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The Anatomy of The Muscle System of Stomphia Coccinea (Müller) and Aiptasia Diaphana (Rapp)

by

 HELEN M. AMERONGEN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the faculty of Graduate Studies and Research, for acceptance, a thesis entitled "The Anatomy of the Muscle System of Stomphia coccinea (Müller) and Aiptasia diaphana (Rapp)" submitted by Helen M. Amerongen in partial fulfilment of the requirements for the degree of Master of Science.

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Date December 7th, 1976

ABSTRACT

An extensive investigation of the fine structure of the muscle system in Stomphia coccinea and Aiptasia diaphana has been carried out. Observations on various aspects of the muscle system are presented. The functional relationship between the muscle system and the mesoglea and between the muscle system and the nervous system is considered. Emphasis is placed on the functional significance of the occurrence in both species, of two types of muscle fiber.

Type A and Type B fibers are distinguished mainly by differences in the thick filaments of the two types. In Type A fibers, the thick filaments have a consistent diameter of 180Å. In Type B fibers, the diameter of the thick filaments is variable and on the average (225Å), is greater than that in Type A fibers. The thick filaments in Type B fibers show an axial striation with a period of 110Å. The differences between Type A and Type B fibers do not result from differences in the state of contraction of the muscle; the two fiber types are therefore considered to represent two morphologically distinct kinds of muscle fiber . The fine structural characteristics distinguishing A and B fibers are similar to those which distinguish fast and slow muscle fibers in higher animals. The distribution of A and B fibers in Stomphia and Aiptasia is consistent with the distribution of fast and slow muscles in these two species. It is proposed that A and B fibers represent two morphologically distinct kinds of smooth muscle, and that the capacity for fast and slow contractions in the muscles of Stomphia and Aiptasia and possibly in all actinians, is due to morphological differentiation in the muscle system.

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INTRODUCTION

Actinians are capable of some relatively complex behavior patterns. These behavior patterns are usually stereotyped but in some cases behavior assumes different forms according to the circumstances in which the activity takes place. The complexity and adaptability of these behavior patterns are surprising considering the apparent simplicity of actinian structure. Examples of such patterns are swimming in Stomphia coccinea (Yertsch and Pierce, 1955; Sund, 1958) and transfer to shells in Calliactis parasitica (Ross and Sutton, 1961a and b) and in Stomphia coccinea (Ross and Sutton, 1967).

The nervous system that controls these behavior patterns in actinians has been studied extensively both from the standpoint of its structure (Pantin, 1952; Robson, 1961 and 1963; Peteya, 1976) and its function (McFarlane, 1969 a and b; Lawn and McFarlane, 1976; Lawn 1976). However, knowledge of the muscular organization of sea anemones is still limited, particularly at the ultrastructural level. This investigation was undertaken to provide information about the structure and organization of the muscles comparable to that available on the nervous system, using both light and electron microscopy.

The first comprehensive studies on the histology of actinians were done by von Heider (1877) and by the brothers Richard and Oscar Hertwig (1879-80). Subsequently, there was a good deal of disagreement about details, especially about the organization of a nervous system. These contradictions between earlier and later works were probably due to differences in the techniques of fixation used by different investigators. The recent study by Peteya (1976) has clarified the situation by

demonstrating the artifactual nature of some of the features previously described and by distinguishing nervous from non-nervous components in the so-called "nerve plexus" of some earlier authors. Nevertheless, a century of studies with the light microscope has firmly established the main features of actinian histology. In particular, the presence of epitheliomuscular cells, first described in Hydra by Kleinenberg (1872), was confirmed; the nature of mesoglea as a type of connective tissue was demonstrated; the basic cell types in the ectoderm and the endoderm were described in detail. With respect to muscle, the observations made by the Hertwigs were confirmed by subsequent workers (Faurot, 1895; Carlgren, 1893;MacMurrich, 1899 and 1901; Parker and Titus, 1916). Moreover,a detailed knowledge of the organization of the muscles was built up and used as a tool in the classification of the Actiniaria (Stephenson, 1928 and 1935; Carlgren, 1949).

More recent studies have been directed towards the functional organization of the actinian muscle system (Jordan, 1934; Kipp, 1939; Batham and Pantin, 1950 and 1951; Robson, 1957). In recognition of the great importance of the mesoglea in the functioning of the muscles, careful analysis of the structure and of the chemical and physical properties of this tissue have been carried ou (Chapman, G., 1953a and b; Gutmann, 1966; Gosline, 1971).

Very little is known of the fine structure of the muscle system in actinians based on observations with the electron microscope. In the course of his study on the nervous system of Stomphia coccinea, Peteya (1976) observed two types of muscle fiber, A and B, which he distinguished by the appearance of the thick filaments. He was unable to

decide whether these represented differences in state of contraction or distinct types of muscle, but he suggested that there was a direct correlation with the distribution of B fibers and the muscles involved in the swimming of Stomphia.

The physiological literature is extensive and it is necessary here only to draw attention to a few questions in the physiology of nerve and muscle which are of particular importance to the micro-anatomist. One of the problems has been the difficulty of dealing separately with nerve and muscles in the physiological analysis of behavior in conducting experiments on intact animals or on preparations.

It is well known that certain muscle fields of actinians are capable of both fast and slow contraction (Pantin, 1935; Batham and Pantin, 1954; Ross, 1957), but the mechanism underlying this functional differentiation is not understood. The nature of the processes involved in synaptic and neuromuscular transmission is not well understood, in spite of detailed studies by Ross (1960a and b) on the excitatory or inhibitory effects of cholinergic and adrenergic substances on the muscles. From kymograph recordings of muscle preparations, it was demonstrated that the character of a contraction, i.e. its speed, size and latency, appears to depend on the frequency of impulses delivered to the muscle field (Pantin, 1935 and 1952; Batham and Pantin, 1954; Ross, 1957; Bullock and Horridge, 1965; Robson and Josephson, 1969). No differentiation into areas of fast and slow muscle fibers was detected (Pantin, 1965), and the concept of a simple, frequency coded nervous system controlling behavior in an unspecialized muscle system was generated.

Recently, particularly through the work of McFarlane and colleagues

(McFarlane, 1969a and b, 1970, 1974, 1975, 1976; McFarlane and Lawn, 1972; Lawn and McFarlane, 1976), the existence of at least three conducting systems in sea anemones has been conclusively demonstrated, and the control of several behavior patterns has been explained in terms of interactions among these conducting systems (Lawn, 1976; Lawn and McFarlane, 1976; McFarlane, 1969b; McFarlane, 1975; McFarlane, 1976; McFarlane and Lawn, 1972). This information forces a reconsideration of the anatomical substrates for conducting pathways. As outlined by Josephson (1974), there are three alternatives for independent conducting systems in the Cnidaria: 1) mechanical transmission of excitation via the muscle fibers themselves; 2) transmission through a nerve net in which several conducting systems may exist in parallel capable of being independantly excited; 3) non-nervous conduction through epithelial cells. This last possibility is an old concept which has come to the fore recently. It was first introduced by Kleinenberg (1872) working with Hydra, but it was later discounted by the Hertwigs (1879-80). Parker (1919) re-introduced the concept as a feature which must have been present in the elementary nervous system. Experimental evidence for epithelial conduction only became available when Mackie (1965) was able to detect impulses passing in nerve-free epithelia in certain Hydrozoa. Pantin (1965) has suggested that the capacity for fast and slow muscle contractions in actinians might be explained by the presence of neuronal and myoidal conducting pathways, respectively. However, an anatomical substrate for a non-nervous pathway has never been demonstrated. Moreover, the influence of intrinsic properties of the muscle in determining the character of a contraction has not been investigated.

The objectives of the investigation presented in this thesis can be summarized as follows: 1) to increase our knowledge of the structure of actinian muscle at the subcellular level; 2) to study the fine structure of actinian muscle in different states of contraction in order (a) to shed some light on the nature of the two types of fiber described by Peteya (1976) and (b) to gain an understanding of the organization of the muscle system in relation to the different functions it must perform; 3) to approach some questions in neuromuscular physiology from the standpoint of micro-anatomy.

MATERIAL AND METHODS

Collection and Maintenance of Animals:

The swimming sea anemone, Stomphia coccinea (Müller), was dredged in the San Juan Channel of Puget Sound at depths of about 200 feet and maintained in artificial sea water (Instant Ocean) at 12°C. for up to two weeks prior to use. The average size of specimens was 1.5 cm. in diameter in the mid-column region. Aiptasia diaphana (Rapp) was originally obtained off the coast of Bermuda and multiplied in the laboratory by pedal laceration. It was maintained in artificial sea water at room temperature and fed weekly with frozen Artemia. The average size of specimens was 0.35 cm. in diameter in the mid-column region. Approximately 30 specimens of each species were used in the study. Except where mentioned, all observations were made on at least two individuals (of either sex). Hereafter, Stomphia coccinea will be referred to as Stomphia and Aiptasia diaphana will be referred to as Aiptasia.

Anaesthesia:

Prior to fixation for light or electron microscopy, the sea anemones were anaesthetized in equal parts of 6.7 percent $MgCl_2.6H_2O$ and sea water for five to six hours. It is possible to narcotize Stomphia by introducing the anaesthetic at full strength during the refractory period which follows swimming (Sund, 1958), thus eliminating the necessity for gradual addition of anaesthetic (Robson, 1961). Aiptasia could also be introduced to full strength anaesthetic without causing full retraction, since the sphincter is very weak and cannot close over the oral disc and tentacles.

Electron Microscopy;

Full expansion of the anemones was achieved by inflation through the pharynx using a hypodermic syringe during narcotization, and subsequently, during fixation. Whole animals were fixed for two hours in 2.5 percent glutaraldehyde in phosphate buffer at pH 7.6 (Dunlap, 1966; Cloney and Florey, 1968), then dissected and postfixed in two percent OsO_4 in the same buffer for one hour. The tissue was dehydrated in an ethanol series and embedded in Epon or Araldite. One micron thick sections were cut for light microscopy and stained with methylene blue. These were used to facilitate orientation within the tissue when working with restricted fields of view on the electron microscope. Thin (silver-gray) sections were mounted on uncoated grids, stained in uranyl acetate and lead citrate (Reynolds, 1963), and examined with a Phillips EM201 at 60 or 80 KVs. All measurements were made on the printed micrographs.

Light Microscopy;

For light microscopy, the anemones were fixed in Bouin's or in Flemming's strong formula (after Mueller, 1950). The tissue was dehydrated in an alcohol - xylol series and embedded in wax. Sections were stained in Masson's Trichrome, after Bouin's fixative, or in acid fuchsin and light green SF yellowish, according to Mueller (1950), after Flemming's.

Contraction State Experiments;

The retractor and the column circular muscles of Stomphia were chosen for the experiments because they can be isolated without excessive difficulty, and because both muscles are comprised of A and B fibers. In the retractor, the majority of fibers are of one type (A),

and they can be easily distinguished from the Type B fibers on the basis of size, as well as myofilament patterns. In the column circulars, the epithelial muscles are invariably Type B and the mesogleal muscles are invariably Type A. Here again, the two types can be easily distinguished on a basis other than the myofilament patterns and can be studied in relation to one another.

Retractor muscles and pieces of column circulars were isolated from Stomphia having mid-column diameters of 1.0 to 2.5 cm. All dissection was done with anemones which were narcotized for at least five hours, according to the technique described above.

To obtain muscles at resting length or stretched, the isolated pieces were pinned out onto wax using porcupine quills and fixed while still narcotized. Pieces pulled out to 15 - 20 percent more than resting length were considered stretched. For isometric and isotonic contraction, the muscles were allowed to recover from the anaesthetic for one hour (full responsiveness returns in as little as 15 minutes) in normal sea water, following dissection. Contraction was induced either by slow introduction of fixative or by exposure for five minutes to sea water having 20 percent of the sodium ions replaced by potassium (Ross (1960a), demonstrated the excitatory effect of excess potassium ions on muscle preparations of Calliactis and Metridium). To obtain isometric contractions, muscles were held at resting length by porcupine quills; to obtain isotonic contractions, the quills were removed from one end of the muscle to allow free shortening.

Preparation of the tissue for electron microscopy, and for light microscopy of one micron sections was done according to the procedure described above.

Terminology:

There is some confusion in the literature regarding the use of the terms "fiber", "fibril" and "filament". The terms were first used extensively in the description of vertebrate skeletal muscle with the following strict denotations: (a) "filament" (i.e. myofilament) refers to the filamentous proteins, actin (70Å) and myosin (150Å) which together make up the contractile unit; (b) "fibril) (i.e. myofibril) refers to a discrete group of filaments usually bundled together in some way; (c) "fiber" (i.e. myofiber) refers to an entire muscle cell, composed of many myofibrils. The last term, "fiber", is also used to describe the fibrous protein of connective tissue, collagen (diameter = 200 - 1000Å (Leghissa and Mazzi,1969). In this thesis, the terms "fiber" and "fibril" will be used as equivalent terms, since there is usually only one fibril per fiber. The term "fiber" will also be used to refer to collagen. The term "filament" will be used to refer to any small filamentous element with a diameter less than 200nm. Whenever necessary, the prefix "myo" will be used in connection with the muscles, to avoid confusion with non-muscular fibers (i.e. collagen) and non-muscular filaments (i.e. filaments of supporting cells).

RESULTS

I. ANATOMY AND HISTOLOGY

A brief summary of the anatomy and histology of actinians is given here for the sake of orientation. For a more detailed account, see Stephenson (1923) and Hyman (1940).

The main body of a sea anemone is made up of a hollow cylinder, the column, closed below by the pedal disc and above by the oral disc which bears a variable number of hollow tentacles around its circumference (Fig. 1). The oral disc is perforated in the center by the mouth, which leads into the pharynx. The cavity enclosed by the body and tentacles is the gastrovascular cavity, or coelenteron. It is filled with fluid, the amount of which can be varied, and constitutes a hydrostatic skeleton. The gastrovascular cavity is divided into compartments by the septa (mesenteries), thin sheets of tissue which extend radially from the column to the pharynx, and longitudinally from the oral disc to the pedal disc. The septa are produced in pairs; the space between the members of a pair is termed the endocoel; the space between pairs is the exocoel. In most species, the younger pairs of septa do not connect with the pharynx at their median edge. The free edges of these, and of the complete septa below the level of the pharynx are specialized into thin gastric filaments which in several important families, continue as a long thread or acontium and hang freely in the gastrovascular cavity.

Only two tissue layers are present; a true mesoderm is lacking. The two layers are separated by the mesoglea, a layer of connective

tissue produced by the epithelia (Fig. 2a and b).

The greater part of the endodermal epithelium is made up of epitheliomuscular cells in a single layer. These have three parts: a cell body, a narrow peduncle, and a muscle fiber which lies on the mesoglea. Virtually the whole endoderm therefore, forms a continuous sheet of muscle. Briefly, the major muscle fields in the endoderm are the retractors, parietals, parietobasilars (in some species) on the septa, and the circular muscles of the column, pedal disc, pharynx, oral disc and tentacles. The retractors are powerful longitudinal muscles (present only on the complete and larger incomplete septa) which pull down the oral disc in response to adverse stimuli. The parietals, located at the junction of each septum with the column, constitute the main longitudinal musculature acting on the column. At the top of the column the circular muscles are often developed into a sphincter which closes over the tentacles after retraction of the oral disc. In some muscle fields, the epitheliomuscular cells are modified such that all or most of the cell bodies associated with the muscle fibers are reduced and the nucleus and organelles are located adjacent to the myofibrils. In this case the muscle cells are usually located at the base of the epithelium or below it, embedded in the mesoglea. Muscle fields in which the fibers maintain connection with the epithelium will be referred to as epithelial muscles. Fields in which the fibers are located in the mesoglea will be called mesogleal muscle. This is a simplification of the terminology introduced by Carlgren (1949).

Interspersed among the epitheliomuscular cells are gland cells, sensory cells and amoebocytes. The amoebocytes are small wandering

cells, unspecialized or weakly phagocytic, which can be found inserted anywhere in the anemone tissue. The gonads are located on the retractor bearing septa, between the retractor and the gastric filament. The gastric filaments are tri-lobed structures consisting of gland cells, nematocytes and supporting cells. If the anemone contains symbiotic algae, these are located inside endodermal epithelial cells which have no muscle fiber.

The ectoderm is made up mainly of tall, narrow supporting cells. Interspersed among these are at least two types of gland cell, sensory cells and the nematocytes. In the ectoderm of the tentacles, oral disc and pharynx, and also in the column and pedal disc of some primitive species, modified epithelial cells form a muscle layer which lies directly on, or is embedded in the mesoglea. In the siphonoglyph, a groove formed by a fold in the wall of the pharynx, the ectoderm is ciliated.

The nervous system forms part of a plexus of fibers located between the cell bodies and muscle fibers of the epitheliomuscular cells in the endoderm, and similarly, at the bases of the supporting cells in the ectoderm. The plexus, composed of nervous and non-nervous fibers, reaches its greatest development in the ectoderm of the tentacles, oral disc and pharynx, and in the endoderm at the base of each septum, above the parietal muscles.

The mesoglea is composed of a network of fibers which are probably collagenous, embedded in a matrix whose chemical nature is as yet unknown. Amoebocytes, small mesogleal cells, mesogleal muscle cells and possibly nerve fibers, are the only cell types in the mesoglea.

II. THE MUSCLE FIELDS

Organization of Epithelial and Mesogleal Muscle:

The organization of epithelial and mesogleal muscle fields is illustrated in Fig. 3. In epithelial muscle (Fig. 3a and b), the fibers form a two dimensional sheet which lies on the mesoglea. The muscle sheet is separated from the epithelium proper by the nerve plexus. Mesogleal muscle fibers are located in bundles embedded in the mesoglea (Fig. 3c). In contrast to epithelial muscle, muscle bundles embedded in the mesoglea are relatively isolated from the nervous system.

Increase in strength of the muscle field is accomplished by increasing the number of fibers. In epithelial muscle, this increase is accomodated by folding of the muscle sheet, thus forming lamellae which extend into the mesoglea (Fig. 3b); the fibers maintain connection with the epithelium through their peduncles. The area inside each lamella contains the peduncles of muscle fibers as well as any nerve fibers which may descend from the plexus and thus constitutes a deep layer of the plexus. Increase in size of a mesogleal muscle is accomplished simply by increasing the number and/or size of the fiber bundles.

Robson (1957) observed a considerable interstitial space in the area of the plexus in the retractor of Metridium. She postulated the existence of a hydrodynamic fluid in this sub-epithelial space which would facilitate adjustment of the epithelium to changes of shape accompanying contraction in the muscle field. The size of the sub-epithelial space shows no clear correlation with the size of the

adjacent muscle field in Aiptasia and Stomphia. The space is small in all fields except in the endoderm of the oral disc and pharynx, where it can be considerable (Fig. 4). In general, the space is larger in Stomphia than in Aiptasia in all fields. In the retractor and column circulars of Stomphia, no detectable change in volume of the sub-epithelial space occurs as a result of contraction.

Special Aspects of the Muscle Fields:

a) The septa;

The retractor in both species is a very large muscle and is predominantly epithelial (Fig. 5). On the exocoelic face of the septum (opposite the retractor), there is a thin sheet of epithelial muscle fibers which is longitudinally oriented over much of the septum; at the base of the septum, the fibers are transversely oriented, forming the basilar muscles, and at the level of the pharynx, on the complete septa, there is an additional transverse component to this field. The parietal muscles are epithelial, and are located on both sides of the septum at its junction with the column. In Aiptasia, the parietals are equally developed on both faces of the septa. In Stomphia, the parietal of the exocoelic face fans out toward the base of the septum and forms the parietobasilar muscle. The medial edge of the parietobasilar is marked by a fold in the septum which runs diagonally from the column to the center of the pedal disc. Although the parietobasilar is predominantly an epithelial muscle at the base of the parietobasilar fold, there is a small mesogleal component (Fig. 5b).

b) The column;

The column circulars in Aiptasia are composed of a continuous, unfolded sheet of epithelial muscle. According to Carlgren (1949),

Aiptasia has a weak mesogleal sphincter but this was never observed. In Stomphia, the column circulars have a mesogleal as well as an epithelial component. At the top of the column, the mesogleal component is developed into a strong sphincter.

The Tentacles, Oral Disc and Pharynx:

The tentacles, oral disc and pharynx in both species have a circular muscle field contributed by the endoderm and a longitudinal field contributed by the ectoderm. In Stomphia, at the oral end of the siphonoglyph, there appears to be a local development of the ectodermal muscles. In this area, the orientation of ectodermal fibers is shifted from longitudinal to circular, forming a sphincter (Fig. 6). The ectodermal muscles are epithelial except in the tentacles and oral disc of Stomphia, where they are sunk into the mesoglea. The circular muscles in both species are epithelial.

At the base of the tentacles, ectodermal muscle fibers have been observed crossing the mesoglea and contacting the retractor muscles of the septa in both species, and the sphincter in Stomphia (Fig. 7). Similarly, the oral disc ectodermal fibers have been observed crossing the mesoglea and contacting the retractor muscles (Fig. 8).

The Pedal Disc:

The pedal disc muscles in both species are epithelial. In Aiptasia, the muscle is a flat sheet, as in the column; in Stomphia, the field is more strongly developed, particularly towards the center of the disc. At the junction of the septa with the pedal disc in both species muscle fibers have been observed crossing the mesoglea and contacting the pedal disc ectoderm (Fig. 9). These fibers appear to

originate from the basilar muscle of the septa.

