane of fig. 1, C (*b*)) showing mucus-cell lying in intercellular space between stems of musculo-epithelial cells.

B, osmic-phosphotungstic fixation. Longitudinal section of part of extended retractor muscle-fibre in tension. Note filaments and tendency to beading at about 240Å intervals.

C, formol-phosphotungstic fixation. Transverse section of retractor muscle-fibre. Filaments partially dispersed and showing interfilamentar bridges.

D, formol-phosphotungstic fixation. Transverse section of retractor muscle-fibre to show partly ordered packing.

E, osmic-phosphotungstic fixation. Transverse section of retractor muscle-fibre at 50% extended length, partly relaxed, showing muscle filaments.

owing to imperfect fixation of the contents, the nuclei show little other organized structure in our preparations.

The cytoplasm, on the other hand, seems well fixed in both osmic and osmic-phosphotungstic preparations, though there may be some separation of the individual cells through shrinkage. Fig. 5, A, G, of the epithelial surface of the radial face of a perfect mesentery, shows the numerous vacuolar structures set in the cytoplasmic matrix. Some of these can be identified as mitochondria. There are also:

(1) other smaller vacuoles and laminate membranes such as may be seen at the bottom of fig. 5, G below the root of the flagellum. Presumably these represent the Golgi region (Sjöstrand and Hanzon 1954 *b*);

(2) densely staining bodies within a vacuole (fig. 5, H);

(3) thick-walled vacuoles (fig. 4, A).

The surface of the epithelial part of the cell is exposed to the gastric cavity. It appears naked, and is thrown into a remarkable series of membranes seen as free processes in all the preparations (fig. 5, G). There seems no reason to suppose that these are artifacts. They recall similar villous processes illustrated by Hess and others (1957) in the endodermal cells of *Hydra*.

Each cell bears a typical flagellum, with the usual 9 : 2 fibrous structure (figs. 5, B, C, D, E). After osmic-phosphotungstic staining the fibres appear to consist of pale rods surrounded by electron-dense material. Each outer fibre contains two such rods. In the inner pair, each contains one rod, so that this pair resembles somewhat one of the double-rodded fibres of the outer ring (fig. 5, B, C, D, E).

The flagellar membrane is continuous with that of the cell and small vacuoles occur between this and the fibres. The outer filaments run continuously into a densely staining basal corpuscle, corresponding to that seen with the light microscope. From this a fibrous root with marked striations (at

FIG. 5 (plate). All osmic-phosphotungstic fixation.

A, longitudinal section through flagellum arising from crater on surface epithelium of radial muscle face. Note basal body and banded fibrous root, flagellar 'crater', also flagellar fibres and vesicles within cytoplasmic sheath of flagellum (plane of fig. 1, c (*b*)).

B, C, transverse sections of flagella. Note 9 : 2 fibre pattern, cell membrane, vesicles.

D, E, oblique section of base of flagellum and transverse section. Both enlarged from G. Note 9+2 fibre pattern and clear inner rod of each fibre. Outer fibres are double, each with two clear rods. Note vesicles.

F, horizontal section (as in fig. 1, A (*a*)) of retractor muscle-fibre, half extended; showing right muscle-fibre of musculo-epithelial cell with left mitochondria in cytoplasmic crest.

G, section in plane of fig. 1, c (*b*) through surface epithelium over radial muscle-sheet. Note flagellum rising from crater, basal body cut tangentially, membranes below root of flagellum. Note also cytoplasmic villi at epithelial surface, thickened cell membranes with intercellular bridges between epithelial cells.

