FINE STRUCTURE OF EPINEURIAL MUSCLE CELLS IN *APLYSIA CALIFORNICA*

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INTRODUCTION

In the course of an electrophysiological investigation of the visceral ganglion of *Aplysia californica*, Strumwasser (20) noted that stimulation of an input nerve to the ganglion caused marked *shortening* of other nerves leading from the ganglion. Contraction of nerves had not been reported previously in this animal, and no ready explanation for the phenomenon was suggested by existing anatomical studies of *Aplysia*.

It was found subsequently that the connective tissue sheath, or epineurium, surrounding the nerves and ganglia of *Aplysia* contains numerous elongated cells (21), which, upon electron microscopic examination, proved to have the characteristics of molluscan muscle fibers. These muscle fibers undoubtedly account for the contraction observed, and it is their ultrastructure which is the subject of the present paper.

MATERIALS AND METHODS

The visceral ganglion was fixed either *in situ* by perfusion through the aorta, or by immersion after excision. The fixative consisted of buffered 1 to 2 per cent osmium tetroxide in either artificial sea water or a saturated solution of calcium chloride. Specimens were dehydrated in methanol and embedded in Epon 812 (10). Thin sections were stained with lead (8) or uranium (22) salts and examined in an RCA EMU 3E electron microscope or in a Siemens Elmiskop I. 1 to 2 μ sections from the same blocks were studied by means of phase contrast microscopy, or, after being stained with toluidine blue (13), by ordinary light microscopy. Further details about the preparative procedures have been presented elsewhere (16).

OBSERVATIONS

In light micrographs, the fibrous capsule of the visceral ganglion can be seen to contain many dense, elongated, spirilliform strands, which parallel the surface of the ganglion (Fig. 1). Some of these appear helically coiled while others are straight with periodic kinks. Sometimes short, slender projections extend obliquely from the dense strands producing a fern-like pattern (Fig. 2). Opposite projections do not come off at the same level. Generally each strand with its projections is embedded in a low-density shell whose external surface is gently scalloped (Fig. 2).

A low-power electron micrograph of the capsule (Fig. 3) shows that it is composed of elongated cells lying parallel to one another in a matrix of collagen fibrils and fibrillar ground substance the texture of which resembles that of the basement membrane surrounding the parenchyma of the ganglion. The cells contain bundles of dense filaments whose orientation is slightly oblique to that of the cells themselves and which appear to enter and leave the plane of the section periodically.

Most of the intracellular filaments are thick, approaching 500 A in diameter, and are clearly cross-banded (Figs. 4, 5). Thin dark bands alternate with wider light bands with a repeating period of ~150 A. This figure is probably not significantly different from the period of paramyosin (145 A) derived previously (18) from x-ray diffraction

and electron microscopic studies. In some preparations (Fig. 5) the light band appears to be divided in half by a slender dark line. Thin (50 to 100 A) filaments, which are not striated, can sometimes be seen between the thick filaments (Fig. 5). Dense particles, which undoubtedly represent glycogen (12), are also discernible in the filament bundles (Fig. 4).

In longitudinal sections the surfaces of the cells are undulant and give the impression of having buckled. The pattern of the undulation corresponds to the coiling of the intracellular filament bundles and is related also to a specialization of the cell surface consisting of dense patches which resemble half-desmosomes (Figs. 6 and 7). Characteristically these occur where intracellular filaments approach the cell surface closely. The plasma membrane at such a patch is convex inwards, forming a shallow depression in the cell surface, while in adjacent areas the plasma membrane tends to pout outwards, sometimes forming shelf-like projections which extend obliquely from the cell into the surrounding shell of ground substance. These cytoplasmic protrusions together with their covering of ground substance probably account for the fern-like pattern visible in light micrographs.

At a dense patch the plasma membrane is easily resolved into its three components, the innermost of which is somewhat denser than the same component elsewhere along the plasma membrane (Fig. 7). Basement membrane is especially prominent over the patch. A band of diffuse osmiophilic material underlies the cytoplasmic surface of the plasma membrane here and myofilaments appear to insert into this substance. In this last respect the dense patches are comparable to the desmosomes that occur along the lateral surfaces of turtle atrium muscle fibers (4) and to the intercalated discs of other vertebrate cardiac muscles (19). The patches also resemble the "dense bodies" of the oyster adductor, some of which are adherent to the plasma membrane (6), and the "dense areas" which occur at the surfaces of avian smooth muscle fibers (2).