III. THE MESOGLEA

Organization of the Mesoglea;

The mesoglea can be divided into two parts on a structural basis: a superficial layer and a deep layer. In Stomphia, the superficial layer adjacent to a muscle field is 100 - 400 nm. deep and consists of collagen fibers oriented parallel to the myofilaments (Fig. 10a and b). The collagen fibers can sometimes be seen inserting into the basement membrane of the muscle, which is approximately 90nm. thick (Fig. 10b). In Aiptasia, a similar situation is seen only in the retractor. In other fields the basement membrane is much thicker, approximately 150nm, and there is no layer of collagen fibers running parallel to the long axis of the muscle (Fig. 11). The basement membrane lies directly on the deep layer of the mesoglea, and is considered to be the equivalent of the superficial layer in Stomphia.

In the deep layer of the mesoglea in both species the collagen fibers are organized into bundles which in turn form a complex network (Fig. 12). The pattern of the network has been studied in detail in the column of Stomphia, through examination of thick and thin plastic sections. In Stomphia, the predominant network is made up of two sets of folding lattices. In the column, the two sets of lattices are oriented at 45 degree angles to the long axis of the muscle and at right angles to each other, and run diagonally across the mesoglea from endoderm to ectoderm. In addition to the double lattice there are bundles of collagen fibers interspersed in the net-

work which are oriented parallel to the muscle fibers. Also, along the junctions of the septa with the column, bundles of collagen fibers are inserted into the column mesoglea and fan out as they extend across to the ectoderm. All of the bundles in the mesoglea appear to interchange some collagen fibers with one another and in this way the whole framework is loosely spliced together.

Although the organization of the network in the deep layer of the mesoglea is basically similar throughout, the density and size of the deep layer is variable. The mesoglea in Stomphia is much thicker than that of Aiptasia in all fields. In both species the mesogleal fibers are packed very densely in most regions except in the oral disc, pharynx and retractor. In these areas the fibers are packed more loosely and the network is less rigidly organized.

The Effect of Contraction on the Mesoglea:

Changes in the mesogleal network of the column of Stomphia, resulting from circular muscle contraction were studied. In the whole animal, when contraction of the circular muscles of the column occurs with loss of hydrostatic fluid, the result is a decrease in diameter without a corresponding increase in height. This represents an isometric contraction. In the deep layer of the mesoglea, it results in a decreased angle between the two lattices of the network and a corresponding increase in the thickness of the column wall (Fig. 13a). It is more usual, however, that contraction occurs without loss of hydrostatic fluid from the coelenteron. In this case hydrostatic pressure is generated by the circular muscle contraction; in the mesogleal network this causes a decrease in the angles within the two lattices along the oral aboral axis and results in extension of the column (Fig. 13b).

In either case, in the deep layer the effect of contraction is on the angles within the network; the fibers do not bend appreciably and the basic organization is maintained regardless of the contraction state of the muscles. Contraction seems to affect the superficial layer of the mesoglea very little, and the collagen fibers of this area remain parallel to the long axis of the muscle.

IV. THE EPITHELIOMUSCULAR CELL

Differentiation in the muscular system of actinians takes the form of variations on a simple organization which has as its basic unit the epitheliomuscular cell. A diagram illustrating the characteristic features of this cell type, as it occurs throughout the sea anemone, is given in Figure 14. Typically, the cell is in three parts: the muscle fiber, which lies on the mesoglea, the cell body, in the epithelium, and the peduncle, which connects the two running through the sub-epithelial nerve plexus. The fine structure of each part will be described separately.

The Muscle Fiber:

The muscle fiber contains the myofilaments and the organelles associated with contraction. Both thick and thin filaments are present. The filaments are not arranged into discreet sarcomeres, and therefore fall under the general classification of "smooth" myofibrils. Dense bodies, a characteristic structure in many smooth muscles, have not been observed among the myofilaments and except for a few microtubules and vesicles, the myofilament area is generally free of inclusions. Two different types of muscle fiber have been observed in both Stomphia

and Aiptasia.

1) In Type A fibers (Fig. 15 and 16a), the diameter of the thin filaments is 75Å and that of the thick filaments is 180Å. The length of the thick filaments is approximately 1.5μ. The overall ratio of thin to thick filaments is approximately four to one, and the average number of thin filaments associated with one thick filament is six. The average distance between thick filament centers is 490Å. Although there is no distinct pattern to the myofilament organization as seen in striated muscle, it is evident in cross section that the distribution of thick filaments is not entirely random. There are many areas within the fibril that contain only thin filaments (Fig. 15b). In longitudinal section, this appears as a faint and loosely organized striation (Fig. 15a).

2) In Type B fibers (Fig. 16b and c), the diameter of the thin filaments is again 75Å. The diameter of the thick filaments is highly variable, ranging from 180 to 1100Å, and on the average (225Å), is greater than in A fibers. The length of the filaments is also variable; the filaments with the greatest diameter tend to be the l[illegible]ngest and can be up to 7μ in length. In longitudinal section, a [illegible]lar banding with a period of 110Å is visible in the thick filaments of greatest diameter (Fig. 16c). The filaments are not as densely packed as in A fibers; the average distance between thick filament centers is 650Å. The overall ratio of thin to thick filaments is approximately three to one, and the average number of thin filaments associated with one thick filament is five. The regular, clumped distribution of thick filaments which was observed in A fibers is only rarely seen in B fibers. Rather, the thick filaments tend to be

distributed irregularly with the thin filaments in association, leaving filament free areas within the fibril.

The basic pattern of myofilaments in A fibers is very consistent. In B fibers, however, the myofilament pattern is not fixed, but highly variable, even within one muscle field in different individuals. The main variation is in the number, distribution and diameter of the thick filaments, as illustrated in Figure 17. In spite of this, the B fibers are distinguishable from the A fibers in all cases.

The distribution of Type A and Type B fibers in Stomphia and Aiptasia is given in Table I. The distribution is similar in the two species, with the possible exception of the tentacle and oral disc longitudinal muscles and the pedal disc circulars. The tentacle and oral disc circular muscles in Stomphia appear to be composed entirely of A fibers, while in Aiptasia they have both A and B components. In Stomphia, the bases of the support cell peduncles often contain longitudinally oriented thick and thin filaments although in some areas only thin filaments have been observed. Where thick filaments are preserved the support cells appear to be intermediate in structure between epitheliomuscular cells with Type B fibers and the support cells found in the column and pedal disc ectoderm. It is possible that the bases of these cells contribute a B fiber component to the tentacle and oral disc longitudinal muscle.

The distribution of the two fiber types in the pedal disc circulars of Stomphia and Aiptasia is not entirely clear. In Stomphia, the circular muscles of the peripheral part of the disc are composed of A fibers. At a distance of two to three millimeters from the column there is an abrupt changeover and the circular muscles from that point to at

least three millimeters inward are composed of B fibers. The muscle fiber composition of the center of the disc is as yet undetermined. The pedal disc of Aiptasia has not been studied intensively; although only B fibers have been observed in this region, it is possible that A fibers are also present.

Inclusions of the muscle fiber other than the myofilaments are located adjacent to the myofibril (Fig. 14 and 18). These are the mitochondria, microtubules, numerous, small electron dense particles which are probably glycogen, small, electron dense vesicles, and a system of clear tubules tentatively identified as the sarcoplasmic reticulum. Although this identification can not be confirmed without the demonstration of the presence of free calcium in the tubules, the system is very similar to the sarcoplasmic reticulum described in other invertebrate smooth muscles (MacRae, 1965; Rogers, 1969).

The sarcoplasmic reticular system is well developed only in Stomphia. In Aiptasia it is generally limited to individual membrane sacs, and a "reticulum" is rarely seen (Fig. 18b). In Stomphia, the tubules occur in clusters (Fig. 18a and Fig. 19); they have an average diameter of 110nm and are aligned roughly parallel to the myofilaments. The inner surface of the membrane is coated with electron dense material and the lumen of each tubule is clear. The reticulum is present in most muscle fields of Stomphia, and is well developed in the retractor, parietal, parietobasilar and sphincter muscles (Fig. 19). The amount of reticulum in a muscle does not appear to be related to the type of fiber present.

The muscle fibers are larger in Stomphia than in Aiptasia and in both species A fibers are larger, on the average, than B fibers. The

average cross sectional area of A fibers in Stomphia and in Aiptasia is $3.2\mu^2$ and $1.6\mu^2$ respectively. The average cross sectional area of B fibers is $1.5\mu^2$ and $0.6\mu^2$, respectively, except in the retractor where it is $0.6\mu^2$ and $0.2\mu^2$ respectively.

Individual muscle fibers are interconnected laterally and at their ends by desmosomes (Fig. 20). The desmosomes have a constant intercellular gap of 340Å, and a fibrous layer on either side. Thin filaments and thick filaments of B fibers can sometimes be seen entering the end desmosomes, but do not appear to contact the lateral desmosomes. No other type of specialized intercellular junction has been observed between the muscle fibers.

Small, finger-like processes of the sarcolemma, approximately 80 to 140nm in diameter, are present on many of the muscle fibers (Fig. 14 and 21). In epithelial muscle these sarcoplasmic extensions run parallel to the muscle layer in the plexus and are intimately associated with the nervous system. The sarcoplasmic extensions in the mesogleal muscle of Stomphia are located in the core of each bundle of fibers. They are infrequent in the mesogleal muscle of the tentacles and oral disc; in the sphincter and in the column mesogleal muscle the extensions are numerous and often form a spiral in the core of the muscle bundle (Fig. 21c and d).

The Peduncle;

The peduncle is a narrow stand of cytoplasm, 80 to 200nm in diameter, which passes through the plexus and connects the muscle fiber to its cell body (Fig. 14 and 22). In both A and B fibers, the peduncles and sarcoplasmic extensions have a granular cytoplasm and contain microtubules, small mitochondria, thin (75Å) filaments,

and occasionally, elements of the sarcoplasmic reticulum. The peduncles of B fibers also contain thick (170Å) filaments, in addition to the other inclusions.

The Cell Body;

The fine structure of the cell body varies with the function of the epithelium of which it is a part. Endodermal epitheliomuscular cell bodies are generally digestive cells containing lysosomes in various stages (Fig. 23). The cells form microvilli at their apical surface and have one (rarely more) flagellum with a well developed striated root. In some endodermal cells, an accessory striated structure of variable periodicity has been observed in association with the striated root (Fig. 24). The long axis of the structure is composed of parallel filaments 80 to 150Å in diameter, separated from each other by 250Å. The major axial period varies from 0.14 to 0.22μ. At the distal end of the structure, the filaments of the long axis appear to insert onto the cell membrane, which is generally curved inward at the point of insertion. There is no consistent orientation of the structure within the cell, either with respect to the cell surface or to the striated root (which forms an angle of approximately 60 degrees with the cell surface). The structure has been found in epitheliomuscular cells of the mid-column of Aiptasia, and above the retractor in both Stomphia and Aiptasia.

The Effects of Contraction on the Fine Structure of the Muscle Fiber;

The effect of contraction state on the fine structure of the muscle fibers was studied, using the retractor and column circular muscles of Stomphia (Figs. 25-27). These muscles contain both A and B fibers, and therefore provide an opportunity for investigating the

possibility that the two types might represent differences in contraction state of a single kind of muscle.

In general, the effects of isotonic and isometric contractions were the same, except that in isotonically contracted muscle the myofilaments were thrown into considerable disarray in some areas. Although finger-like folds of the sarcolemma appeared in some areas, particularly in isotonically contracted muscle (Fig. 27d), the contour of the muscle fiber usually remained regular and unfolded. The cross sectional area of A and B fibers from the column, and of A fibers from the retractor increased with contraction and decreased with stretch. The cross sectional area of B fibers from the retractor increased, both with contraction and with stretch.

In Type A fibers (Fig. 25 and 27) the areas free of thick filaments which were observed in the resting state, disappeared with contraction but were not affected by stretch. No change was detected in the diameter or the length of the thick filaments. In Type B fibers of the column (Fig. 26) the number of very large diameter thick filaments increased, both with contraction and with stretch, resulting in an overall increase in the average diameter of the thick filaments from 225Å to 290Å. The average length of the thick filaments increased from 3.0μ to 4.0μ. In the retractor the filaments of Type B fibers were sometimes poorly preserved, both in the myofibril and in the peduncle, and could not be distinguished from the dark sarcoplasm which appeared in contracted muscle (Fig. 27c and d) and to a limited extent in stretched muscle (Fig. 27b). Nevertheless, some very large diameter thick filaments were observed in B fibers from contracted and stretched retractor. It seems likely that the thick filaments in these fibers

respond in a similar way to contraction state as those in Type B fibers from the column.

V. NERVOUS INTERACTION

An extensive description of the nervous system of Stomphia has recently been completed by Petreya (1976). This study, therefore, will include only a brief report on observations of synapses and neuromuscular junctions in Aiptasia and Stomphia.

Synapses and neuromuscular junctions can be recognized in Stomphia and Aiptasia at a fine structural level by several characteristics: 1) an aggregation of vesicles closely apposed to the pre-synaptic membrane; 2) a synaptic cleft with a uniform gap of approximately 200Å; 3) an increased electron density in the pre- and post-synaptic membranes of an interneural junction. Neuromuscular junctions may occur on the peduncles, the sarcoplasmic extensions, or on the muscle fiber directly. The size and density of synaptic vesicles will be used almost exclusively as the basis for characterization of particular types of synapses and neuromuscular junctions.

Neuromuscular Junctions;

In Aiptasia, only one type of neuromuscular junction has been observed. The junction occurs on the sarcoplasmic extensions or directly on the myofibril of the longitudinal (ectodermal) muscles of the tentacles and oral disc (Fig. 28). The synaptic vesicles are 48nm in average diameter (range 42 to 79nm) and contain light granular material.

In Stomphia, three different neuromuscular junctions have been observed. 1) A junction occurs on the peduncles or sarcoplasmic

extensions of the A fibers of the retractor (Fig. 29). The synaptic vesicles are 74nm in average diameter; they are dense cored or uniformly light and sometimes appear collapsed. The junction is observed frequently, and on two occasions, a single nerve fiber was observed innervating at least six muscle fibers (Fig. 29a). 2) Neuromuscular junctions have been observed on the peduncles or sarcoplasmic extensions of fibers from the parietals, the column epithelial circulars, and the oral disc and pharynx circulars (endodermal) (Fig. 30). All of these muscle are composed of Type B fibers. The synaptic vesicles of the junctions all have a similar average (71nm) and range (58 to 110nm) of diameters and similar appearance. The vesicles make up a mixed population, having dense, dense cored or light contents. The largest vesicles tend to be the most dense. Although it is possible that the junctions observed in these fields represent input from several different nerves, they are considered here as a single type of junction on the basis of the morphology of their vesicles and of their occurrence exclusively on B fibers of the endoderm. 3) In the ectodermal muscle of the tentacles, a nerve fiber from the ectodermal plexus descends into the muscle bundles and innervates the fibers (Fig. 31). The neuromuscular junction is most frequently observed directly on the muscle fiber, although it occurs occasionally on a sarcoplasmic extension. The vesicles are dense cored or light and have an average diameter of 65nm.

Synapses;

Four different synapses have been observed in Aiptasia (Table II). 1) and 2) Two different synapses have been observed in the column endoderm. The first (Fig. 32a and b) is a reciprocal synapse with many

clear vesicles, 60nm in average diameter. The second (Fig. 32b) is polarized, and has light granular vesicles with an average diameter of 67nm. The post-synaptic fiber of this synapse is the first fiber, described above. 3) In the oral disc and tentacle ectoderm a reciprocal synapse having clear vesicles with an average diameter of 78nm has been observed frequently. 4) The fourth synapse has been observed only once, in the plexus of the parietal, near the retractor (Fig. 32d). It is polarized and has light, granular vesicles with an average diameter of 49nm.

Seven different synapses have been observed in Stomphia (Table III). 1) and 2) Two synapses are present in the ectodermal plexus of the tentacles and oral disc (Fig. 33). The first has dense core vesicles with an average diameter of 68nm and is polarized. The second has dense or dense core vesicles with an average diameter of 80nm and can be polarized or unpolarized. 3) In the endoderm of the oral disc and pharynx, a reciprocal synapse having granular synaptic vesicles with an average diameter of 55nm has been observed frequently (Fig. 34). 4) In the septa above the retractor there is an unpolarized synapse with clear or granular vesicles having an average diameter of 68nm (Fig. 35). 5) and 6) In the plexus above the parietal muscles there are two synapses (Fig. 36 and 37). The first (Fig. 36) is reciprocal and has granular synaptic vesicles with an average diameter of 51nm. The vesicles in this fiber, as well as in the Type 4 fiber described above, are susceptible to fixation damage and their appearance is therefore quite variable (Fig. 35 and 36). The second synapse of the parietal plexus has been observed only once. It is polarized and has lightly granular synaptic vesicles with an average diameter of 68nm (Fig. 37).

7) In the plexus overlying the sphincter, a reciprocal synapse has been observed; the synaptic vesicles are clear or lightly granular and have an average diameter of 78nm. (Fig. 38).

A nerve fiber has been observed in the deep plexus of the pedal disc circular muscle passing out between the muscle fibers into the mesoglea (Fig. 39). The fiber contains many microtubules and a population of dense or dense core vesicles with an average diameter of 98nm. The fiber has been observed in the endodermal plexus of the pedal disc, and in both the endodermal and the ectodermal plexus of the oral disc. It is possible that the fiber is also present in the ectodermal plexus of the pedal disc, however, this has not yet been confirmed. Although no synapse of this fiber has ever been observed, it is mentioned here because it possibly represents a nervous connection between the ectoderm and the endoderm in the oral disc and the pedal disc.

DISCUSSION

Discussion of the results will be limited to three topics; 1) the fine structure of the mesoglea; 2) the function of the sarcoplasmic extensions; 3) the nature of Type A and Type B fibers, and their significance in the interpretation of actinian behaviour.

1) The Mesoglea:

The muscle fibers of actinians operate over their length, by deforming the adjacent mesoglea, rather than over their ends, as in insect and vertebrate skeletal muscle. Each muscle field, therefore, forms a unit with the underlying mesoglea with which it is functionally inseparable (Batham and Pantin, 1951; Gutmann, 1966). This intimacy of functional contact between the muscles and the mesoglea imposes very specific demands on the fibrous organization of the mesoglea and the relationship of mesogleal fibers with epitheliomuscular cells.

A superficial and a deep layer can be recognized in the mesoglea. The function of the superficial layer is to provide a surface for attachment of the muscles and to convert shortening in the muscles to an integrated change in the network of the deep layer. Batham and Pantin (1951) suggested that the superficial layer of the mesoglea must be fiberless, in order to follow the folding (buckling) of the muscle layer which occurs as a result of contraction in an opposing muscle field. Gutmann (1966) argued that the superficial layer must be composed of densely packed fibers, if it were to effectively transmit tension from the muscle layer to the fiber network.

The results of the present study indicate that there is some variation in the structure of the superficial layer.

In Aiptasia, except in the retractor, the layer is composed of a fine filamentous net. In Stomphia and in the retractor of Aiptasia, it is composed of densely packed collagen fibers aligned parallel to the axis of the muscle fibers. The significance of the variation in the structure of the superficial layer is unclear. It is tempting to suggest that the difference might relate to the degree of development of the muscle field. In Stomphia, virtually all of the muscle fields are relatively strongly developed, such that the muscle does not form a flat sheet, but is folded into the mesoglea. In Aiptasia, only the retractor muscle is permanently "buckled" and all other fields form flat sheets lying on the mesoglea.

If this association holds in other genera, it could be proposed that a fibrous superficial layer can serve to increase the effectiveness of the muscle fibers located at a distance from the deep layer, so that the buckled field can function as an integrated unit in deforming the deep layer. In the weaker, sheet muscle fields, all of the muscle fibers are closely apposed to the deep layer and can deform it without the specialized fibrous superficial layer. If the fibrous layer is a requirement of a buckled muscle field, one might ask why it could not be fulfilled by a filamentous superficial layer. It can be assumed that this layer in the muscle fields of Aiptasia, where it occurs, effectively transmits the contraction of the muscles to a tension on the mesogleal network, and it seems that similar forces would be involved in integrating the contraction of a buckled muscle field. Unfortunately, there is not enough known about the forces involved in contracting actinian muscle fields to be able to answer this question satisfactorily.

The function of the deep layer of the mesoglea is to resist the hydrostatic pressure of the coelenteron and in so doing to give support to the animal, at the same time allowing free and effective movement of the attached muscle fields. The deep layer is composed of a network of interconnected fiber bundles embedded in a fluid or semifluid matrix. According to Chapman (1953a) and Leghissa and Mazzi (1959), the network is organized as a double crossed helix of fibers, in parallel through the depth of the mesoglea. Gutmann (1966) pointed out that since the muscle fibers act by deforming the network, it is essential for the effectiveness of the muscles that tension in the network be maintained at all times. In this regard, he suggested that any model of the mesogleal network must include a major fiber component going across, as well as around the mesoglea. The analysis of the fine structure of the mesoglea in the column of Stomphia indicates that the network is made up of two crossed lattices oriented at right angles to each other, running obliquely from endoderm to ectoderm. Contraction in the muscle results in a change in the angles of the network in the oral-aboral direction (with contraction against hydrostatic pressure) or across the body wall (with contraction and loss of hydrostatic fluid). The organization of the network is such that tension in the fibers of the network is maintained, regardless of the extent of contraction in the muscle, and thus effectiveness of muscle fiber shortening is preserved over a wide range of lengths of the muscle.