H, epithelial section, same place as D. Junction between epithelial cells. Note double cell membranes, thickened distally with intercellular bridges between outer components of membrane. Note that where the cell membranes are cut tangentially (near the surface and near the right side of the picture) the bridges appear as bars parallel to the epithelial surface. Note two mitochondria (bottom right).
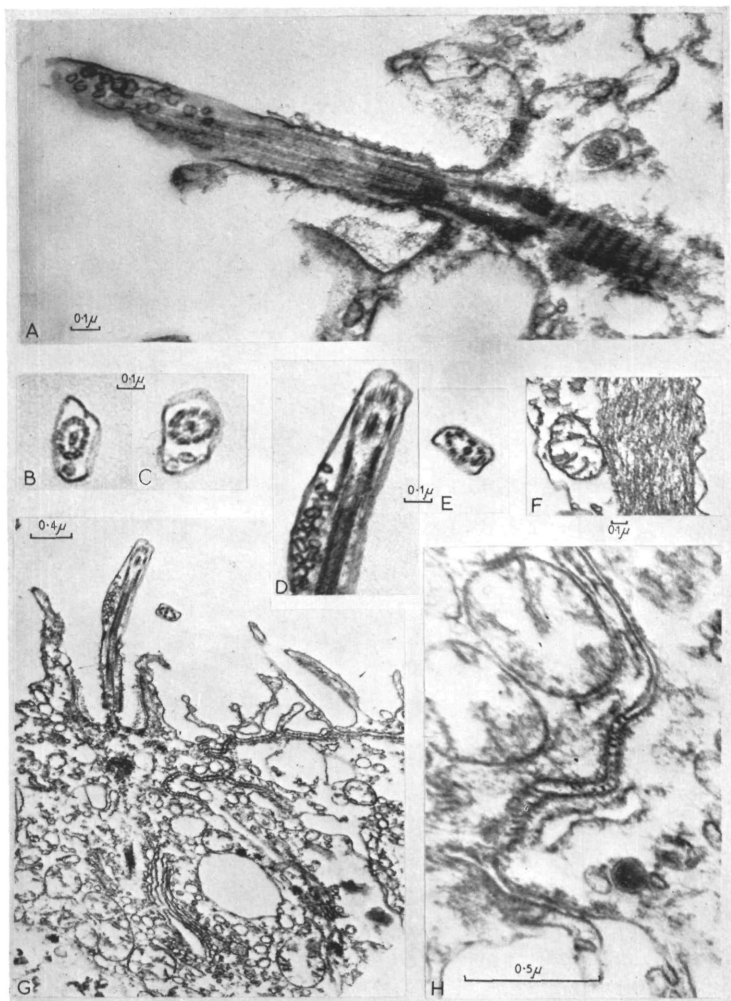
FIG. 5

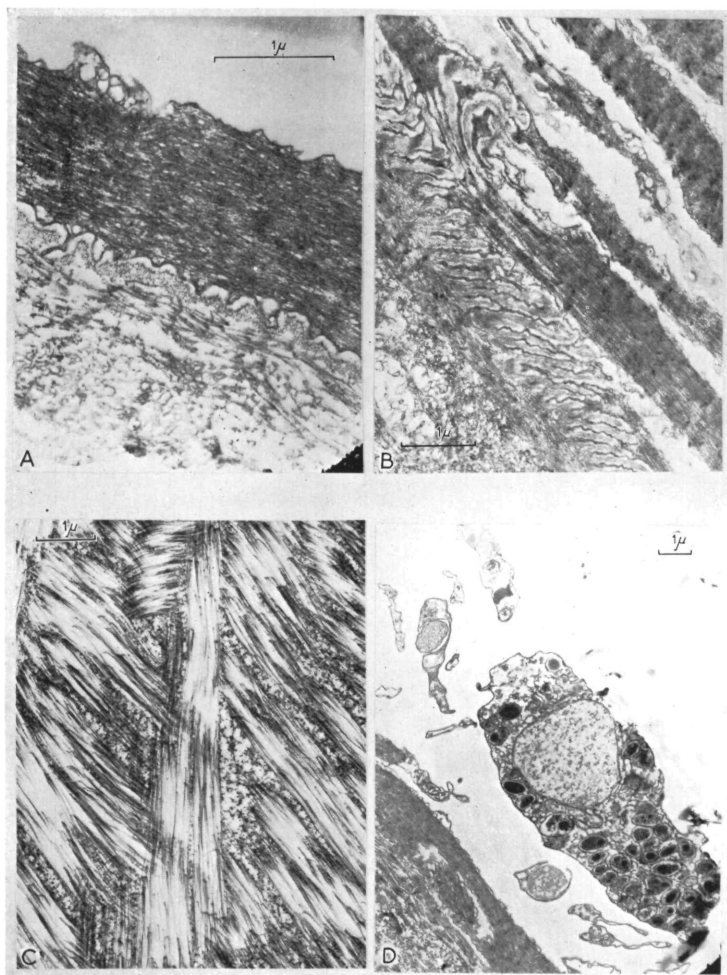A. V. GRIMSTONE, R. W. HORNE, C. F. A. PANTIN, *and*
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FIG. 6

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about 520 Å) passes down into the cytoplasm. At present we have no evidence for more than one root.