A second specialization of the cell surface consists of membranous cisternae in close apposition to the plasmalemma (Fig. 4). Such vesicles have been described in other muscle fibers, both vertebrate and invertebrate, and have been implicated in the intracellular transmission of stimuli (1, 6). Comparable vesicles occur in neurons as well (15).

DISCUSSION

The obliquity of the muscle filaments with respect to the long axis of the cell containing them, the fact that filament bundles appear to pass in and out of the plane of section at regular intervals, and the spirilliform appearance of the muscle fibers at low magnification are all consistent with a helical arrangement of filaments in each muscle fiber. The cross-banding of the thick filaments suggests, in addition, that this muscle is of the paramyosin type (5).

FIGURE 1

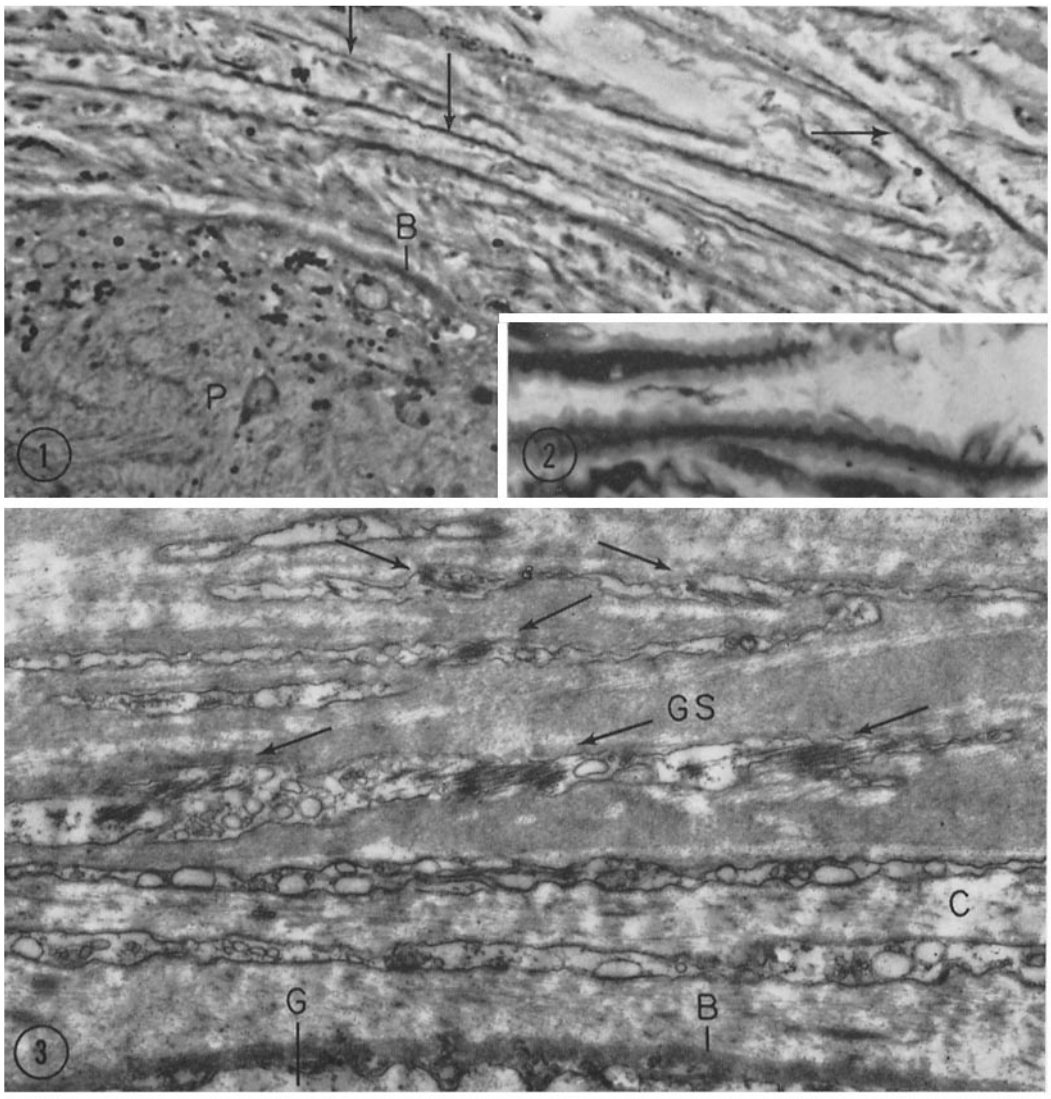
Phase contrast photomicrograph of the capsule of the visceral ganglion. The parenchyma (*P*) of the ganglion is separated from the capsule by a basement membrane (*B*). Several elongated muscle fibers are visible in the capsule. Some of these (horizontal arrow) appear to be coiled uniformly, while others (vertical arrows) are intermittently kinked. × 800.