The question has been raised of whether or not the mesoglea undergoes changes of shape at constant volume, and it has been implied that during contraction of the muscle, loss of fluid from the mesoglea may occur (Batham and Pantin, 1951). In the mesoglea of the column of

Stomphia, the possibility seems to be unlikely, although it is difficult to answer this question, since investigation requires dissection, and thus distortion of the net - fluid system. However, examination of tissue sections combined with observations on the whole animal allow some speculation. It has been observed that in the column of Stomphia the mesoglea either increases in thickness (with a loss of coelenteric fluid) or increases in height (with retention of coelenteric fluid) in proportion to the decrease in its circumference, resulting from contraction in the circular muscle field. This suggests that the mesoglea itself acts as a hydrostatic system, operating at constant volume. Moreover, there appears to be no outlet for a fluid loss from the mesoglea. Robson (1957) observed a considerable subepithelial space in the area of the nerve plexus of the ectodermal musculoepithelium in Metridium; she suggested that movement of fluid between this space and the mesoglea might provide a means for accomodation of the mesoglea to contraction, and also might facilitate adjustment of the overlying epithelium to changes in shape of the muscle layer. This relationship between musculoepithelium and mesoglea seems unlikely. With the demonstration by Peteya (1976) of a dense fiber plexus in the area of the sub-epithelial space, it is clear that the space cannot be nearly as large as Robson had first considered (previously, recognition of much of the plexus was prevented by fixative-induced damage). In any case, no possible connection between this space and the mesoglea has been observed; the two are effectively sealed off from each other by the muscle layer.

2) The Sarcoplasmic Extensions:

The sarcoplasmic extensions are narrow strands of cytoplasm which come off the muscle fiber and extend into the fiber plexus in the case of an epithelial muscle field, or into the core of a muscle bundle, in a mesogleal muscle field. The extensions of epithelial muscle fields have often been observed receiving innervation, and it is possible that they have developed for this specific function. The occurrence of this type of neuromuscular junction, where the muscle sends a process out towards the nerve, rather than the reverse, is not uncommon among higher animals. It has been reported in platyhelminths (MacRae, 1963; Chien and Koopowitz, 1972), annelids, (Wissocq, 1970; Mill and Knapp, 1970), nematodes (Debell, 1965; Rosenbluth, 1965), echinoderms, (Cobb and Laverack, 1967), cephalochordates (Flood, 1966), and in arthropods (Atwood, et al, 1969). It has been suggested that the extensions might allow or compensate for a sparse or distant nervous system, and/or that they might be the site of integration of incoming signals to the muscles (Chien and Koopowitz, 1972). The former explanation seems likely for the sarcoplasmic extensions in epithelial muscle fields, particularly in regions other than the retractor, such as the column, where the nerve net is quite sparse (Batham et al, 1960; Chapman, et al, 1962).

In the sphincter and column mesogleal muscle of Stomphia, however, no neuromuscular junctions have been observed, in fact nerve fibers are rarely seen in these muscle bundles, yet the sarcoplasmic extensions can be well developed. In the bundles, the extensions are shorter and stouter (in one dimension) than they are in epithelial muscle. They form foldlike extensions which lie in the core of the bundle, where the

extensions of all of the muscle fibers mingle, and often form a spiral configuration. The function of the extensions in this arrangement is unclear. It is possible that they serve a mechanical function, and aid in the maintenance of the integrity of the muscle bundles during contraction or distortion of the muscle layer.

It is clear that the area of contact between muscle fibers in a bundle is greatly increased by the sarcoplasmic extensions. It is tempting to suggest that the extensions may provide an increased surface area for electrical transmission of impulses within the muscle. It is generally accepted that the presence of gap junctions (nexuses) is required for direct electrical coupling between cells to occur (Barr and Jakobsson, 1976). No gap junctions have been observed in any actinian tissue examined so far, although a variety of fixatives have been employed (Grimstone, et al, 1958; VanPraet and Doumenc, 1975; Amerongen and Peteya, 1976; Peteya, 1976). This would argue against the possibility that non-nervous conduction occurs in actinians. However, it has recently been demonstrated that electrical coupling occurs in the absence of specialized junctions in some vertebrate smooth muscles, and it has been suggested that "nexus-like" structures invoked in electrical coupling in some tissues have been produced by swelling damage (Daniel, et al, 1976). It is perhaps possible then, that electrical coupling occurs between the muscle fibers of actinians, and the sarcoplasmic extensions may be the site of coupling in the sphincter and the column mesogleal muscles. However, where epithelial conduction has been demonstrated in the Cnidaria, it involves slow conduction (Josephson, 1974) and the sphincter and column mesogleal muscles are capable of fast contractions. Until more is known of the

factors involved in the excitation of the muscles in actinians, the role of non-nervous conducting systems, if they exist at all, cannot be evaluated.

3) The Nature of Type A and Type B Fibers;

It is well established that some muscles of actinians are capable of fast and slow contractions, while others are capable of only slow contractions (Batham and Pantin, 1954; Ross, 1957; Pantin, 1964 and 1965; Robson and Josephson, 1969; Josephson, 1974). The sphincter of Metridium and of Calliactis, and the retractor of Metridium will give a slow contraction in response to low frequency stimulation and a fast contraction following high frequency stimulation. On the other hand, the column circular muscles of Metridium will only contract slowly, regardless of the frequency of stimulation. The mechanism underlying the capacity for quick and slow contractions in a single muscle field is unknown. The possibility that the different contractions were due to the presence of two kinds of muscle was dismissed early; the histologists could recognize only one type of muscle in tissue sections (Batham and Pantin, 1951; Robson, 1957) and with direct observation of the contracting muscle field it appeared that the entire field was involved in both types of contraction (Batham and Pantin, 1954; Ross, 1957; Pantin, 1965). Further support for the idea of a single muscle type came from the first electron microscope study on actinian muscle by Grimstone, et al (1958). In the mesenteries of Metridium they observed a single type of muscle, however, their fixatives failed to preserve the thick filaments. Recently, Peteya (1976) observed two different muscle types in Stomphia, which he distinguished on the basis of thick filament diameters. He was, however, unable to state whether

these two muscle types represented differences in contraction state of a single kind of muscle, or two morphologically distinct kinds. In this study, the presence of the two types of muscle in Stomphia has been confirmed, and has been established for a second species, Aiptasia, and the distibution of the two types in the various muscle fields of both species has been mapped. In addition, the effect of contraction on the fine structure of the two types has been investigated, and there is now strong evidence which suggests that the two types represent two morphologically distinct kinds of muscle.

The major difference between the two types of muscle is in the structure of the thick filaments of the myofibril. The thick filaments of Type A fibers have a relatively constant diameter of 180A, and are approximately 2u in length. In Type B fibers, the thick filaments have a highly variable diameter, ranging from 180 to 1100A (average = 225A) and can be very long (up to 7u). In longitudinal sections, an axial striation with a period of approximately 110A is visible in the filaments. It is unlikely that the differences occur as a result of fixation artifact. The two types of muscle have been observed side by side, under identical conditions of anaesthesia and fixation. Furthermore, they have been observed repeatedly, in the same muscle fields, in different individuals, in different species, and with several different fixatives (Peteya used several fixatives and did most of his work with a modified Cavey fixation).

The possibility that Type A and Type B fibers might represent different contraction states of a single kind of muscle has been considered. Since both types occur when the tissue is fixed after magnesium narcotization, this would mean that one of the muscle types

was being stimulated by a mechanism insensitive to magnesium.

It has now been demonstrated that there is no change either of A fibers into B fibers, or of the reverse, as a result of contraction in the retractor or the column circulars of Stomphia. Both types of fiber, however, do show some change with contraction. This is especially marked in B fibers, where the number of large diameter thick filaments increases. A similar change has been reported in the longitudinal muscle of the tentacles of Chrysaora (Scyphomedusae) and has been attributed to the effects of contraction state (Perkins, et al, 1971). In A fibers, no difference was observed in the dimensions of the thick filaments from relaxed and contracted muscles. It can be concluded, therefore, that A and B fibers do not result from different contraction states of a single kind of muscle. Although changes do occur as a result of contraction, and in Type B fibers these changes involve the thick filaments, no change from one type of fiber into the other occurs.

It is very likely then, that A and B fibers represent two morphologically distinct kinds of muscle. Before discussing the functional significance of this difference, the nature of the thick filaments in A and B fibers must be considered. The thick filaments of Type A fibers are similar in fine structure to the thick filaments of a variety of other muscles from both vertebrates and invertebrates. These include the muscles of Hydra (Haynes, et al, 1968; Slautterback, 1967; Chapman, 1974), the muscles of Notoplana (Platyhelminthes) (MacRae, 1965), the fast adductor of Pecten (Nisbet and Plummer, 1968) and the mantle muscle of Octopus (Hanson and Lowy, 1957; Kawaguti, 1962), (Mollusca), some muscle cells in the body wall of Lumbricus (Annelida) (Mill and Knapp, 1970), many somatic muscles of insects (Maruyama, 1965), the blood

vessel muscle in Siboglinum (Pogonophora) (Jensen and Myklebust, 1975), some muscles of Parastichopus (Echinodermata) (Jensen, 1975), and vertebrate skeletal muscle (Huxley and Hanson, 1960). The thick filaments from these muscles have a relatively constant length and diameter, both of which are small, and they show no marked axial striation; it is this type of thick filament which is present in most cross-striated muscles. Wherever these filaments have been isolated and characterized, it has been demonstrated that they are composed of myosin (Maruyama, 1965; Hasselbach and Schneider, 1951; Huxley and Hanson, 1957).

The thick filaments of Type B fibers closely resemble the thick filaments of the tentacle muscle of Chrysaora (Perkins, et al, 1971), as mentioned above, the tonic adductor muscle of many molluscs (Hanson and Lowy, 1960; Sobieseck, 1973; Cilloteaux, 1976) the body wall muscles of nematodes (Waterson, et al, 1974), the body wall muscles of annelids (Mill and Knapp, 1970), the somatic and visceral muscles of some echinoderms (Bacetti and Rosati, 1968; Dolder, 1972), the longitudinal muscle in the tentacles of a pogonophoran (Gupta and Little, 1969; Gupta, et al, 1966), and some slow muscles of arthropods (Fahrenbach, 1967). The thick filaments of these muscles have large and variable diameters, are usually quite long, and show a characteristic axial periodicity of approximately 140Å. Wherever these filaments have been characterized, it has been shown that they are composed of paramyosin (Tropomyosin A) and myosin (Hodge, 1952; Locker and Schmitt, 1957; Laki, 1971). Szent-Gyorgyi, et al (1971) have demonstrated that the paramyosin forms the core of the filaments and myosin is present as a surface layer. The amount of paramyosin in the core is variable, and accounts for the wide

range in the average diameters of the paramyosin filaments in different muscles. The fine structural characteristics of thick filaments from A and B fibers from Stomphia and Aiptasia coincide very closely with the characteristics of thick filaments composed of myosin and paramyosin. Therefore, it seems likely that the filaments of A fibers are composed of myosin and those of B fibers are composed of paramyosin.

Several interrelated structural features are known to influence the intrinsic properties of contraction in a muscle, and these must be taken into account in an interpretation of the functional implications of A and B fibers in Stomphia and Aiptasia. 1) The sarcomeric organization of myofilaments, as in obliquely striated and cross striated muscles, rather than a comparatively random organization, as in smooth muscles, allows a more rapid development of tension. 2) The presence of a paramyosin core in the thick filaments enhances the ability for maintenance of tension over prolonged periods, with little expenditure of energy (Rüegg, 1971; Toida, et al, 1975; Szent-Gyorgyi, et al, 1971; Cohen, et al, 1971; Jewell, 1959).

The ATPase activity of myosin influences the rate of tension development in both myosinic and paramyosinic muscles (Rüegg, 1971; Barany, 1967). In general, the ATPase activity of thick filaments in myosinic muscles is relatively high and tension development is rapid. The ATPase activity of myosin in paramyosinic muscles is relatively low, possibly due to an inhibitory effect on the enzyme activity by paramyosin (Szent-Gyorgyi, et al, 1971). 3) The length of the thick filaments influences the speed of tension development and release; with longer thick filaments, the rate of tension development is less (Szent-Gyorgyi, et al, 1971; Rüegg, 1971). 4) The amount of sarcoplasmic reticulum

present, and the extent of its association with the myofilaments influences the latency (time between stimulation and onset of contraction) and the speed of relaxation (McGuffee and Bagby, 1976; Tomanek, 1976).

Three of the factors listed, when applied to the structural features of A and B fibers lead to the suggestion that A fibers are likely to be capable of phasic contractions, while B fibers are perhaps more tonic. 1) Although both A and B fibers fall into the category of "smooth" muscles, the myofilaments of A fibers are arranged in a more orderly way than those of B fibers and show a faint sarcomeric organization. 2) As described above, it seems that the thick filaments of A fibers are composed of myosin, while those of B fibers contain paramyosin. 3) The thick filaments in B fibers are longer, on the average, than those in A fibers. Since the amount of sarcoplasmic reticulum in the muscles of Stomphia and Aiptasia is not related to the presence of A and B fibers, this factor must be considered independantly in drawing correlations between the fine structure and the function of the various muscle fields.

The distribution of A and B fibers in Stomphia and Aiptasia is, for the most part, consistent with the distribution of fast and slow muscles. All actinian muscles so far investigated are capable of slow contraction; some muscle fields are also capable of fast contraction (Batham, et al, 1960). In Aiptasia, the retractor muscles, and the tentacle and oral disc longitudinal muscles are capable of fast contraction (King, personnal communication; personnal observation). All three of these muscle fields are composed mainly of Type A fibers, while the remaining fields are composed mainly or entirely of Type B

fibers. In Stomphia, the retractor, the sphincter and the tentacle and oral disc longitudinal muscles are capable of fast contraction (Hoyle, 1960). Both the retractor and the sphincter have A and B fiber components. Although the tentacle and oral disc muscles appear to be composed entirely of A fibers, it is possible that a B fiber component is contributed by the (myo?)-filaments in the bases of the support cell peduncles.

The specialized swimming behavior of Stomphia requires fast contractions in muscle fields which in other species, are generally capable of slow contractions only. Prior to swimming in Stomphia, the pedal disc detaches rather suddenly from the substratum. This release involves the formation of a ridge between the pedal edge and the center of the disc; a turgid cone is formed by the center of the disc, and persists during swimming (Robson, 1961; Ellis, et al, 1969). The pedal disc circular muscles are composed of Type A fibers on the periphery and Type B fibers towards the center. It is possible that the A fiber component contributes to the rapid release of the disc, while the B fiber component is responsible for the turgidity of the central cone. Following the detachment of the anemone from the substratum, a rapid peristaltic wave passes down the column, and causes elongation of the animal (Robson, 1961); the mesogleal circular muscles are composed of A fibers, and probably effect this rapid contraction. The epithelial circular muscles are composed of Type B fibers; prolonged tonic contraction in these muscles can account for the maintenance of high hydrostatic pressure throughout swimming. The parietobasilar muscles in Stomphia are thought to be responsible for the quick flexions of the column during swimming (Sund, 1958; Robson, 1961). Hoyle

(1960) demonstrated the capacity for fast and slow contraction in the parietobasilars, although he mentioned that it was not possible to distinguish the reactions in these muscles from those of the parietals. The parietobasilars are composed mainly of B fibers, suggesting a tonic function; however, the B fibers contain a large amount of sarcoplasmic reticulum, comparable to that seen in the retractor and the sphincter. Thus, it seems that a combination of factors may be in operation, determining the overall pattern of contraction in this muscle.

The correlation between the distribution of A and B fibers with that of many known fast and slow muscles in Stomphia and Aiptasia can explain, to some extent, the nature of the differentiation within the muscle system of these two anemones. Further study is required before the functional significance of this differentiation can be fully understood. It would be most profitable to develop a wider knowledge of the fine structure of neuromuscular systems in other species of actinians, particularly in Calliactis parasitica, since both the behavior and the neurophysiology of this species have been so well studied.

TABLES, PLATES AND FIGURE LEGENDS

TABLE I. The distribution of Type A and Type B fibers in Stomphia and Aiptasia.

		Stomphia		Aiptasia	
		% A	% B	% A	% B
tentacle	longitudinal (ectoderm)	100?		80	20
	circular (endoderm)		100		100
oral disc	radial (ectoderm)	100?		80	20
	circular (endoderm)		100		100
pharynx	longitudinal (ectoderm)	30	70	30	70
	circular (endoderm)		100		100
column circulars	epithelial		100		100
	mesogleal	100			
	sphincter	100			
septal muscles	retractor (endocoelic)	90	10	90	10
	parietal (both faces)		100		100
	parietobasilar (endocoelic)		100		
	basilar (both faces)		100		100
pedal disc	circular	outer part of disc	inner part of disc		100?

TABLE II. Synapses in Aiptasia.

	Vesicles			Location	Figure #
	Average Diameter	Range of Diameters	Contents		
1	60nm	56-67nm	clear	column endoderm	32a and b
2	67nm	57-65nm	granular light	column endoderm	32b
3	78nm	42-91n	clear	oral disc and tentacle ectoderm	32c
4	49nm	-	granular light	septum, above parietal	32d

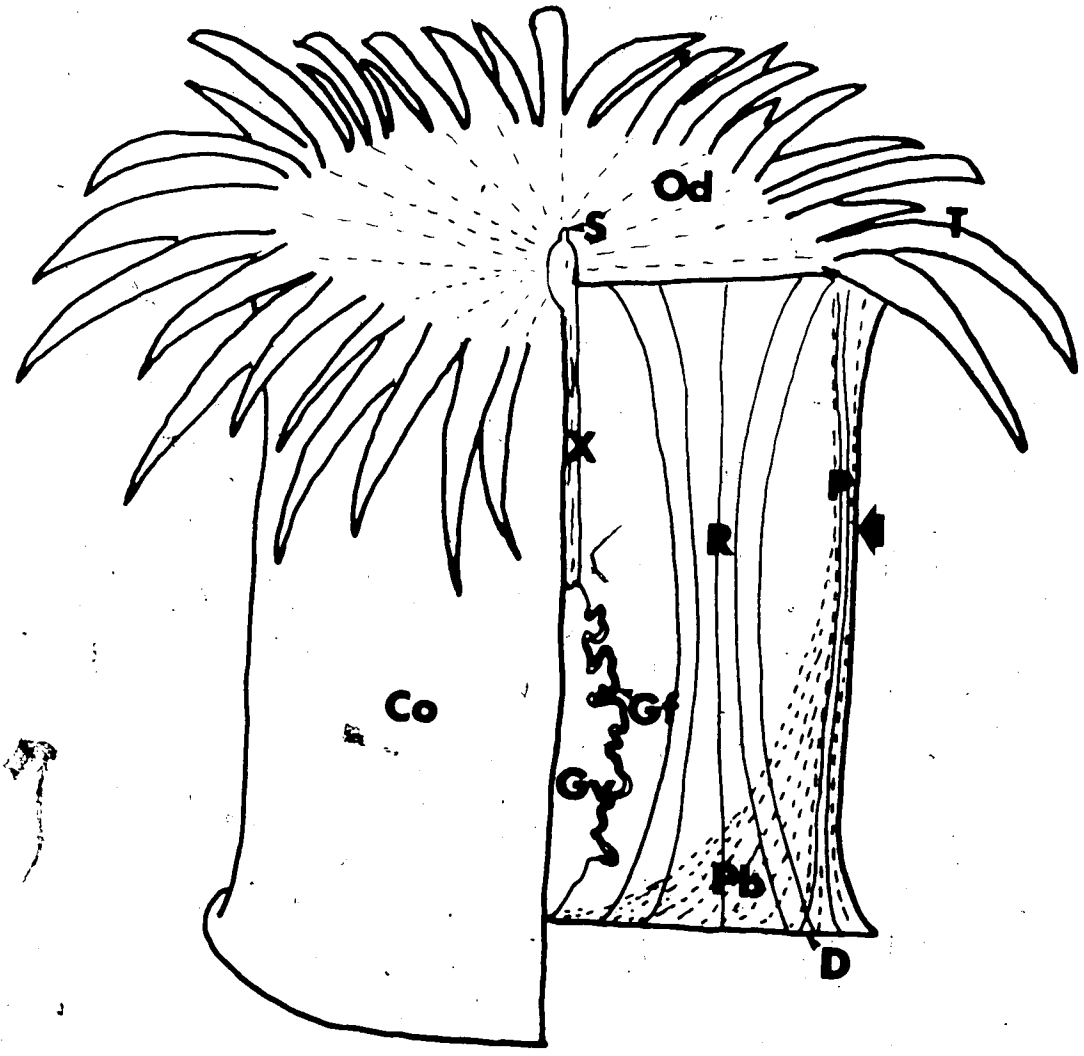
Note: The information included in this table and in Table III is based on limited data and may be subject to revision after further study.

TABLE III. Synapses in Stomphia.

	Vesicles			Location	Figure #
	Average Diameter	Range of Vesicles	Contents		
1	68nm	55-76nm	dense core	oral disc and tentacle ectoderm	33a
2	80nm	52-95nm	dense or dense core	oral disc and tentacle ectoderm	33b
3	55nm	4[illegible]-65nm	dark granular	oral disc and pharynx endoderm	34
4	68nm	63-78nm	clear or granular	septum, above retractor	35a and b
5	51nm	41-77nm	dark granular	septum, above parietal	36a and b
6	68nm	58-82nm	light granular	septum, above parietal	37
7	78nm	46-81nm	clear	column endoderm, above sphincter	38

PLATE 1

Figure 1. Diagram illustrating the general anatomy of an actinian; Co, column; D, pedal disc; Gf, gastric filament; Gv, gastrovasular cavity (coelenteron); Od, oral disc; P, parietal; Pb, parietobasilar; R, retractor; S, siphonoglyph; T, tentacle; X, pharynx; arrow in mid-column region indicates the location of the longitudinal sections drawn in Figure 2.