At the surface each flagellum is surrounded by a cytoplasmic 'crater' (figs. 5, A, G). Sometimes fine strands run between the crater and the base of the flagellum just before it enters the body of the cell.

The cell membranes of each cell are well defined. They are about 80 Å thick and appear double. Within about 0.5μ of the outer surface of the epithelium the stained cell membranes become much denser to electrons. In this outer region there are regular ladder-like cross-connexions between the membranes of adjacent cells (fig. 5, G, H). Where the cell membrane is cut almost tangentially these cross-connexions appear as elongated bars parallel to the epithelial surface, rather than isolated pegs.

The densely staining outer region of the cell membrane may correspond to the cell boundaries which Robson has illustrated in stained preparations (Robson, 1957, fig. 1, F), and may perhaps be taken to represent secreted 'intercellular matrix'. Possibly also the 'basal granules' of Robson's light microscope figure may represent the basal craters of the flagella.

Mucus-cells and amoebocytes

In addition to musculo-epithelial cells, the general surface of the mesentery contains mucus-cells and amoebocytes. The region of the 'mesenterial filament' with its abundant cnidae and digestive and absorptive cells (Stephenson, 1928) was not examined by us.

Fig. 4, A shows a longitudinal section through a mucus-cell lying in the epithelium. The histological appearance easily enables it to be identified with such cells seen in fixed preparations under the light microscope. The ill-fixed nucleus is seen lying above the granular basal part of the cell which tapers downwards; whilst above are seen numerous mucus vacuoles in varying degree of dispersion about a central granule. Their appearance recalls that of the goblet cells of the intestine of vertebrates (Florey, 1955).

Amoebocytes are common in the immediate neighbourhood of the muscle-sheet, both in the intercellular space (fig. 6, D) above and in the mesogloea just below. There is no evidence that the amoebocytes secrete material in the mesogloea in any of our preparations, and a remarkable feature of the cells is their naked appearance.

That cells like those shown in fig. 6, D are the amoebocytes can be inferred

FIG. 6 (plate). A, osmic-phosphotungstic fixation. Approximately vertical section along retractor muscle-fibre, 50% contracted. Note contact with basement layer of mesogloea, partially buckled.

B, osmic-phosphotungstic fixation. Almost horizontal section in plane of muscle (as in fig. 1, A (*a*) and C (*a*)). Muscle-fibres contracted to about one-fifth of extended length. Note extreme buckling of attachment layer of mesogloea with folds at about 0.25μ.

C, formol-phosphotungstic fixation. Mesogloea. Note warp and woof structure, darkly stained amorphous material.

D, osmic-phosphotungstic fixation. Horizontal section through retractor (fig. 1, A (*a*) and C (*a*)), showing amoebocyte in the intercellular space. Note dark granules, and Golgi membranes above nucleus.

from their position and varied shape, from the absence of alternative objects with which they could be identified, and from their cytological appearance. Like the amoebocytes seen under the light microscope, these wandering cells are highly granular. The granules stain deeply, both with the two modes of osmic fixation and with the formalin-phosphotungstic method. In histological preparations under the light microscope granules of the same order of size are seen which stain strongly with acid fuchsin in Mallory's triple stain. Above the nucleus in the figure may be seen a system of canals or membranes which may represent Golgi membranes.

At the magnification employed, sensory cells and nerve-axons are few and far between (Pantin, 1952). The probability of a cut axon appearing in one of the sections is low, and we found no object that at present could certainly be identified as one.