FIGURE 2

Toluidine blue–stained muscle fibers. Each dense strand is surrounded by a scalloped shell of ground substance. Note that the scallops at opposite sides of the same fiber are not lined up over each other. This apparent phase difference also applies to the projections that extend laterally from the central strands. × 800.

FIGURE 3

Parts of seven attenuated cells are visible in the capsule. These are separated from one another by ground substance (*GS*) and collagen fibrils (*C*). Several of the cells contain bundles of filaments at intervals along their length (arrows). The axis of the filaments is slightly oblique to that of the cells. The capsule is separated from the parenchyma of the ganglion by a basement membrane (*B*), below which are glial processes (*G*) containing glycogen particles. × 16,000.



The dense patches along the plasma membranes of these cells probably represent attachment areas for muscle filaments inasmuch as filaments appear to merge with the dense material under the plasma membrane in these regions. In addition the plasma membrane here is indented inwards as if an inwardly directed force is being applied to it. These observations suggest that helical filament bundles are attached to the surface of the cell at intervals along its length, and that their contractile force is applied directly to the plasma membrane in these specialized regions. Buckling of the plasma membrane presumably results from contraction of the fiber with consequent shortening of the distance between successive attachment areas.

The importance to the animal of epineurial muscle fibers is not clear. It has been suggested (20) that their function may be simply to adjust the lengths of the nerves to the length of the animal as it shortens and elongates during locomotion. This view is supported by the fact that epineurial musculature has also been reported to occur in two other snails, *Helix* (9, 17) and *Archachatina* (11), in an earthworm (7), and in a leech (14). All of these animals also undergo marked changes in body length during locomotion. It would be interesting to know whether epineurial muscle fibers also occur in invertebrates whose body length is fixed by the presence of a rigid exoskeleton.

A second possibility is that this muscle is concerned with the circulation to the nervous system. Eales (3) has pointed out that the nervous system in *Aplysia* is supplied by a blood sinus lying under the investing fibrous capsule. Musculature in the capsule might serve to empty and fill the sinus intermittently or to pump hemolymph through it.

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FIGURE 4

Muscle fiber containing cross-banded filaments (*F*), glycogen particles (*G*), and vesicles (*V*) which are in close apposition to the plasma membrane. × 42,000.