1

PLATE 2

Figure 2. Diagram of a longitudinal section through the mid-column region of a) Stomphia and b) Aiptasia illustrating the general histology. Ay, amoebocyte; Ec, ectoderm; Ed, endoderm; Em, epithelial muscle; Fp, fiber plexus; G, gland cell; O, mesoglea; Om, mesogleal muscle; Sc, support cell. The magnification is approximately 400X; cellular and mesogleal detail is exaggerated.

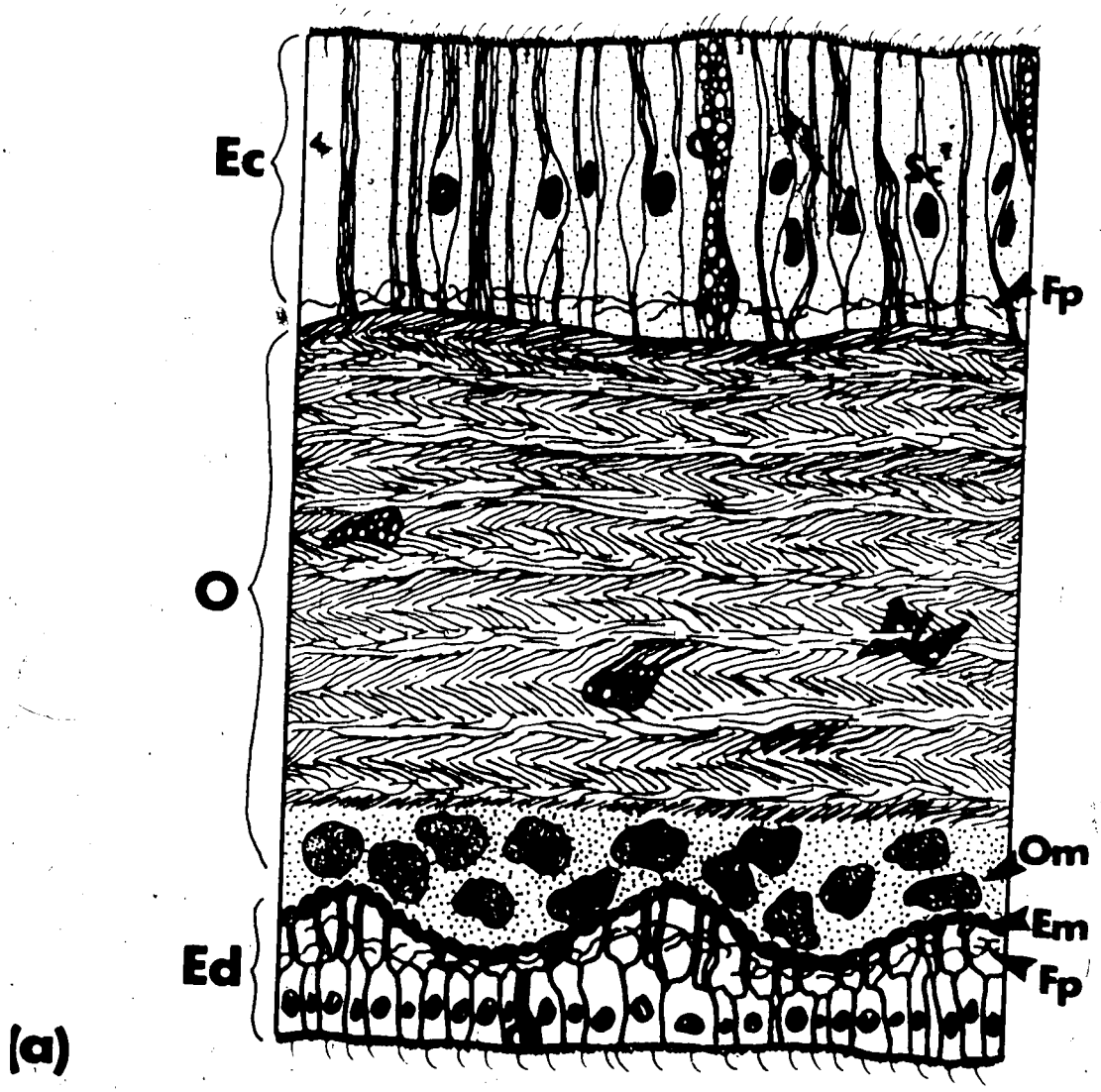

Ec
O
Ed
Fp
Om
Em
Fp
(a)
Ec
O
Ed
Fp
Em
Fp
(b)

PLATE 3

Figure 3. The organization of epithelial and mesogleal muscle fields. a) cross section of epithelial muscle fibers from the column circulars in Aiptasia; b) cross section of folded (buckled) sheet of epithelial muscle fibers from the column circulars of Stomphia: c) cross section of mesogleal muscle fibers from the sphincter of Stomphia. Mf, muscle fiber; O, mesoglea; Bars = 2μ.

PLATE 4

Figure 4. The subepithelial space (asterisks) in endodermal epithelial muscle of Stomphia. a) circular muscle of oral disc; b) epithelial circular muscle of column; c) retractor. Bars = 2μ.

PLATE 5

Figure 5. Diagram illustrating the location of the muscles of the septa in a) Aiptasia and b) Stomphia. The septa are cut in cross section, in the mid-column region. Fp, fiber plexus; Jc, junction of septum with column; Jx, junction of septum with pharynx; O, mesoglea; Om, mesogleal muscle; P, parietal; Pb, parietobasilar; Pbf, parietobasilar fold; R, retractor. The magnification is approximately 500x.

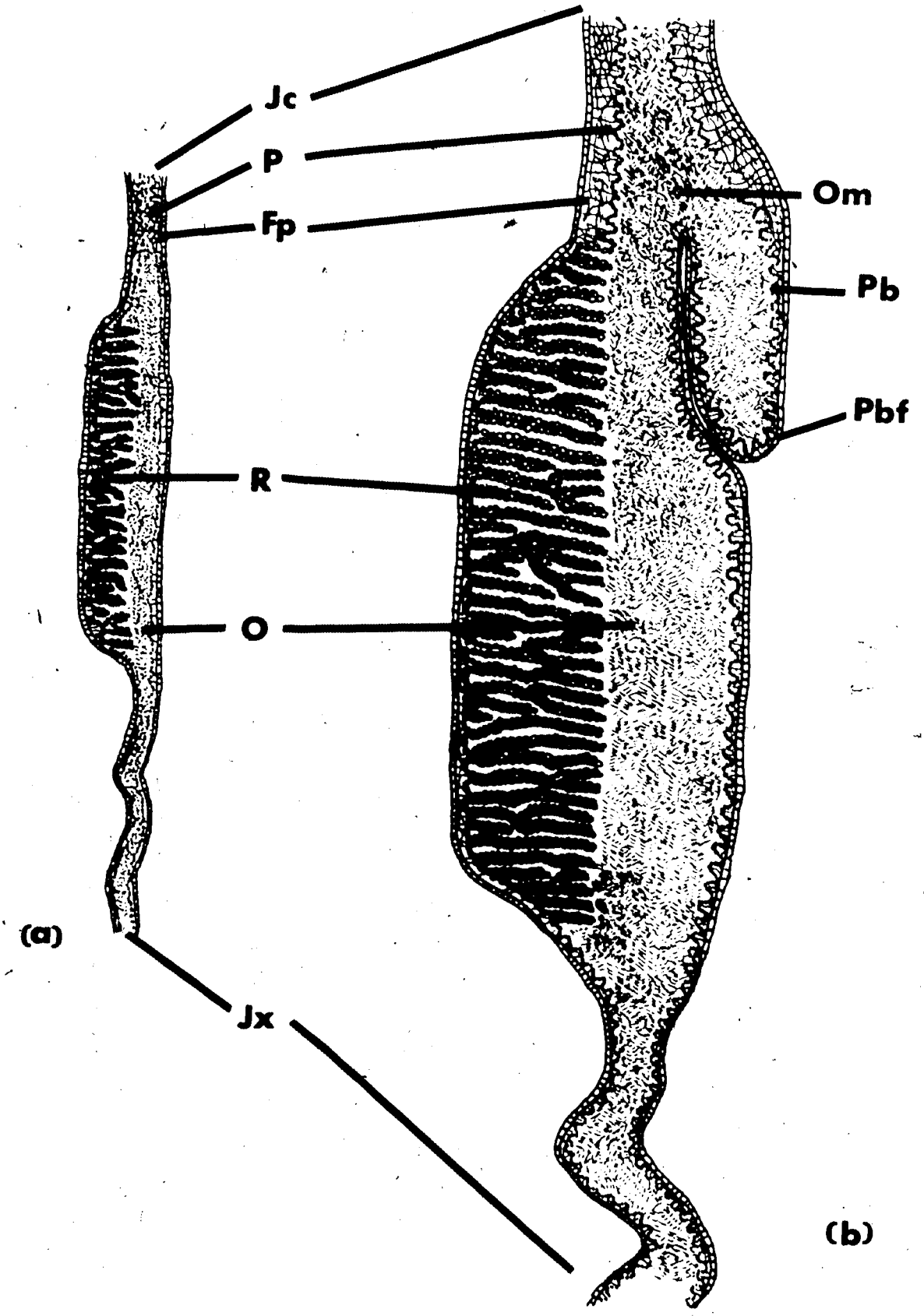

Jc
P
Fp
R
O
Jx
Om
Pb
Pbf
(a)
(b)

PLATE 6

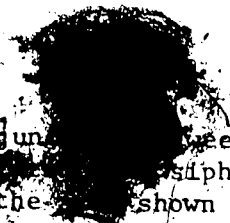


Figure 6. Longitudinal section of the junction between the oral disc and the siphonoglyph, illustrating the siphonoglyph sphincter. To the right of the [illegible] shown on the micrograph and below it, the ectodermal muscle is longitudinally oriented. At the siphonoglyph, there is a three-fold increase in the size of the muscle. The orientation of the fibers is circular, as shown (cut in cross section). Ec, ectoderm of the siphonoglyph; Mf, muscle fibers; O, mesoglea; Bar = 3μ.

Figure 7. Light micrograph of an oblique section through the junction of a tentacle with the column in Stomphia. Note the connection between the tentacle longitudinal muscle (Tl) and the sphincter (S). Bar = 50μ.

Figure 8. Light micrograph of a cross section through the junction of the oral disc with a septum in Stomphia. Note the connection between the oral disc longitudinal muscle (Odl) and the beginning of the retractor (R). Bar = 50μ.

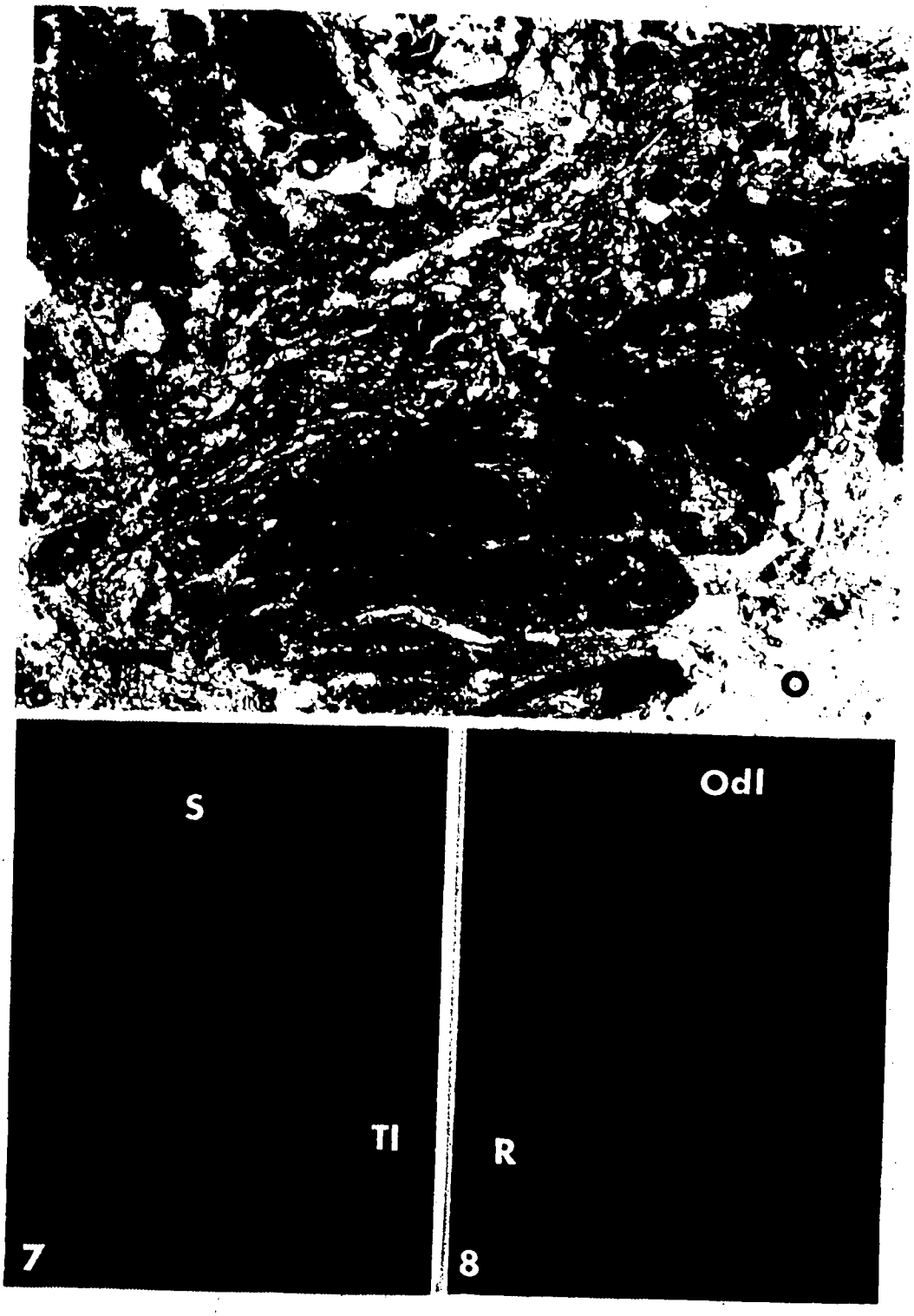

O
S
Tl
7
Odl
R
8

PLATE 7

Figure 9. Light micrographs of cross sections through the junction of a septum with the pedal disc in a) Aiptasia and b) Stomphia. Note the connection between the basilar muscle of the septum (arrow) and the pedal disc ectoderm (Ec). Cm, circular muscle of pedal disc; S, septum Bar = 50μ.

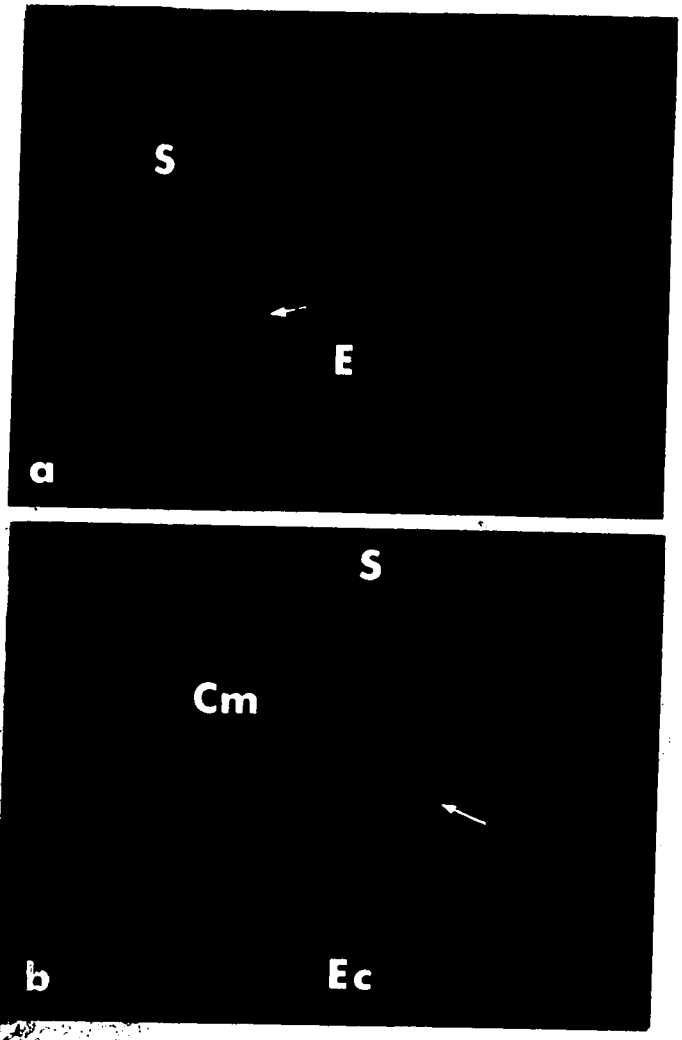

S
E
a
S
Cm
b
Ec

PLATE 8

Figure 10. The basement membrane and the mesoglea in Stomphia. a) mesoglea in the column; note the sharp boundary between the superficial layer (Os) and the deep layer (Od). Bar = 2μ. b) the basement membrane (Bm) of a muscle fiber. Bar = 0.5μ.

Figure 11. The basement membrane and the mesoglea in Aiptasia. Note the thickness of the basement membrane (Bm) in Aiptasia compared to that in Stomphia (Figure 10b). Ay, amoebocyte; Od, deep layer of the mesoglea; Bar = 0.5μ.

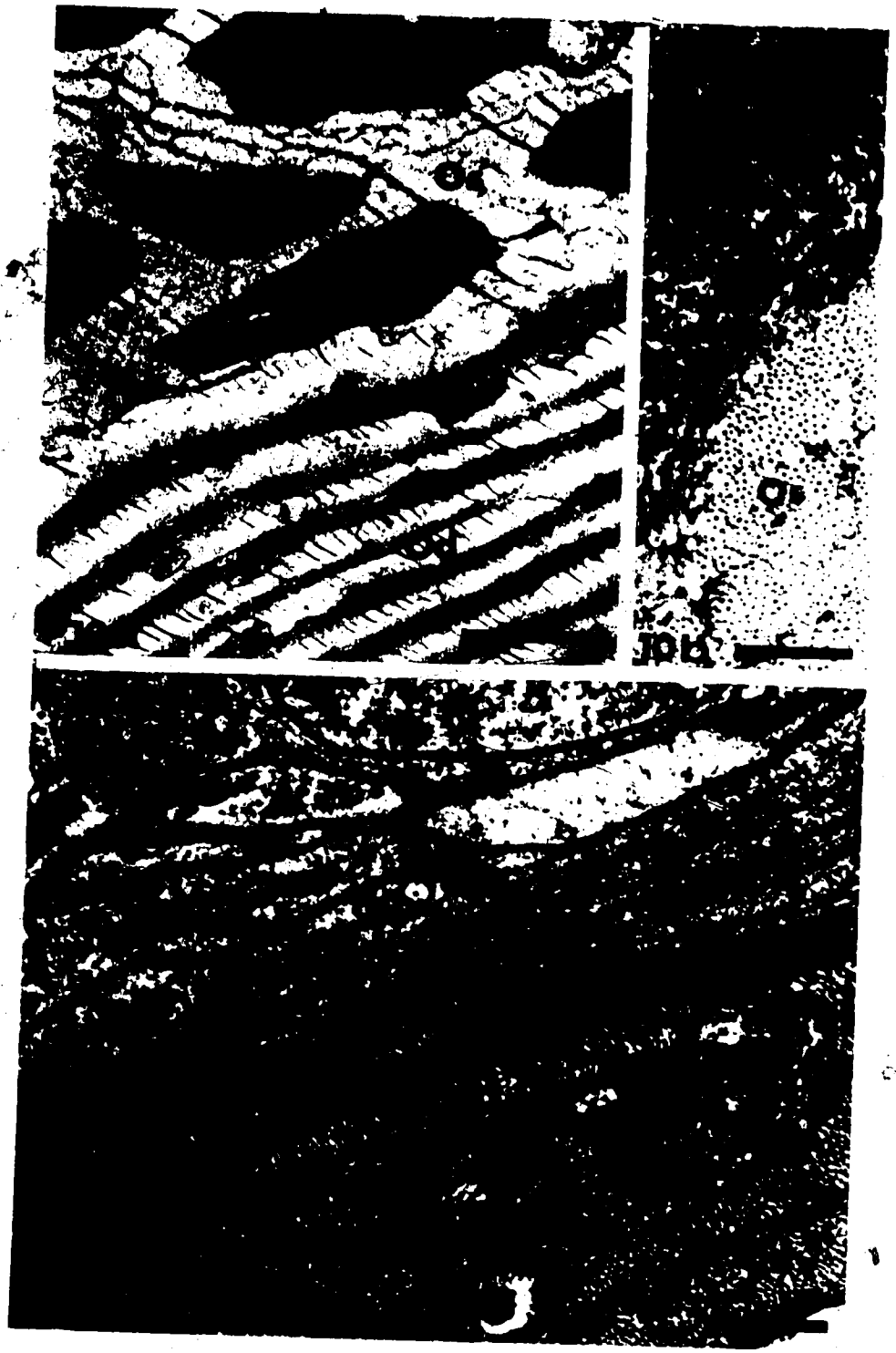


PLATE 9

Figure 12. Diagrammatic representation of the two crossed lattices in the deep layer of the column mesoglea of Stomphia. The orientation of the muscle fibers in the endoderm is indicated (Mf). Ec, ectoderm; Ed, endoderm; O, mesoglea.