Mesogloea

That the mesogloea has an organized fibrous structure of collagen is known from examination of its structure and from its mechanical properties (Chapman, 1953). On both grounds Batham and Pantin (1951) showed that there must be an outer, moderately extensible, portion of the mesogloea to which the muscle-fibres are attached. Fig. 6, A shows clearly the reality of Batham and Pantin's mesogloeal basement membrane to which the muscles are attached. It is a three-dimensional network attached directly to the cell membrane of the muscle-fibre above and it passes rather suddenly, but continuously, into the grosser mesogloeal fibres below. It follows the normal buckling of the muscle-sheet with folds at intervals of the order of 20μ, already described. But this superficial mesogloeal layer also carries folds of the muscle-fibre and its cell membrane. These folds project as pockets into the mesogloea at intervals of the order of 0.2 to 0.3μ. That this is not a fixation artifact is shown both by the relation of these folds to the fibrous structure of the underlying mesogloea and of the muscle, and also by the way they increase with the contraction of the muscle. Fig. 6, B shows the extreme folding of this layer of mesogloea and the muscle-cell membrane under maximal muscular contraction. It is clear that this basement mesogloeal layer and the folds into which it is thrown during contraction provide a firm attachment of the muscle-fibre through its cell wall.

The structure of the fibres of Chapman's mesogloeal fibrous lattice, which composes the bulk of the mesogloea, is of particular interest. Whilst sections show some amorphous fine material in the general mesogloeal spaces, most of the solid material is made up of clusters (figs. 6, C; 7, A) of fibrils. The fibres which make up Chapman's cross-lattice are evidently these clusters of fibrils. Under high resolution it is, however, seen that the clusters are not well-defined entities; fibrils pass from one cluster to another of the lattice.

The fibrils themselves, far beyond optical resolution, have well-defined structure and are of regular size (fig. 7, A. C). Their diameter varies slightly about 100 Å and they show a characteristic pattern of periodic bands spaced
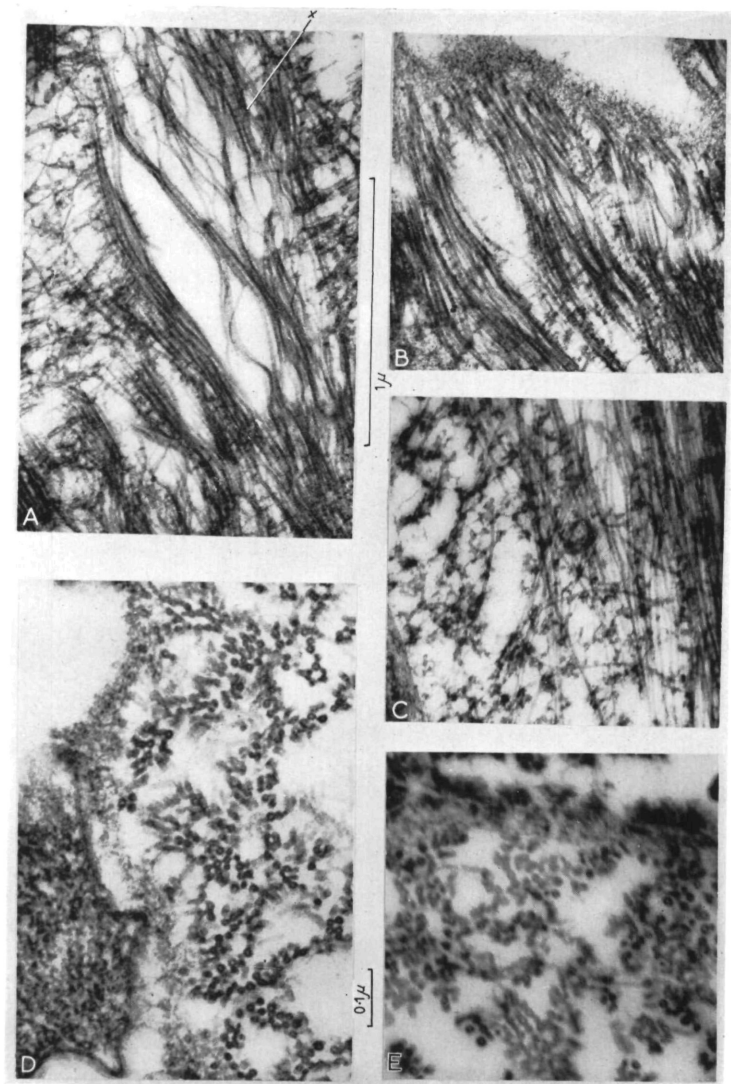
FIG. 7

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at about 260 Å. In osmic-phosphotungstic preparations these fibrils often give the appearance of being hollow tubes. This is strikingly the case when well-focused fibrils are seen precisely in cross-section (fig. 7, D).