FIGURE 5

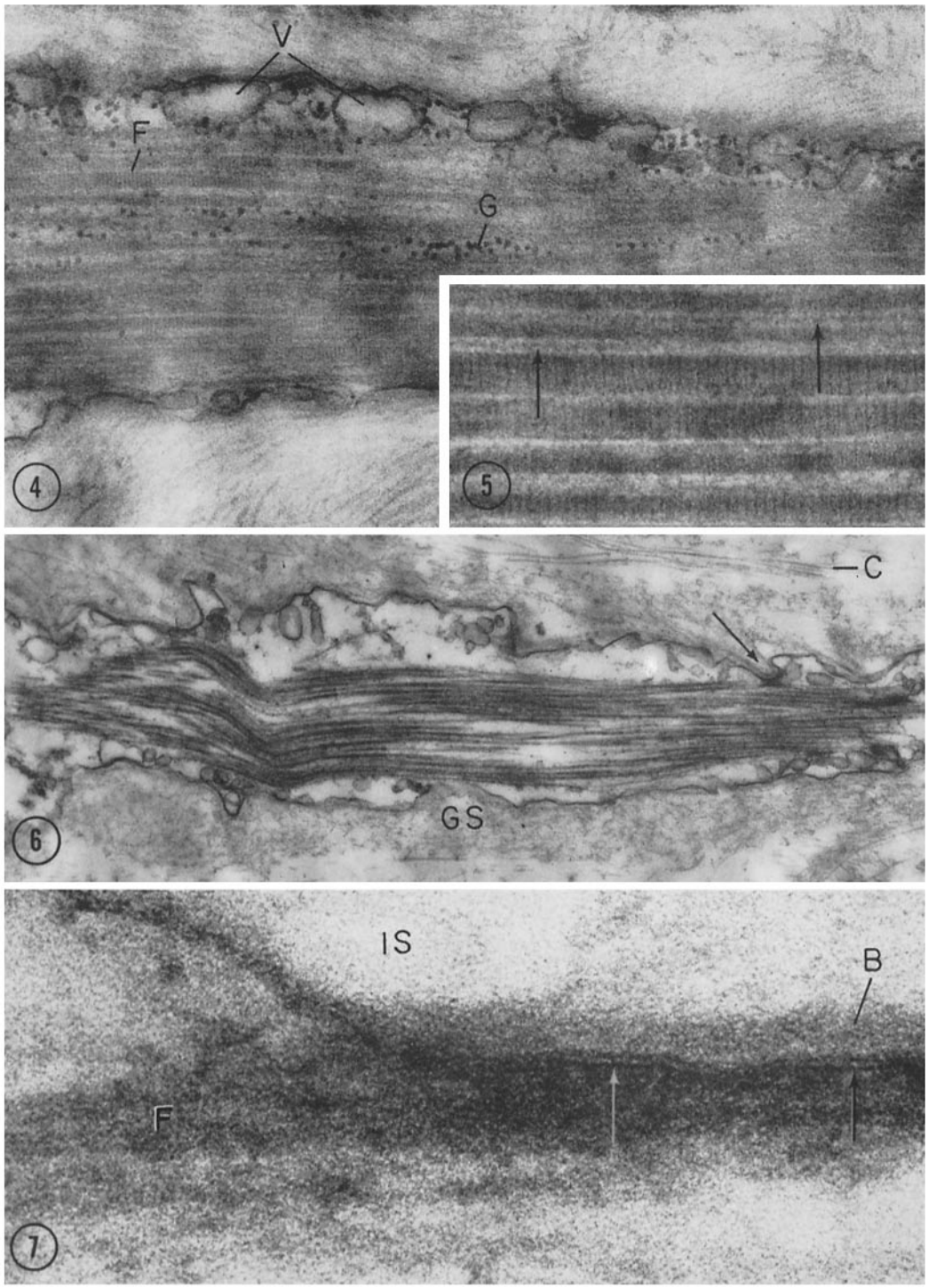
Higher magnification of muscle filaments. The thick filaments are ~500 A wide and are cross-banded with a period of ~150 A. Some of the light bands in the thick filament at the bottom of the figure are bisected by thin dark lines. Thin filaments can be distinguished between the thick filaments in several places (arrows). × 95,000.

FIGURE 6

Muscle fiber. The intracellular filaments follow a straight course across most of the field, but near the left of the figure they bend abruptly and pass out of the plane of the section. Near the right of the figure, a dense patch (arrow) occurs at an indentation in the plasma membrane. Basement membrane is prominent over the outside of this patch, and myofilaments approach its inner surface closely. *GS*, ground substance; *C*, collagen fibril. × 23,000.

FIGURE 7

Higher magnification of a dense patch at the surface of a muscle fiber. The unit membrane structure of the plasmalemma is distinct (arrows), and the overlying basement membrane (*B*) is prominent. Several myofilaments (*F*) appear to insert into the dense material immediately subjacent to the plasmalemma. *IS*, interstitial space. × 240,000.



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