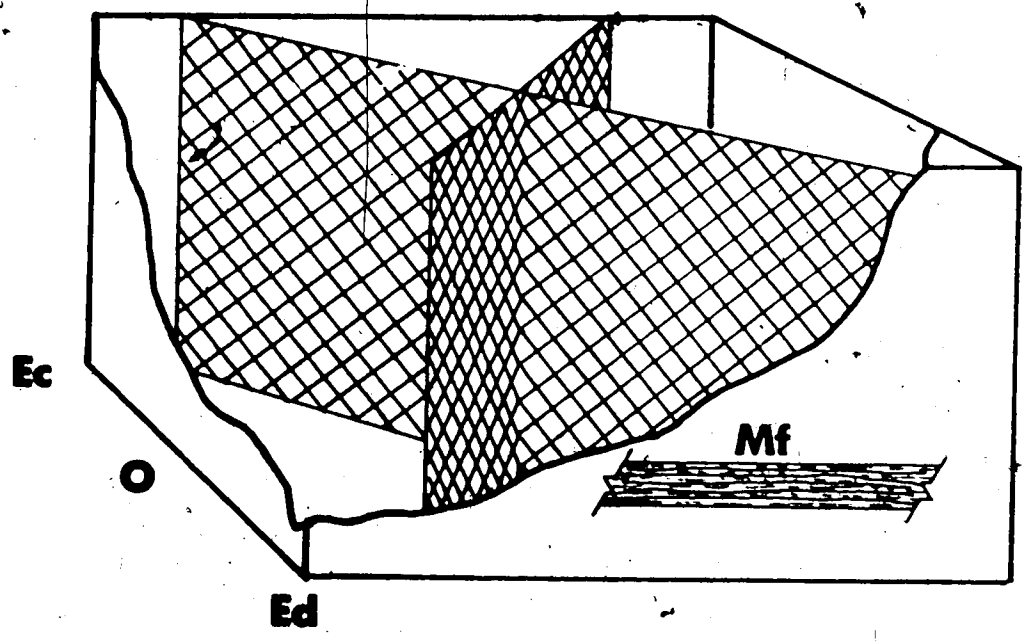

Ec
O
Mf
Ed

PLATE 10

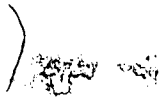
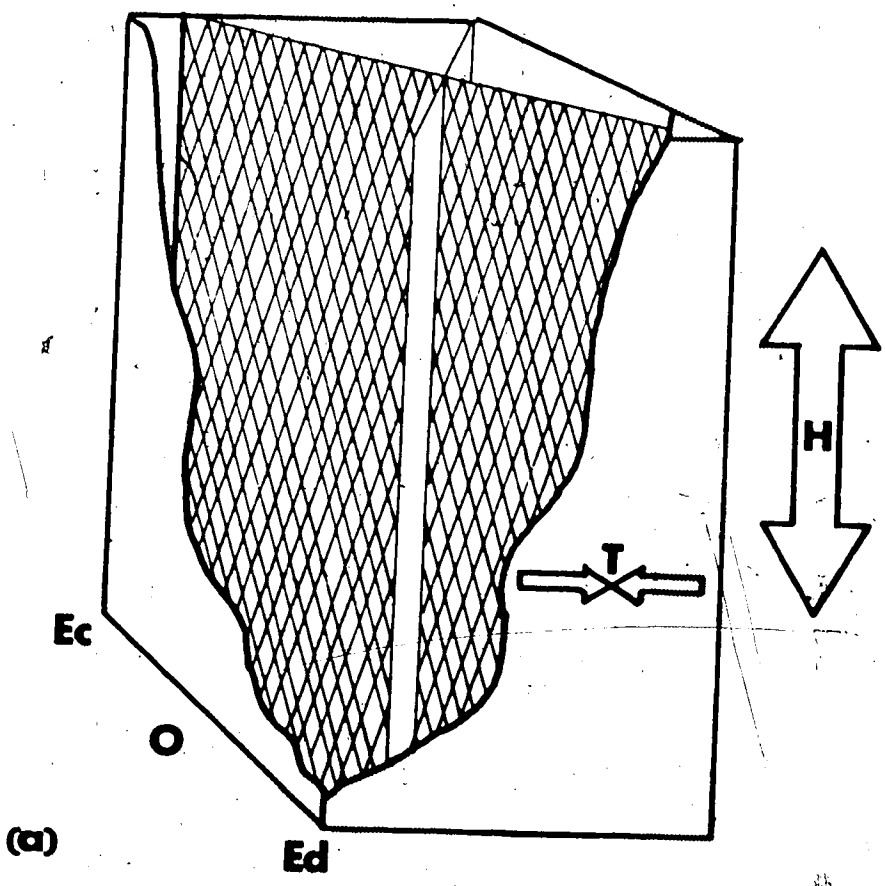
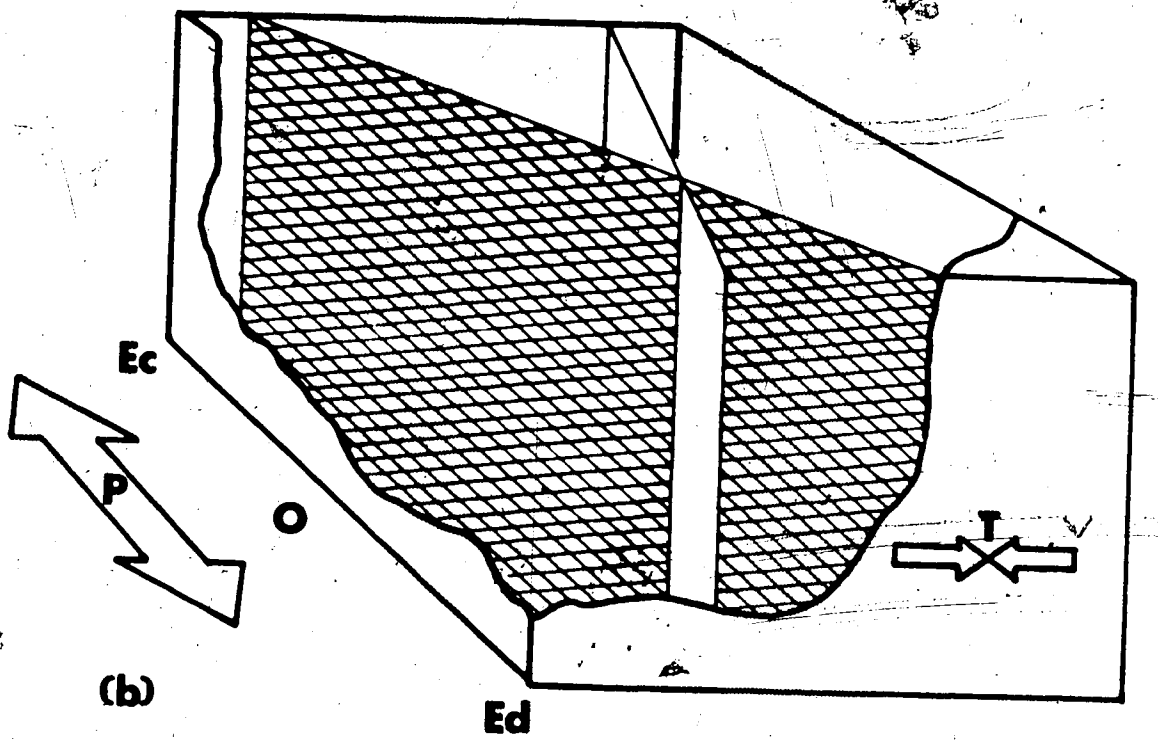


Figure 13. Diagrammatic representation of the effect of contraction on the mesogleal network in the column of Stomphia. a) contraction of the circular muscles against hydrostatic fluid in the coelenteron generates a force (H) along the oral - aboral axis of the animal and results in an increase in height of the column. b) contraction of the circular muscles with loss of fluid from the coelenteron results in an increase in the thickness of the column. P, direction of force in mesoglea; Ec, ectoderm; Ed, endoderm; O, mesoglea; T, direction of pull of circular muscles.



(a)



(b)

PLATE 11

Figure 14. Diagram illustrating the typical features of an endodermal epitheliomuscular cell. The cell is in three parts; the cell body contains the nucleus (N), endoplasmic reticulum (Er), mitochondria (A), lysosomes (Ly) and other vesicular inclusions, a golgi apparatus (Ga), which is usually associated with the striated root (St) of the flagellum (Fm). The cells have a microvillous border (Mv) and are joined apically by septate desmosomes (Ds). The peduncle (P) connects the cell body and the muscle fiber (Mf), and contains mitochondria, small electron dense vesicles and thin filaments (F). The muscle fiber contains the myofilaments (M), mitochondria, glycogen granules (Gy), sarcoplasmic reticulum (Sr), microtubules (T) and small electron dense vesicles. The sarcoplasmic extension (Se) has the same inclusions as the peduncle. Individual muscle fibers are connected at their ends by desmosomes (De).

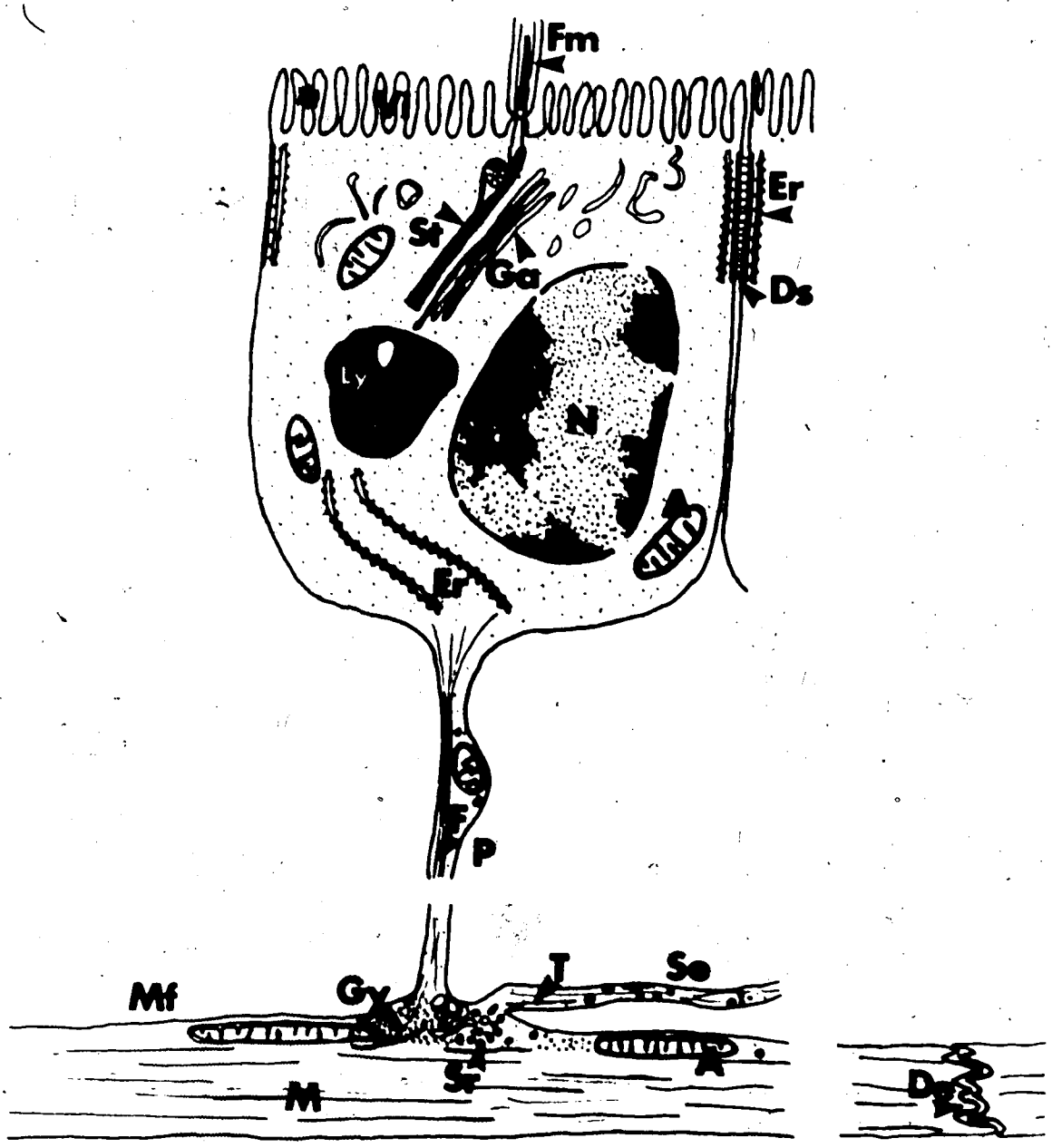

Fm
Er
Sf
Ga
Ds
Ly
N
Er
P
Mf
Gx
J
So
M
Sf
D

PLATE 12

Figure 15. Type A fibers from the tentacle ectoderm of Stomphia: a) in longitudinal section ; b) in cross section. Note the loosely organized striation visible in the fibers in longitudinal section. Arrowheads indicate thick filaments; arrows indicate areas free of thick filaments. Bar = 1μ.

PLATE 13

Figure 16. Type A (a) and Type B (b) muscle fibers. Note the difference in the thick myofilaments of the two fiber types. c) Longitudinal section of a Type B fiber thick filament showing the 110A axial period (arrows). Small arrowheads indicate thin filaments; large arrowheads indicate thick filaments. Bars = 125nm.

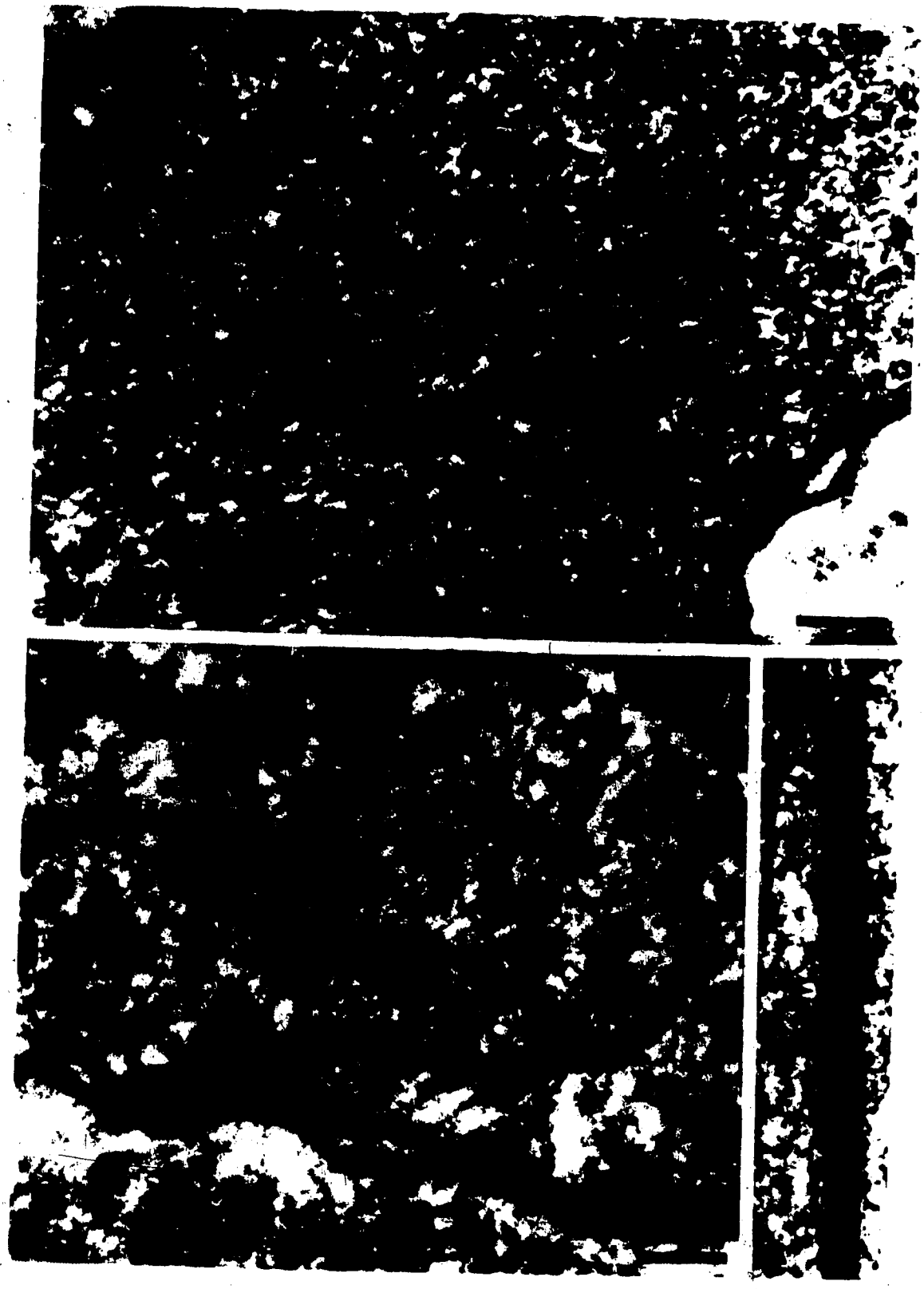


PLATE 14

Figure 17. Type B fibers from a) the oral disc circular muscle of Stomphia, b) and c) the parietal of Stomphia, c) the parietal of Aiptasia. Note the variation in the size and arrangement of the thick filaments (arrowheads), and the variation in the density of the sarcoplasm. Bars [illegible] u.


b
c

PLATE 15

Figure 18. Inclusions of the muscle fibers in a) Stomphia and b) Aiptasia. In Aiptasia, the sarcoplasmic reticulum (Sr) in most fibers is limited to individual membrane sacs (arrows). A, mitochondria; Gy, glycogen granules; M, myofilaments; V, electron-dense vesicles. Bars = 1μ.

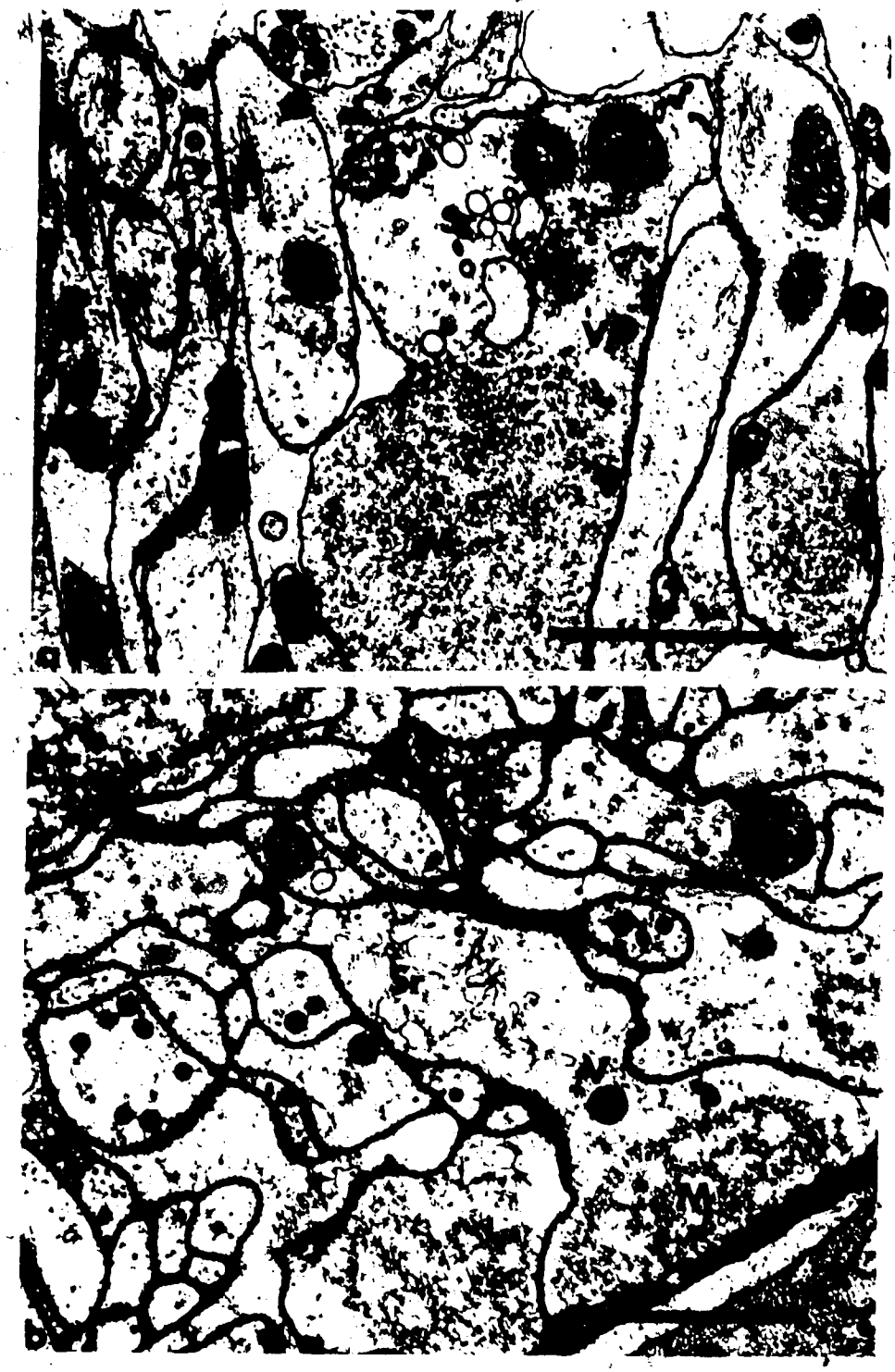

M

PLATE 16

Figure 19. The sarcoplasmic reticulum (arrows) in Stomphia. a) In the mesogleal circulars of the column; b) in the peduncles of the epithelial circulars of the column; c) and d) in the sphincter. Note the electron dense material on the inner surface of the tubule membranes. a) and c) Bar = 1μ; b) Bar = 2μ; d) Bar = 0.25μ.