The fibrils are easily seen after simple osmic fixation though the contrast is not sufficient to show certainly whether these seem tubular. The fibrils seen in the formalin-phosphotungstic preparations have about the same dimensions and the characteristic banding at about the same interval (figs. 6, C; 7, D, E) as in osmic-phosphotungstic preparations.

With all three methods of fixation there is, in addition to the fibrils, some amorphous substance. Its character and quantity differ somewhat with each fixative, so that there is some reason to suppose that it is an artifact of fixation (compare figs. 7, A, C). The amount of amorphous material not organized into fibrils appears least after simple osmic fixation.

But the fibrils survive all three methods of fixation, and do so in similar form and relation. Where contrast is sufficient for detail to be seen, as in both osmic-phosphotungstic and in formalin-phosphotungstic preparations, the fibrils are seen to adhere in bundles along part of their length to give the fibres of Chapman's lattice. As figs. 6, C and 7, A show, when they do so, the periodic banding extends through the whole bundle—the fibrils aligning themselves so that their banding corresponds to that of its neighbours.

DISCUSSION

Before accepting electron micrographs as evidence of structure in living cells, two classes of error must be considered. We must show that the appearance we examine is not of instrumental origin, as in optical diffraction or interference; and we must show that it is not an artifact of fixation or subsequent treatment. In the light microscope the appearance of double membranes at cell surfaces or the tubular appearance of mesogloeal fibrils might raise such suspicion of a diffraction effect. But considering the dimensions of the instrument we used and the exceedingly short equivalent wavelength of the electron beam (about 0·041 Å) it becomes clear that we cannot attribute these appearances in the structures we are considering to an effect analogous to optical diffraction. Effects analogous to optical interference are less easy to dismiss, and, as is well known, are sometimes apparent in electron micrographs. There is, however, a test which can be applied. False images of instrumental origin will vary with instrumental conditions, that is with the applied magnification

FIG. 7 (plate). B and C are on same scale as A. D is on the same scale as E.

A, osmic-phosphotungstic fixation. Note co-ordinated banding of fibril-bundles (at *x*); also amorphous material.

B, osmic-phosphotungstic fixation. Tangential section of mesogloea showing passage of fibrils into surface layer of mesogloea.

C, formalin-phosphotungstic fixation. Mesogloea, showing fibres and amorphous material.

D, osmic-phosphotungstic fixation. Transverse section through retractor (plane of fig. 1, A (*b*)). Note filaments of muscle-fibres, cell membrane, mesogloeal attachment layer, apparently tubular mesogloeal fibrils.

E, formol-phosphotungstic fixation. Transverse section of retractor. Note apparently tubular mesogloeal filaments as in C.

and with the focus; and such false images will be apparent in all objects of the same dimensions in the object examined. Now the dimensions of the features of the cell membranes and of the mesogloeal fibrils which we are discussing are independent of magnification by the instrument and of the focus. Moreover, the appearance of double structure is quite evidently absent in many minute features in micrographs which clearly illustrate it in the cell membrane; whilst a tubular appearance like that of the mesogloeal filament is absent in the cross-section of other filaments and of granules of the same size, and in the same micrograph. We may therefore conclude that these features are not instrumental artifacts.

The possibility of histological artifact is still more difficult to eliminate. Grosser features can be directly identified with structures seen optically in the living cell, but finer detail cannot receive this direct confirmation. However, there is strong circumstantial evidence for the reality of some electron-micrographic structure in the living cell. The fact that the mesogloeal fibres are seen after both osmic and formol fixation lends probability to the supposition that the appearance is not an artifact. Whether they are hollow tubes, as they appear to be after phosphotungstic treatment, or whether for some reason the cortex of the fibres takes stains differentially, cannot at present be determined; though we may note that the very different method of metallic shadowing suggests that the rather similar fibres of vertebrate collagen may be tubes (Wyckoff, 1952).

Further evidence for the reality in life of electron-micrographic structures may be gained by what may be termed 'the principle of functional connexions'. If some feature of a structure seen in an electron micrograph fulfils, and alone fulfils, an evident functional requirement inferred on other grounds there is presumptive evidence for its existence in life. Thus the known contractility of the muscle-fibres implies the existence of contractile structures in them which are in turn attached, directly or indirectly, to the underlying mesogloea. The muscle filaments seen under the electron microscope fulfil this requirement and there is no other evident structure which could do so. On the other hand, any feature that seems attributable to the histological treatment which the specimen has received is a presumptive artifact. That is seen in shrinkage, disruption of membranes, or regional differences in fine structure correlated with the direction of penetration of histological reagents.

By these tests we can obtain quite strong likelihood for the existence in the living cell of structures inferred from electron micrographs. This is especially true if supporting evidence comes from sources of widely different character. Thus, the reality of the differentiated fibrous layer at the surface of the mesogloea and in contact with the muscle-fibres is strongly supported by the fact that Batham and Pantin (1951) predicted the existence of such a layer from the mechanical behaviour of the tissue.

Other kinds of evidence undoubtedly affect the subjective estimate of the observer. Of these the most important is the degree of organization of structure. Whether or not a functional significance can be attributed to it, a

structure that shows well-defined organization both generally and in detail creates a strong impression that it was present in the living cell from which the preparation is derived. It is difficult to justify this impression except on the ground that experience often justifies it. Nevertheless, it is important because the first detection of significant structure often proceeds directly from it; the other kinds of evidence being used to justify what is in fact a tentative subjective hypothesis.

Judged by these criteria, the structures described in this paper can with fair probability be taken to correspond to things in the living cell. We shall therefore briefly comment on them, making that assumption.

The epithelium

The epithelial part of the musculo-epithelial cells shows striking similarities to the ciliated epithelial cells described by Fawcett and Porter (1954) in lamellibranchs and vertebrates. The flagella of *Metridium* endoderm have the familiar 9 : 2 fibrous structure, though in *Metridium* the double nature of the outer fibres is so evident (fig. 5) that it might as easily be called an 18 : 2 structure. The fibres arise from a hollow basal corpuscle about 2,000 Å wide and 3,500 Å long. Unlike molluscan cilia, but like those of the ctenophore *Pleurobrachia pileus* (Bradfield, 1955), the fibres continue into the basal corpuscle. As in other cases a fibrous root, perhaps single in *Metridium*, passes from the basal granule into the cytoplasm, and this root shows well-marked striation at about 520 Å, which may be compared with 500 Å for the root of *Pleurobrachia* cilia. Goreau and Philpott (1956) describe similar structures in two madreporarian corals, though the periodicity of the striation in the root is given by them as 670 Å.

Like other flagella and cilia, the flagellum of *Metridium* is bounded by a membrane continuous with that of the cell proper. In *Metridium* small vesicles are seen in electron micrographs on one side of the flagellar fibres, particularly near its base. This is of some interest. The retinal rods in vertebrates arise as modifications of ciliated cells (Sjöstrand, 1956). The remarkable pile of membranous sacs of the retinal rod apparently arises from the growth and flattening of vesicles on one side of the ciliary fibres (de Robertis, 1956). The developmental stages of the vesicles described by de Robertis are by no means unlike the permanent state of *Metridium* flagella with their lateral vesicles. The musculo-epithelial system of *Metridium* has a general sensitivity to light and the action spectrum somewhat resembles that of vertebrate rods (North and Pantin, 1958). Flagella are known to be light-sensitive in some organisms (Mast, 1938). The possibility that this may be true of *Metridium* flagella warrants investigation.

The remarkable crater round the base of *Metridium* flagella and the membranes and cytoplasmic villi which project from the surface of the epithelium resemble the similar and more numerous epithelial and surface structures described in the ciliated epithelium of animals from several different phyla (Bradfield, 1955; Goreau and Philpott, 1956). In *Metridium* the presence of