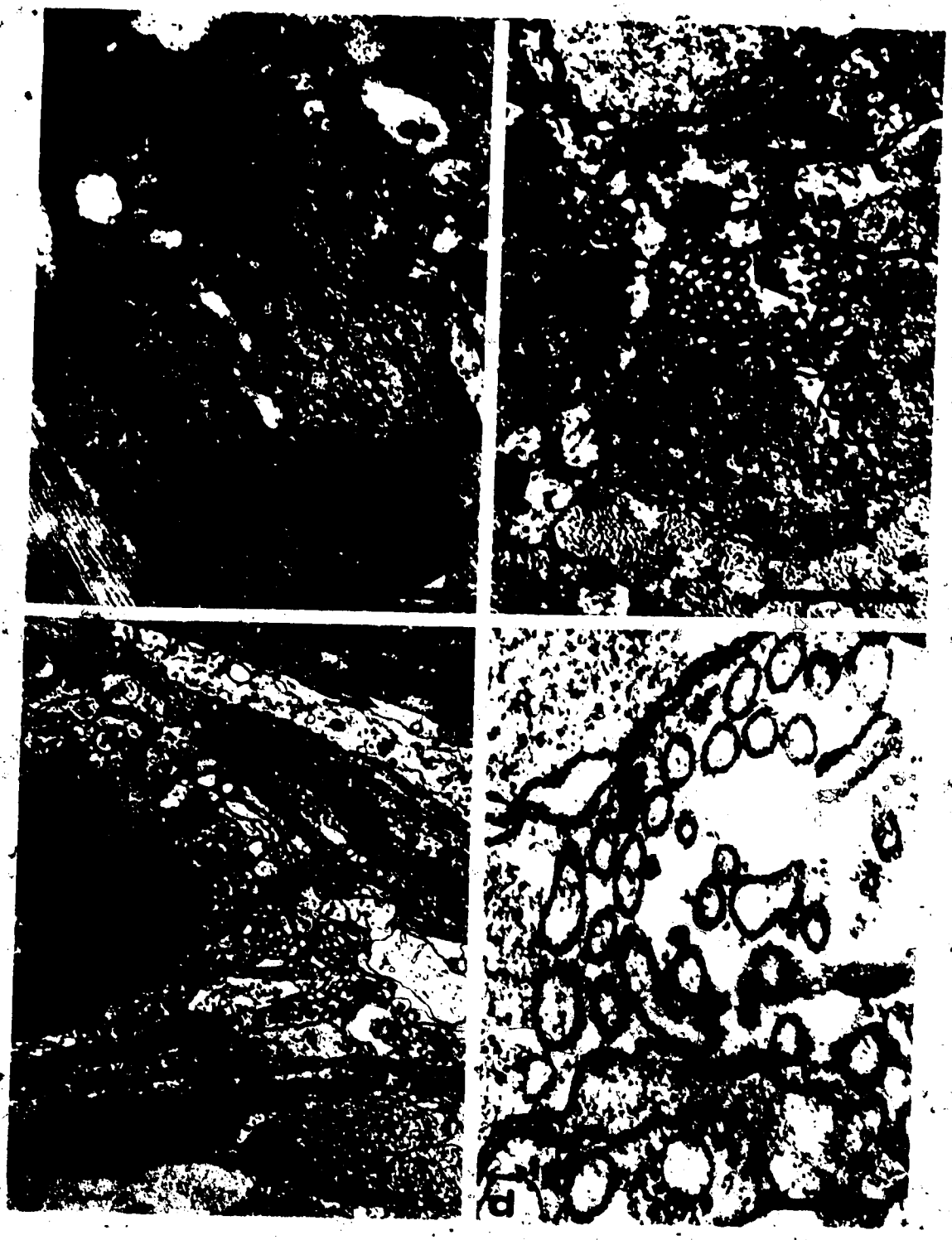

d

PLATE 17

Figure 20. Desmosomes are present at the ends (a and b) and on the lateral surfaces (c and d) of the muscle fibers. The thin filaments (small arrowheads) of A fibers and the thick (large arrowhead) and thin filaments of B fibers can be seen running into the end desmosomes. a) and c) Bars = 0.25μ. b) Bar = 0.5μ. d) Bar = 1μ.

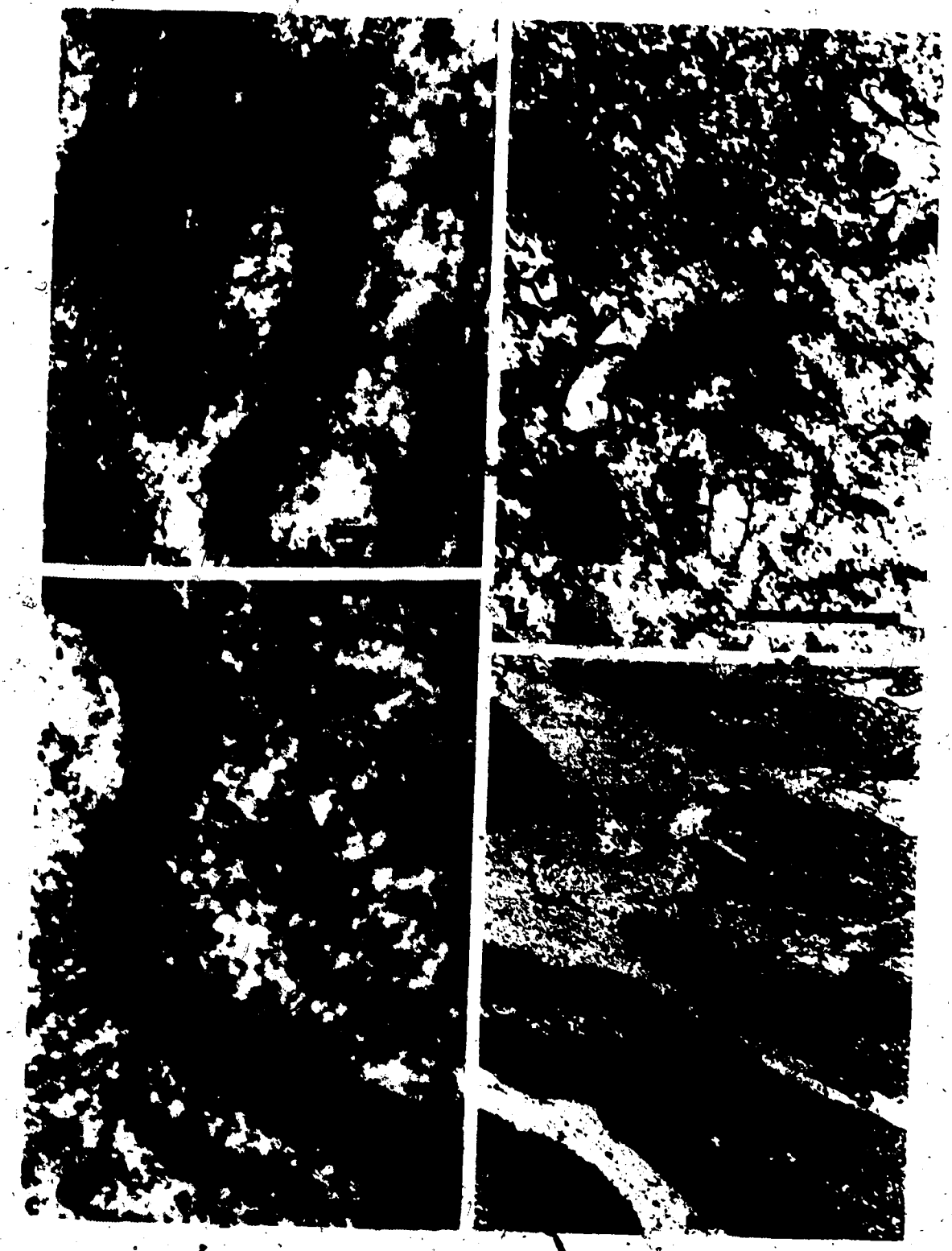


PLATE 18

Figure 21. Sa[redacted] extensions in epithelial muscle (a and b) a[redacted]eal muscle (c and d). a) Aiptasia, column. b[redacted] retractor. c) and d) Stomphia, column. Fp, f[redacted]us; arrows indicate sarcoplasmic extensions. a) [redacted]) Bars = 2μ. c) and d) Bars = 1μ.

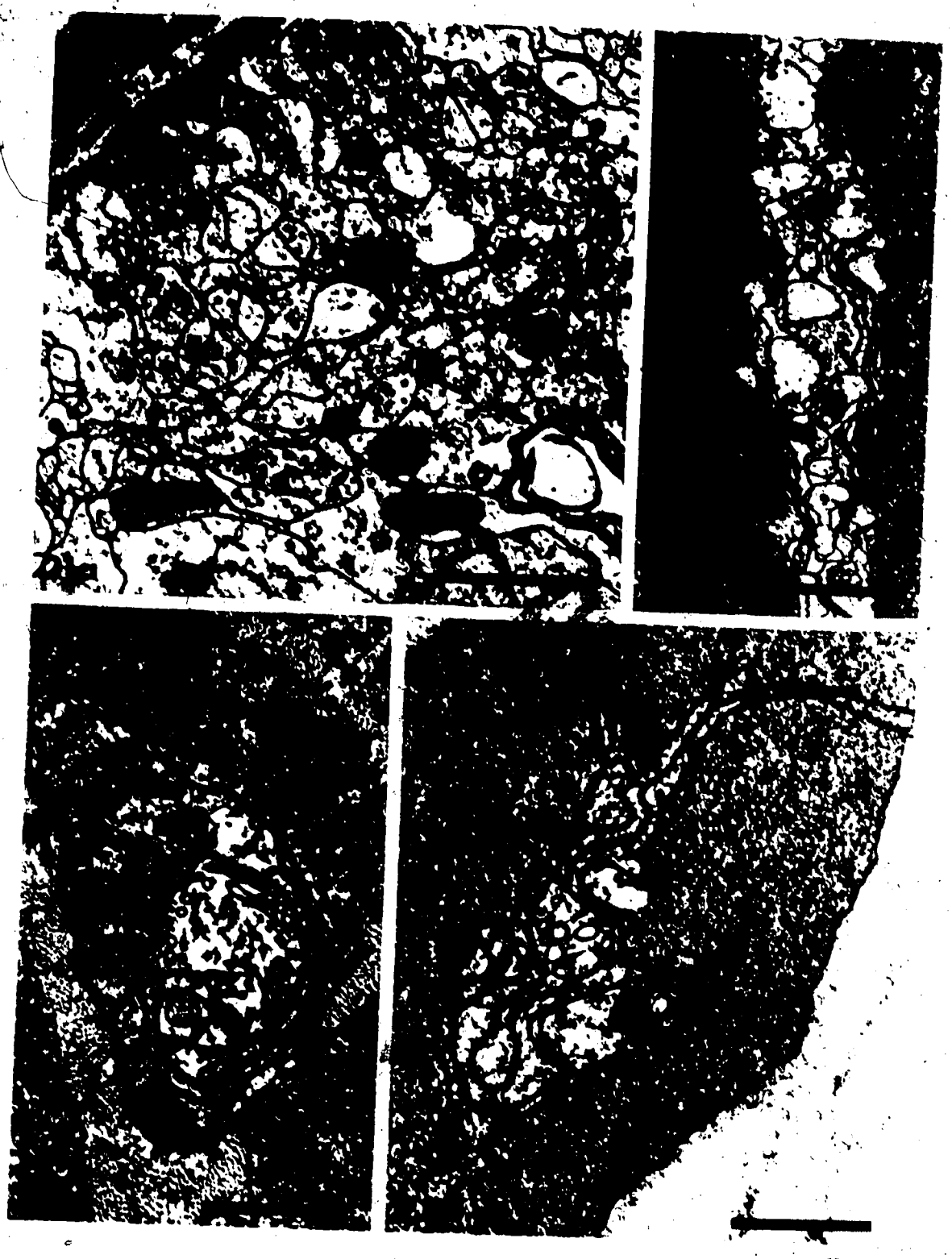


PLATE 19

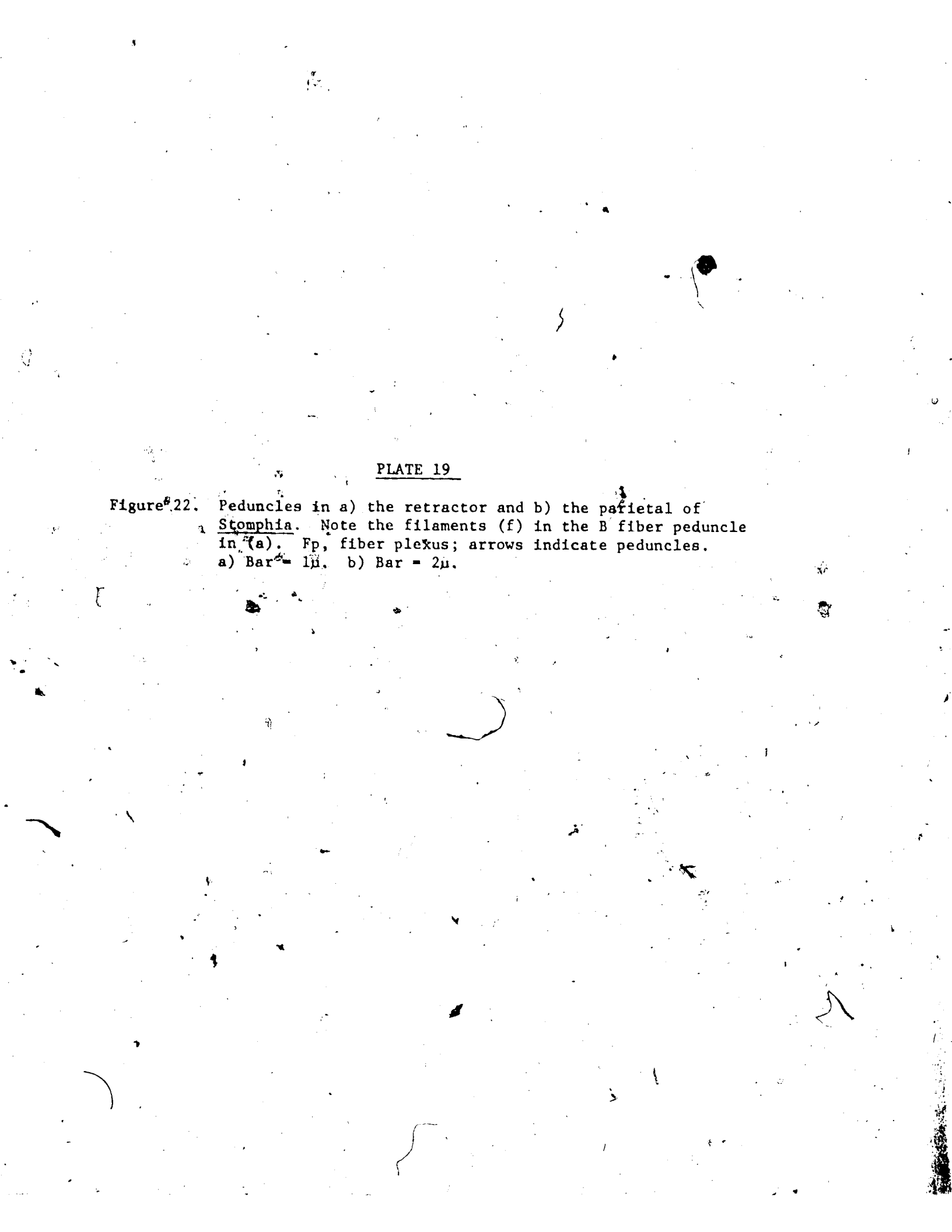


Figure 22. Peduncles in a) the retractor and b) the parietal of Stomphia. Note the filaments (f) in the B fiber peduncle in (a). Fp, fiber plexus; arrows indicate peduncles. a) Bar = 1μ. b) Bar = 2μ.

PLATE 20

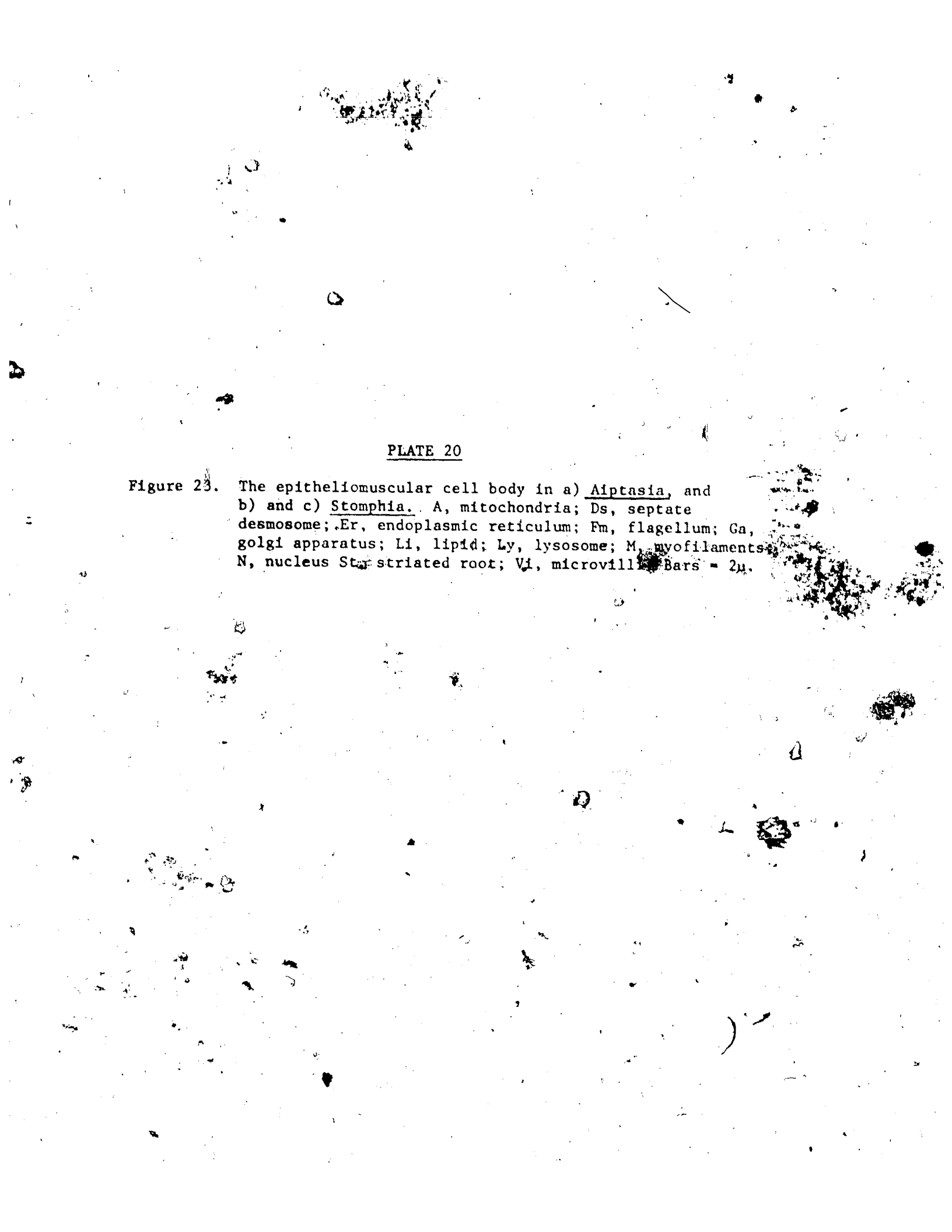


Figure 23. The epitheliomuscular cell body in a) Aiptasia, and b) and c) Stomphia. A, mitochondria; Ds, septate desmosome; Er, endoplasmic reticulum; Fm, flagellum; Ga, golgi apparatus; Li, lipid; Ly, lysosome; M, myofilaments; N, nucleus St, striated root; Vi, microvilli. Bars = 2μ.