these extensions of the cell membrane raises a new problem. The epithelial part of the cells undergoes very great extension and contraction according to the state of expansion and contraction of the animal. How can a villous cell surface of this kind accommodate itself to such extensive deformation? We are very far from being able to consider the epithelial surface as a simple 'surface-tension layer'. It must have reversible extensibility which, nevertheless, permits the retention of these surface features. This feature may be correlated with the peculiar intercellular surface structures of *Metridium* epithelio-muscular cells. In the deeper parts adjacent cells are each bounded by a double cell-membrane which may be in contact with that of its neighbour but which does not adhere to it. Indeed, between the epithelial and muscular parts of the cells the cytoplasm is narrowed to neck-like strands which are separated by intercellular fluid (Robson, 1957). But in the outermost region of the epithelium there is an electron-dense connecting membrane apparently holding the adjacent cells together. As elsewhere the cell membrane is double, though here it is deeply stained; but here and there the outer component of each cell membrane is connected to that of its neighbour by strands like the rungs of a ladder. 'Intercellular bridges' or desmosomes are well known in the tissues of other animals. But they are exceptionally strongly developed near the surface of *Metridium* epithelium. In vertebrate squamous epithelium and many other tissues, all that is to be seen are darkly staining masses in the cytoplasm at corresponding points beneath the cell surface (Fawcett and Selby, 1958). Adjacent cell surfaces are separated by a clear space of 150–200 Å without visible structure. In *Metridium*, whilst there are such dark masses below the cell membrane, in addition bridges actually connect the outer surfaces of the adjacent cell membrane (fig. 5, H). Well-developed cell boundaries would certainly seem a necessity in highly extensible epithelium.

Muscle

Striated muscle from a variety of animals seems to consist of two kinds of filaments, thick myosin filaments in the A band and thinner actin filaments extending through the I band and into the A band. According to a current hypothesis the contraction of such muscles takes place in effect by the sliding of the actin filaments along the spaces between myosin filaments (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954). A cardinal feature of this hypothesis is necessarily the presence of two sorts of filaments. Recently Hanson and Lowy (1957 *a*, *b*) have shown that a double array of filaments comparable to that of striated muscle occurs in a variety of unstriated muscles from various animals; though in these the two kinds of filament occur throughout the length of the muscle and are not aggregated in gross striae along its length. They suggest that a similar sliding mechanism between the two sorts of filament may account for contraction in plain and in striated muscle.

Both the cytological and ultimate filamentous structure of the muscle-fibres of *Metridium* seem rather simpler than those of unstriated muscles so far described, except perhaps for that of the unstriated muscle of the frog's

bladder (Hanson and Lowy, 1957 *b*). Though there is some tendency for regularity of packing of the muscle filaments (fig. 4, C, D), there is none of the regular organization of fine structure seen in some of Hanson and Lowy's molluscan and annelidan unstriated muscles. Nor do they show clear evidence of more than one kind of filament; though these often seem connected by interstitial bridges of other material (fig. 4, C). Clearly, the nature of this interstitial material needs further investigation, but such preparations as we have made so far show only one sort of clearly defined filament.

Whether or not this interstitial material disguises some second kind of filament such as Hanson and Lowy find in molluscs, it seems certain that *Metridium* muscle is physiologically of the same class as other sorts of muscle. Not
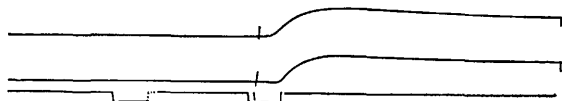

Fig. 8. Facilitated retractor response of *Metridium* to two condenser shocks at 1-sec. interval. Recorded from two opposite points on the disk. Note rapid development of tension. Recorded by a light spring lever.

only are the mechanical properties of the muscle essentially the same (Batham and Pantin, 1950 *a*) but actomyosin-like proteins which can be caused to contract by ATP have been extracted from the actinian *Anthopleura japonica* (Maruyama, 1956).

In comparing the functional organization of an unstriated muscle like that of *Metridium* retractor with that of a vertebrate striated muscle, there is one other point that is perhaps worth bearing in mind. It is still often considered that the chief functional distinction between striated and unstriated muscle is that the latter contracts more slowly than the former. But this distinction is not reliable (Pantin, 1956), and Hanson and Lowy (1957 *b*) point out that the significance of cross-striation is still unexplained. However, certain clear propositions can be made which current hypotheses of muscular contraction do not seem fully to take into account (Pantin, 1956). These are:

(1) Some unstriated muscle can develop tension as swiftly as many unstriated muscles. Despite its repeated evolution in different animals striation is not thus a necessary feature of swift contraction. Fig. 8 shows the response of *Metridium* retractor working under more or less isometric conditions. The contraction phase reaches 50% of the maximum tension in about 0·1 sec whilst maximum tension is often developed in much less than 0·3 to 0·5 sec. Under rigidly isometric conditions unobtainable in *Metridium* the development of maximum tension would be even more rapid. Some other unstriated muscles can contract even faster.