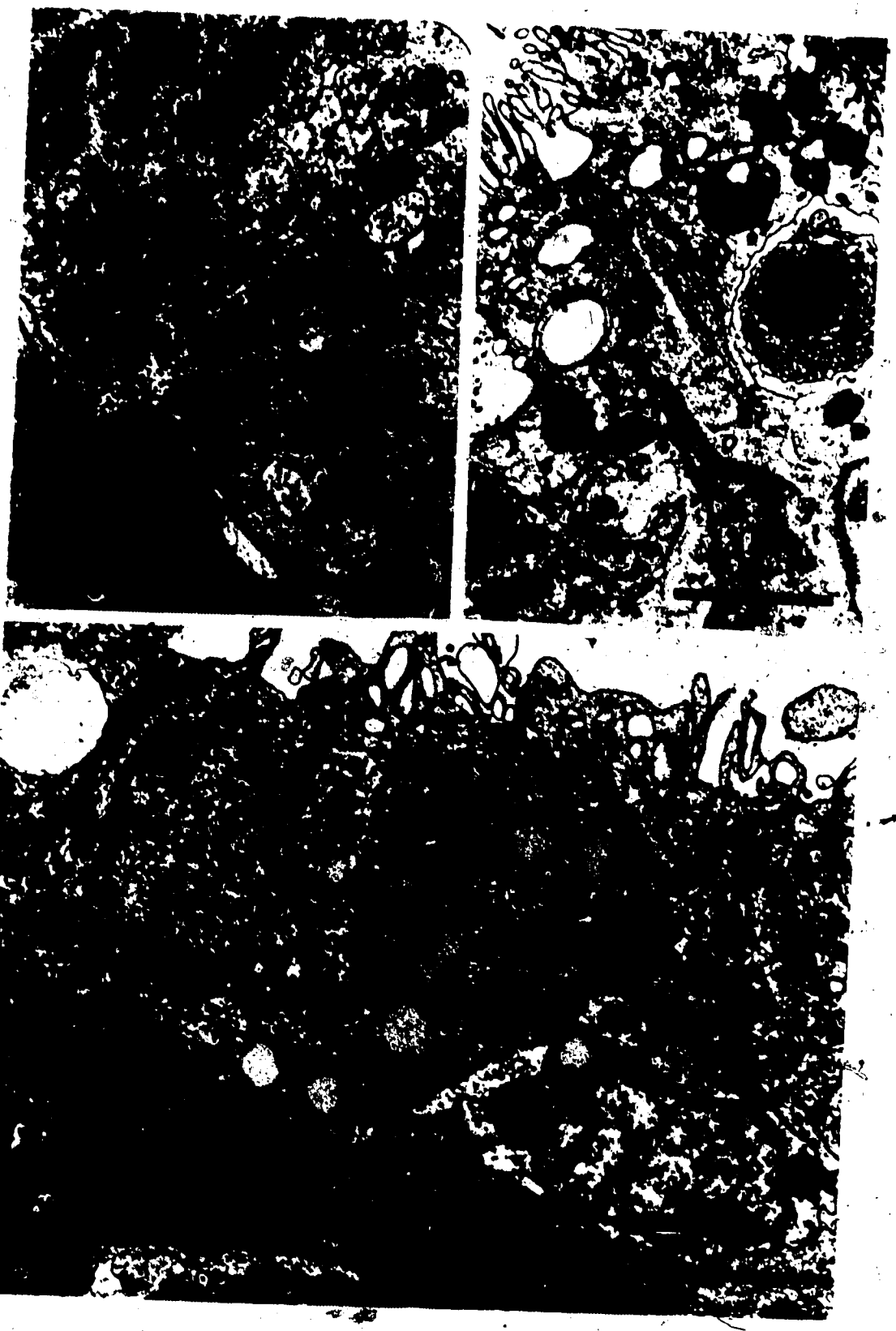


PLATE 21

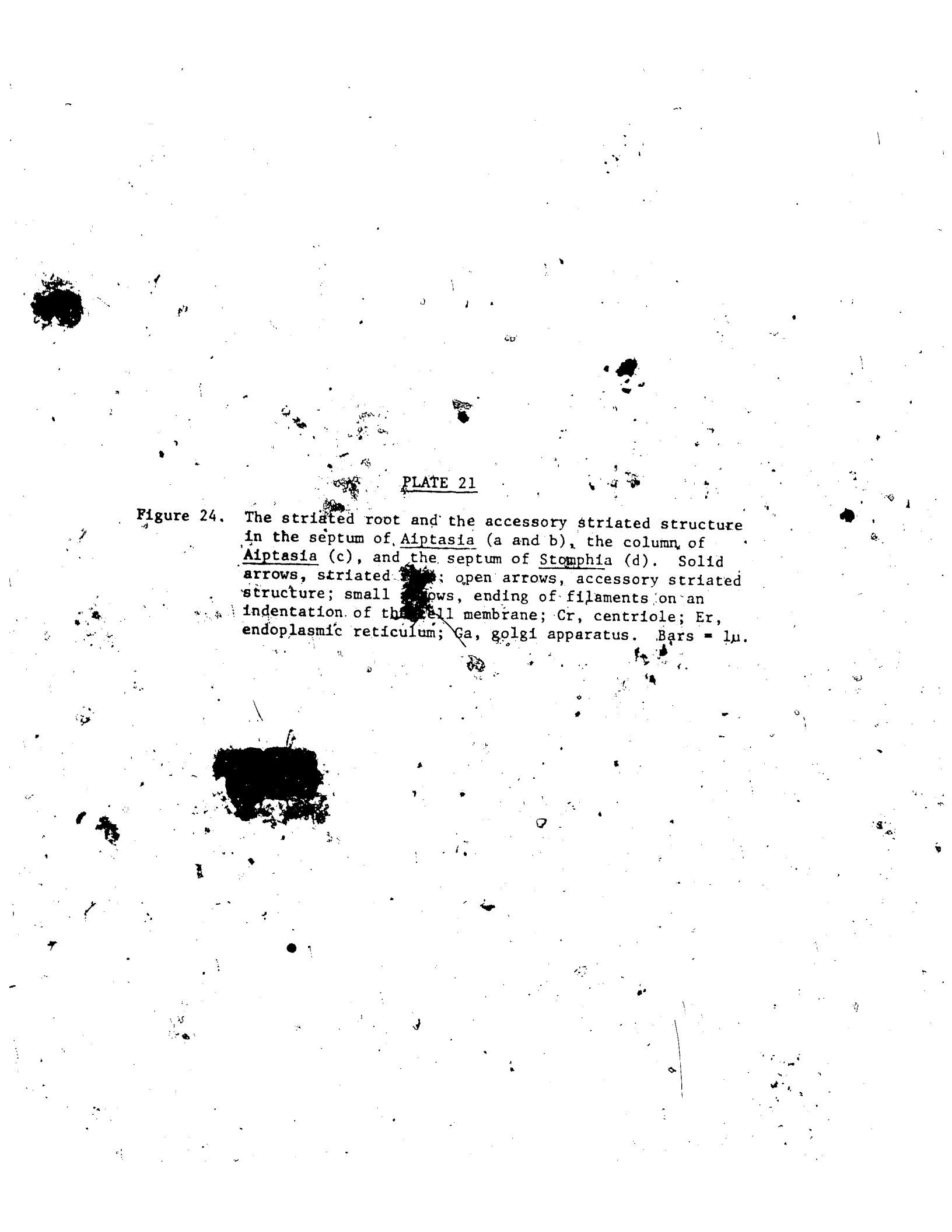


Figure 24. The striated root and the accessory striated structure in the septum of Aiptasia (a and b), the column of Aiptasia (c), and the septum of Stomphia (d). Solid arrows, striated roots; open arrows, accessory striated structure; small arrows, ending of filaments on an indentation of the cell membrane; Cr, centriole; Er, endoplasmic reticulum; Ga, golgi apparatus. Bars = 1μ.

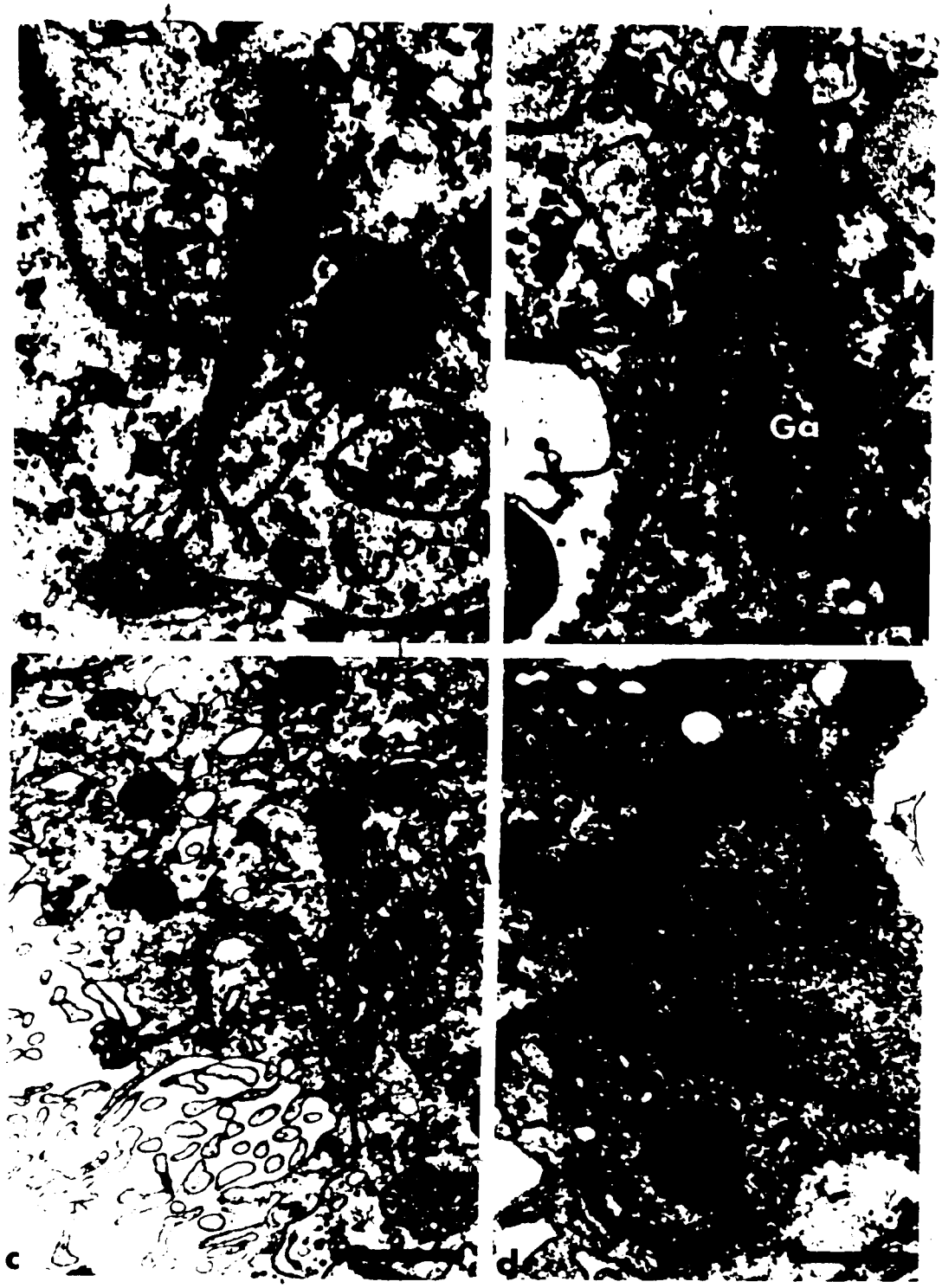

Ga
c
d

PLATE 22

Figure 25. The effect of contraction on the mesogleal muscle (Type A) of the column in Stomphia. a) cross section and b) longitudinal section of the muscle fibers in the resting state. Arrowheads indicate areas free of thick filaments. c) cross section and d) longitudinal section of the muscle fibers during isotonic contraction. Note the absence of areas free of thick filaments in contracted fibers. Bars = 1μ.

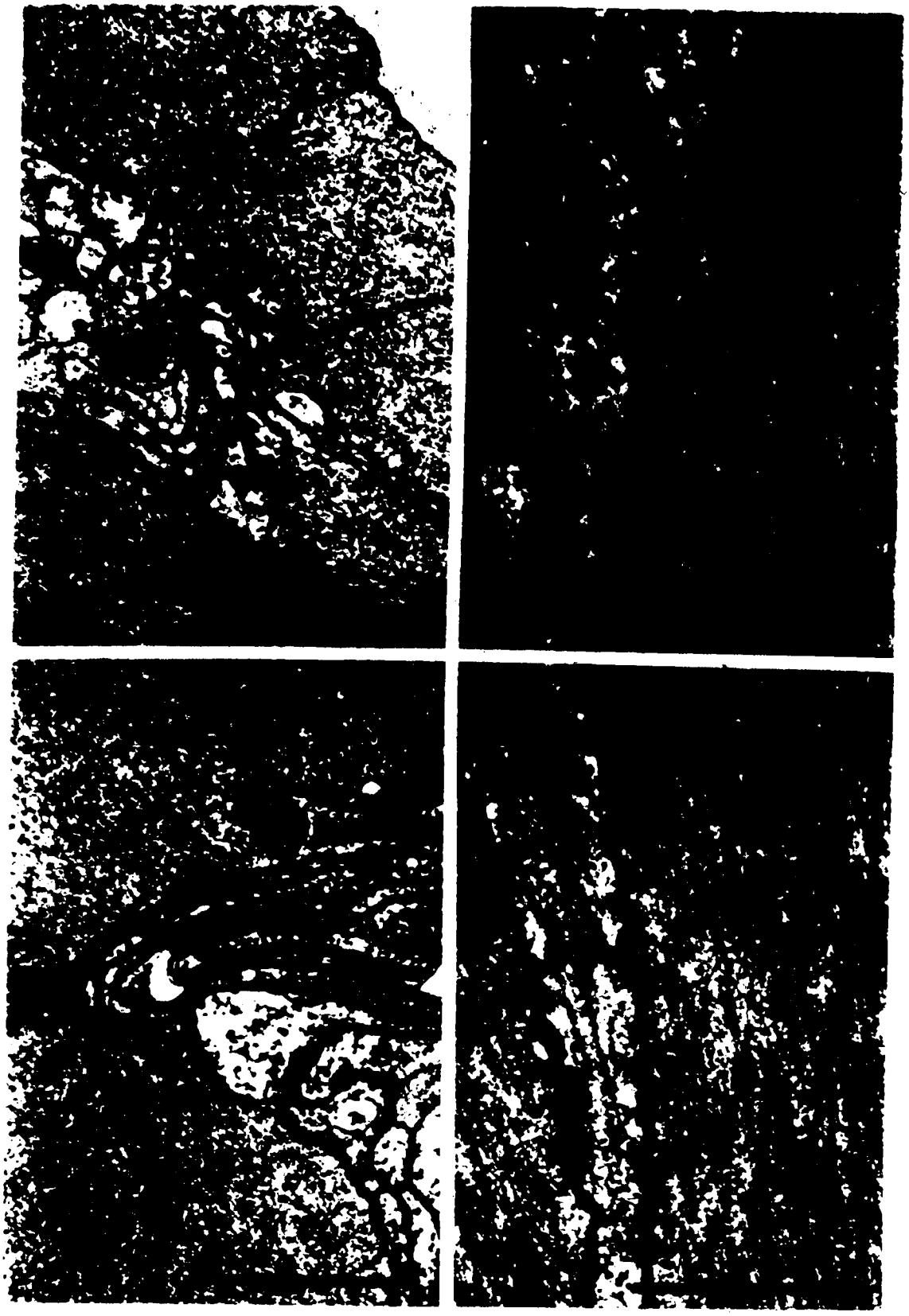


PLATE 23

Figure 26. The effect of contraction on the epithelial muscle (Type B) of the column in Stomphia. a) and b) Longitudinal sections of the muscle fibers in the resting state (a) and during isotonic contraction (b). c) and d) cross sections of the muscle fibers in the resting state (c) and during isotonic contraction (d). Note the increase in the number of large diameter thick filaments during contraction. a) and b) Bar = 1μ. c) and d) Bar = 2μ.

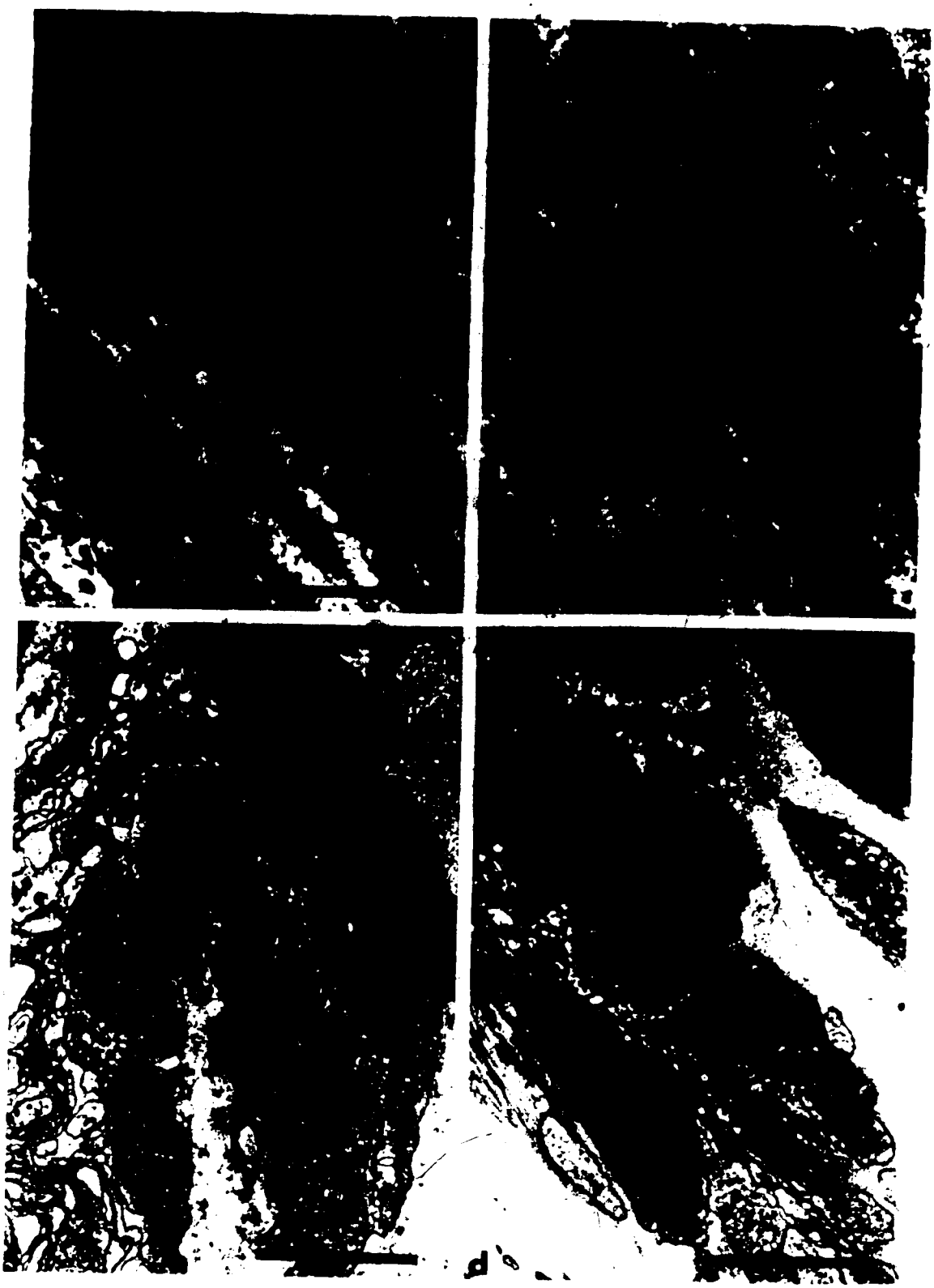


PLATE 24

Figure 27. The effect of stretch and contraction on the retractor of Stomphia. The retractor a) in the resting state; b) while the muscle is passively stretched. Large asterisks, Type A fibers;small asterisks, Type B fibers. Bar = 1μ.

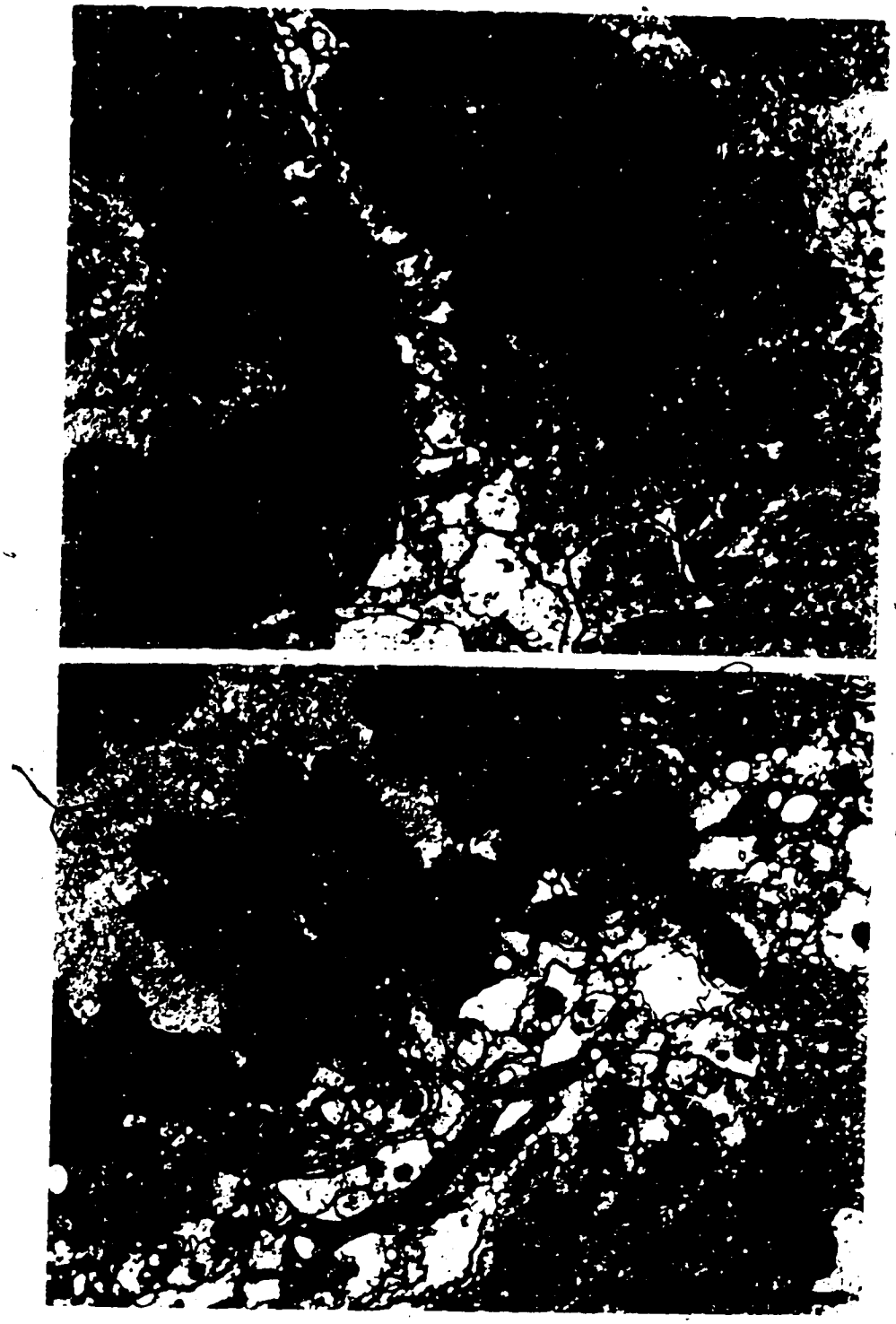


PLATE 25

Figure 27. (cont.) The effect of contraction and stretch on the retractor muscle of Stomphia. The retractor c) during isometric contraction; d) during isotonic contraction. Note the absence of areas free of thick filaments in A fibers during contraction. Large asterisks, Type A fibers; small asterisks, Type B fibers. Bar = 1μ.

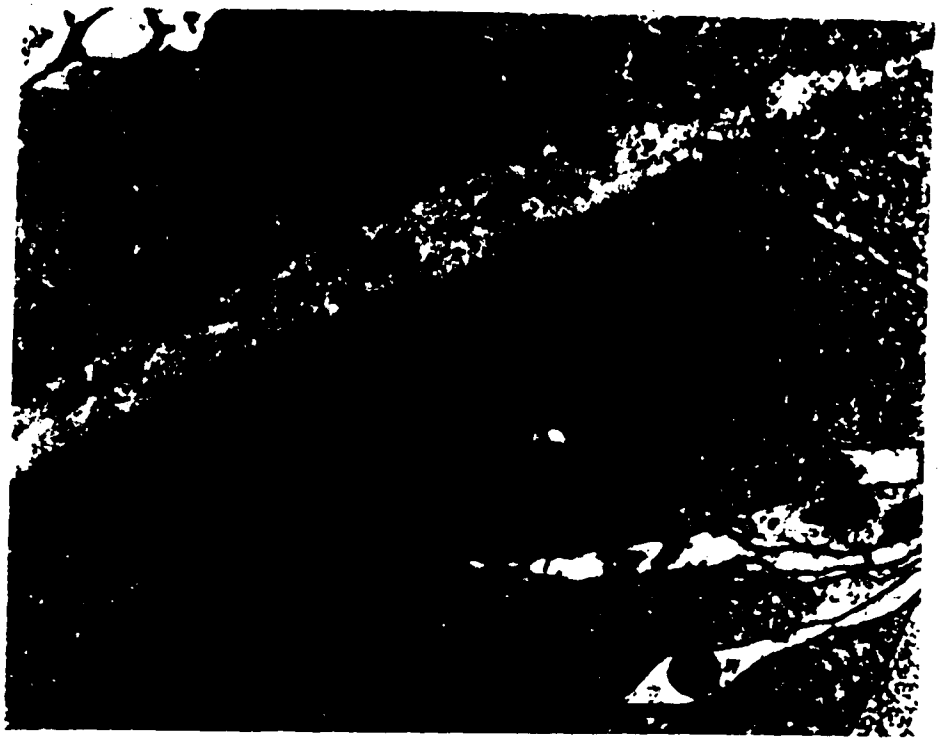


PLATE 26

Figure 28. Neuromuscular junctions (arrows) in the tentacle and oral disc ectoderm of Aiptasia. a) On the sarcoplasmic extension; b) directly on the muscle fiber. Bars = 1μ.


b

PLATE 27

Figure 29. Neuromuscular junctions (arrows) of A fibers in the retractor of Stomphia. Bars = 1μ.

PLATE 28

Figure 30. Neuromuscular junctions (arrows) on the peduncles (P) of a Type B fiber from the column circulars (a) and from the parietal (b) in Stomphia. Bars = 1μ.

Figure 31. Neuromuscular junctions (arrows) in the longitudinal muscle (ectoderm) of the tentacle of Stomphia. Note that the junctions are directly on the muscle fiber. Bars = 1μ.

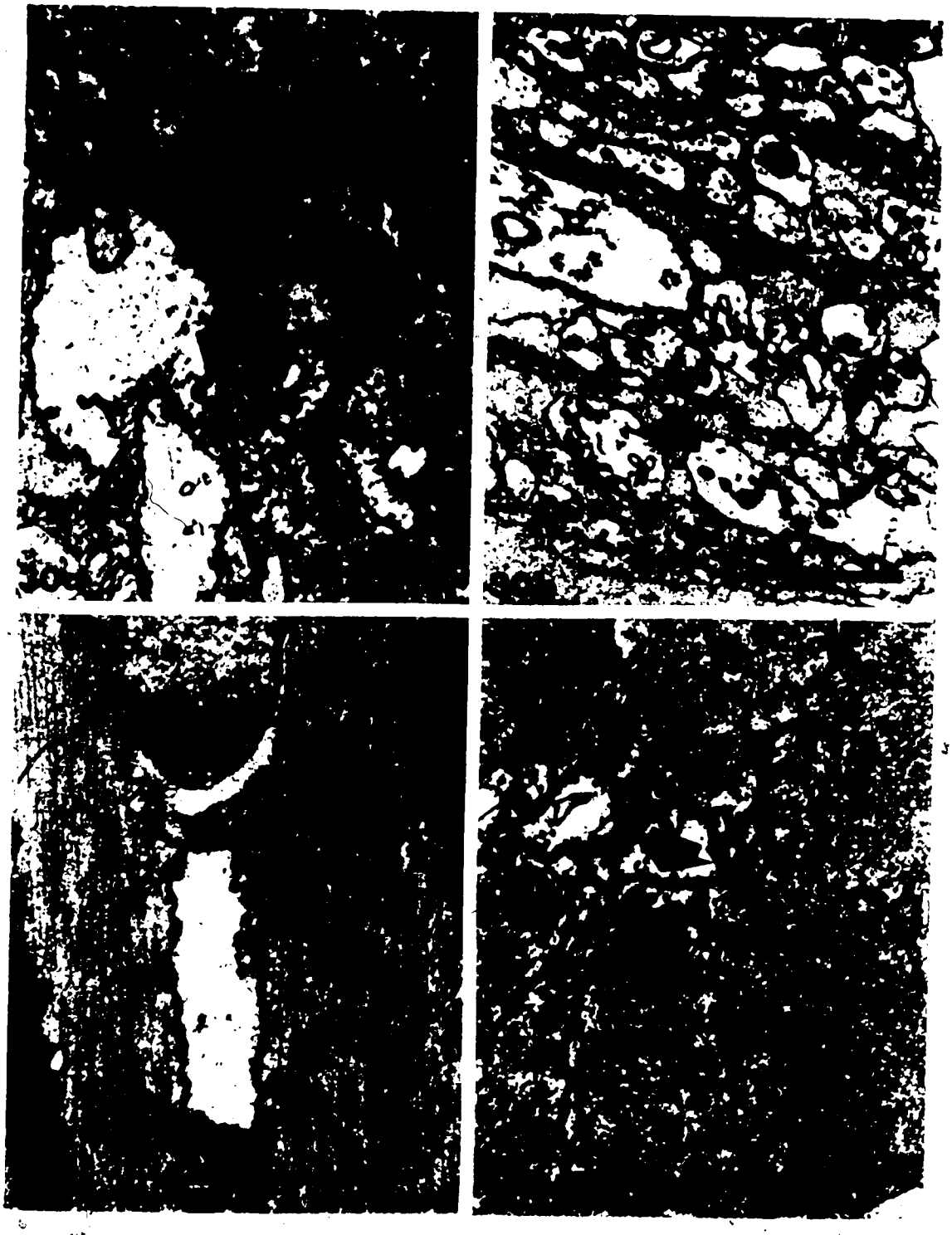


PLATE 29

Figure 32. Synapses in Aiptasia. a) and b) in the column endoderm; c) in the ectoderm of the tentacles and oral disc; d) in the septum, above the parietal. Bars = 1μ.

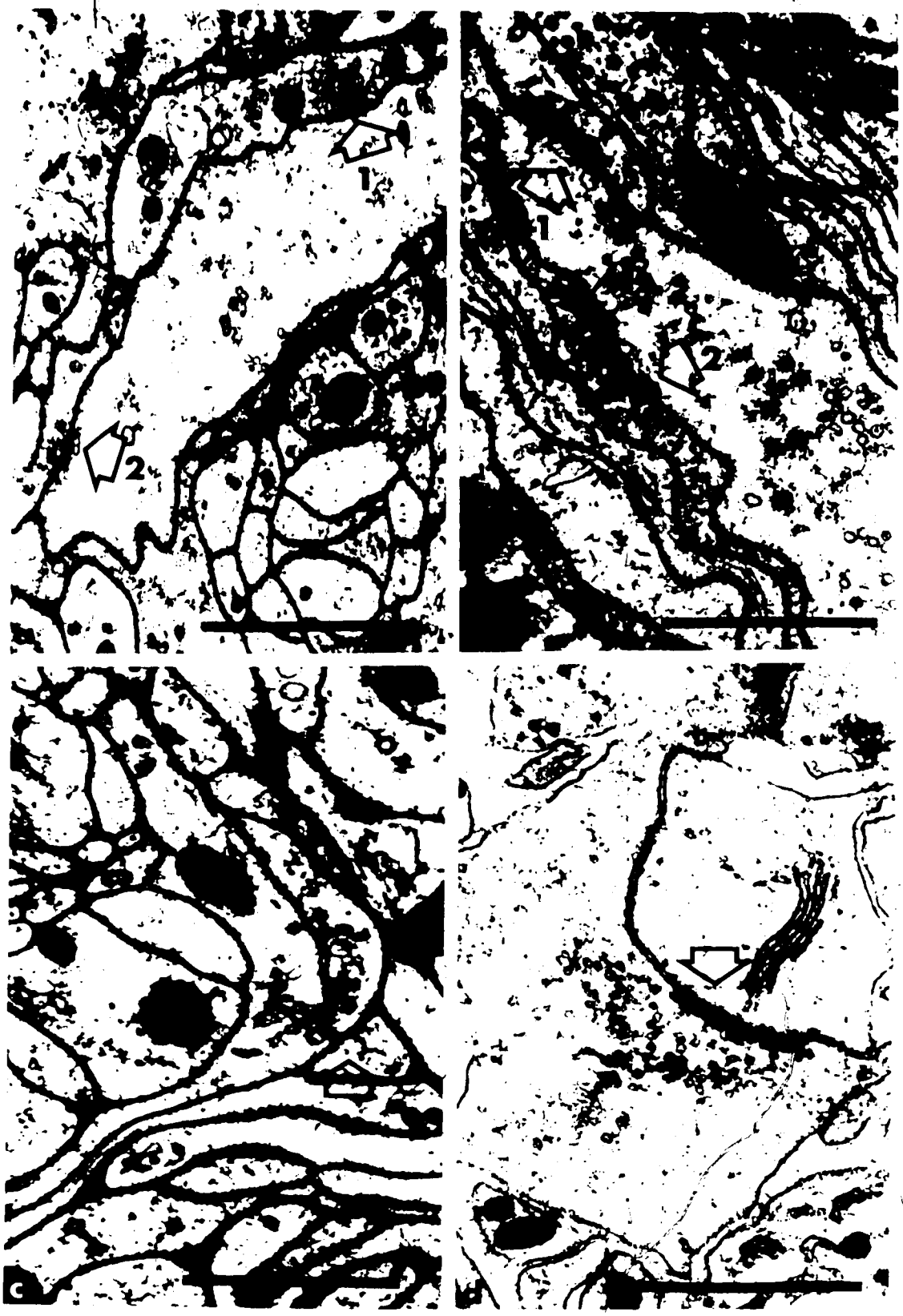

1
2
1
2
C

PLATE 30

Figure 33. Two different synapses (arrows) in the ectodermal plexus of the tentacles and oral disc of Stomphia. a)Bar = 1u; b)Bar = 2u.

Figure 34. Synapse (arrows) in the endodermal plexus of the oral disc and pharynx of Stomphia. Bar = 1u.

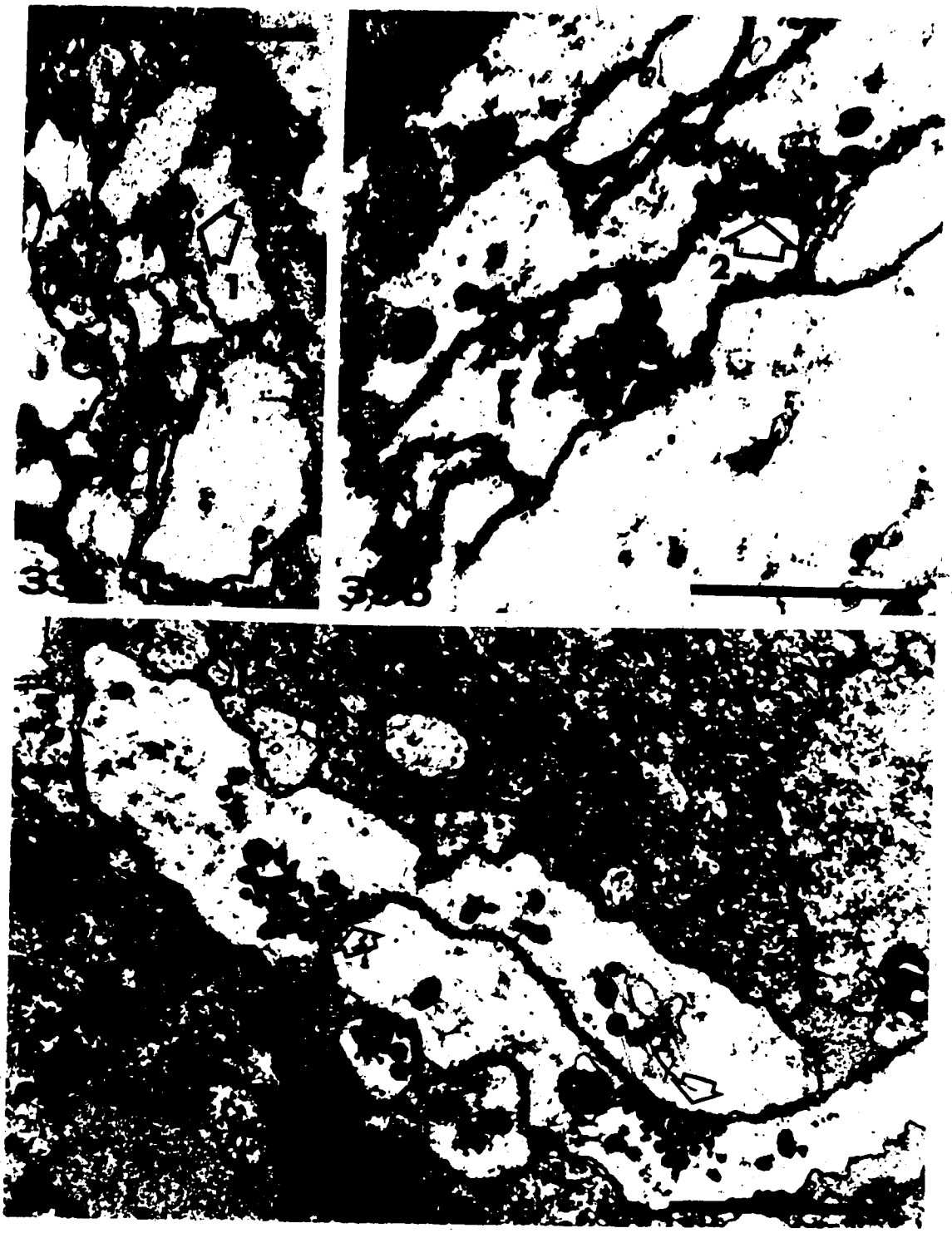

1
2

PLATE 31

Figure 35. Synapses (arrows) in the endodermal plexus overlying the retractor in Stomphia. The synapse in a) and that in b) represent extremes of the range in appearance of this synapse. Bar = 1μ.

Figure 36. Synapses (arrows) in the endodermal plexus overlying the parietal muscle in Stomphia. The synapse in a) and that in b) represent extremes of the range in appearance of this synapse. Bar = 1μ.

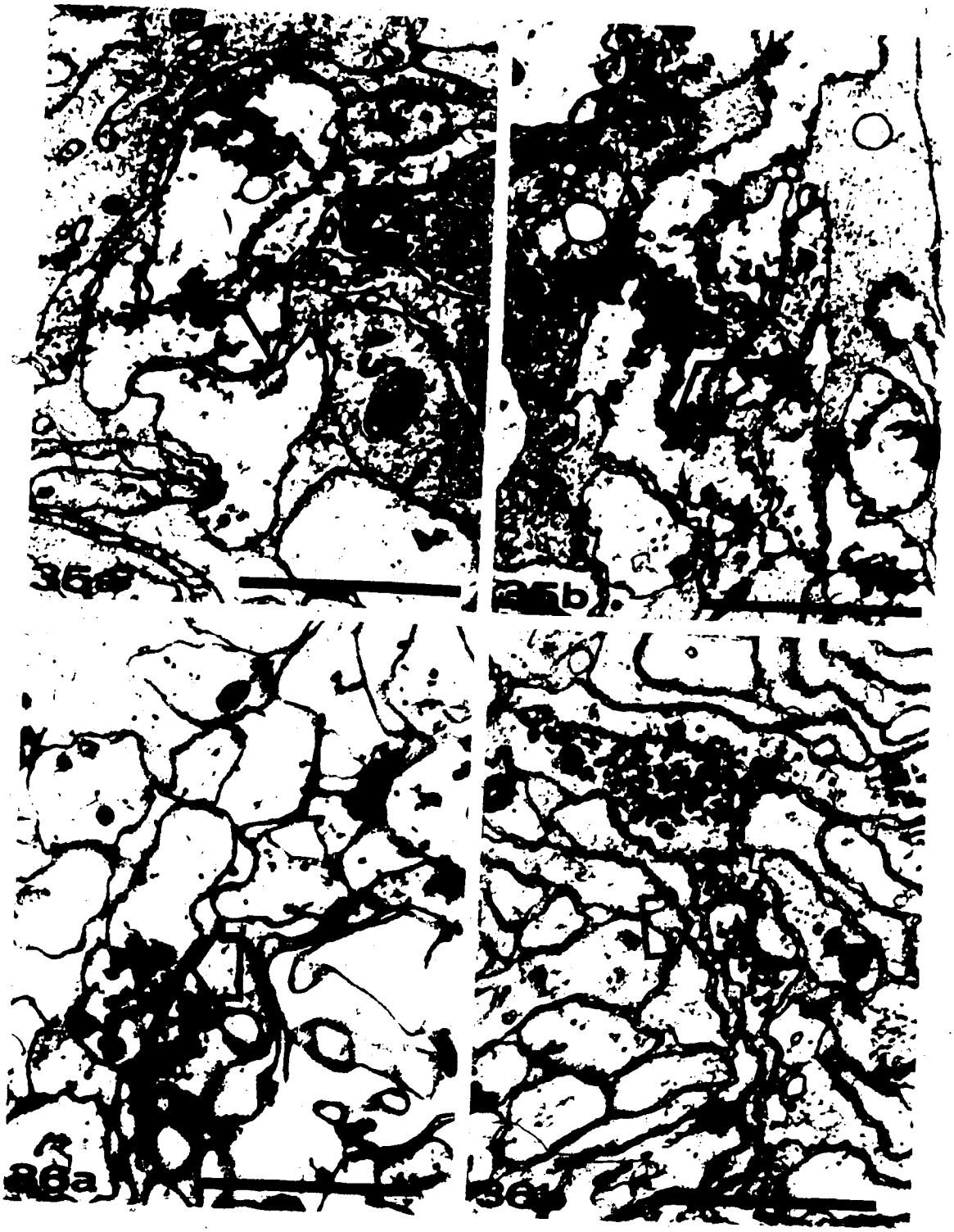

36a
36b
36a
36c

PLATE 32

Figure 37. Synapse (arrows) in the endodermal plexus overlying the parietal. Bar = 1μ.

Figure 38. Synapse in the endodermal plexus overlying the sphincter. Bar = 1μ.

PLATE 33

Figure 39. Nerve fiber (arrowheads) observed in the endoderm of the pedal disc and the oral disc, and in the ectoderm of the oral disc of Stomphia. In the pedal disc, the fiber passes into the mesoglea, as illustrated, and possibly represents a nervous connection between the endoderm and the ectoderm. Bars = 0.5μ.

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