(2) Unstriated muscle has an enormous range of resting length. Fully extended resting retractor muscle in *Metridium* is more than 5 times as

  long as the resting fully shortened muscle. For the longitudinal muscles of *Hydra* tentacles the factor is not 5 but 20.

(3) Compared with striated muscle, all unstriated muscles relax much more slowly then they contract.

These propositions suggest that the development of striation is related to speed and extent of relaxation rather than to speed of contraction: yet none of them seems to arise as a necessary consequence of current hypotheses about the functional significance of striation in muscle.

Mesogloea

Knowledge of the mesogloeal proteins of actinians is at present limited, but there is reason to suppose that they are related to collagen. Chapman (1953) showed many resemblances between actinian mesogloea and vertebrate collagen, histologically, chemically, and in physical properties. He also drew similar conclusions about the mesogloeal fibrous matter of various medusae. That is in agreement with X-ray diffraction studies by various authors of the mesogloea of medusae and Actinozoa (Rudall, 1955). But Bouillon and Vandermeerssche (1956) have concluded that the fibres in medusae are not collagen but elastin. Whether that is so or not—and the authors differ on certain questions of fact—it seems probable that Chapman is right in supposing the mesogloeal fibres of actinians to be a collagen because of the chemical composition, particularly the abundance of proline and hydroxyproline, and because of the contraction under heat and the solubility in dilute acids. Lenhoff and others (1957) record hydroxyproline in collagen fibres from *Metridium*.

Like collagen, the ultimate fibrils of the mesogloeal substance show regular banding. The period, which varies slightly with the preparation, ranges from 220 Å to 250 Å. Comparable periods are known from some vertebrate connective tissue (Wyckoff, 1952), but the characteristic banding of mature collagen at about 640 Å has not been found by us in the mesogloeal fibrils. But other periodicities occur in collagen, as in the 520 Å period in the basement membrane of amphibian larvae (Weiss and Ferris, 1956) and at 210 Å in immature connective tissue from chick embryos (Porter, 1951). In mesogloea from the southern species *M. canum* Dr. E. J. Batham has preparations showing accentuated banding of 440 Å.

Whatever the ultimate nature of the mesogloeal fibrils, their organization into higher units is very like that of vertebrate connective tissue. The fibrils are grouped into fibres and bands as in Wyckoff's (1952) figure for collagen fibres in chick tendon.

Chapman (1953) showed the striking crossed lattice organization of the fibre aggregates in the mesogloea of *Calliactis* and called attention to the great importance of this for the extensibility of the tissue. Weiss (1957) records what seems a quite parallel organization in the basement membrane of amphibian larvae.

The question arises how this crossed lattice pattern originates. The evidence both in vertebrates and in coelenterates suggests that the fibrils arise as extra-

cellular exudates and are only secondarily organized into fibres of the con-
nective tissue lattice (Bretschneider, 1951); Chapman, 1953; Weiss, 1957;
Jackson and Smith, 1955). Chapman points out that crossed lattices are a com-
mon feature of systems exposed to stresses. Our own observations on the fine
structure of the lattice support the idea that the organized aggregation of the
fibrils is imposed on the fibrils, for under the electron microscope the fibre-
aggregates are seen not to be well-defined structures; individual fibrils pass
freely from one fibre-bundle to another.

It is an essential feature of any such process of aggregation that to con-
stitute fibres the fibrils must have the power to adhere to each other, and this
is clearly seen in the manner in which the fibrils in *Metridium* mesogloea aggre-
gate band by band—just as Weiss notes in amphibian basement membrane.

This still leaves the ultimate problem unsolved of how the fibre-bundles of
the lattice come to be arranged in alternating layers obliquely to the axis of the
animal, as Chapman has shown. It is not enough to point out that once it has
arisen such a system, and such a system alone, allows the necessary flexibility
of the tissues. Possibly some kind of 'natural selection' during the normal
muscular movements, longitudinal and transverse, may operate. Adhering
groups of fibres along an axis may tend to be parted during extension along
that axis; whilst obliquely arranged aggregates might survive through their
lattice-like freedom of movement relative to the axis.

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