THE STRUCTURE OF THE SARCOLEMMA OF THE FROG SKELETAL MUSCLE FIBER

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The classical paper of Bowman (1)[1] provides an excellent description of the delicate tubular structure which invests each striated muscle fiber, and is especially replete with elaborate sketches of various muscle fibers from a comparative point of view. Subsequently many papers have been published using the light microscope in conjunction with various histological techniques. In recent years both replica and sectioning methods have provided electron micrographic evidence which has further elucidated the ultrastructure of this region of the fiber. In this brief report we shall attempt to present both light and electron micrographs of the frog muscle fiber so as to bring together in one paper the various levels of observation with regard to the structure of this region which is of interest to both cytologists and physiologists.

MATERIAL AND METHODS

Specimens were obtained from the tibialis anticus muscle of the frog (*Rana pipiens*). Single fibers were prepared by microdissection with the aid of a dissecting microscope. Laidlaw's silver impregnation method for connective tissue was used in photomicrographic studies. For electron micrographic studies the conventional fixation procedure was used with a 1 per cent solution of OsO_4 buffered at pH 7.8 with veronal acetate. Most of the specimens were embedded in methacrylate. Some material was embedded in epon—mixture B of epon 812 as described by Finck (2)—and sectioned at 60°F.

OBSERVATIONS AND DISCUSSION

So as not to become involved in fruitless discussion of "proper" nomenclature, we shall proceed at the outset, as did Bowman (1) in his paper of 1840, to consider a view of the freshly dissected fiber. In Fig. 1 there is seen a typical dissected fiber from the tibialis anticus of the frog in which a "retraction clot" (*cf.* Speidel (3)) has been induced by mild compression with a pair of dissecting forceps. The empty tube (also referred to by some authors as "husk" or "shell," "casing," etc.) was described by Bowman (1) as follows:

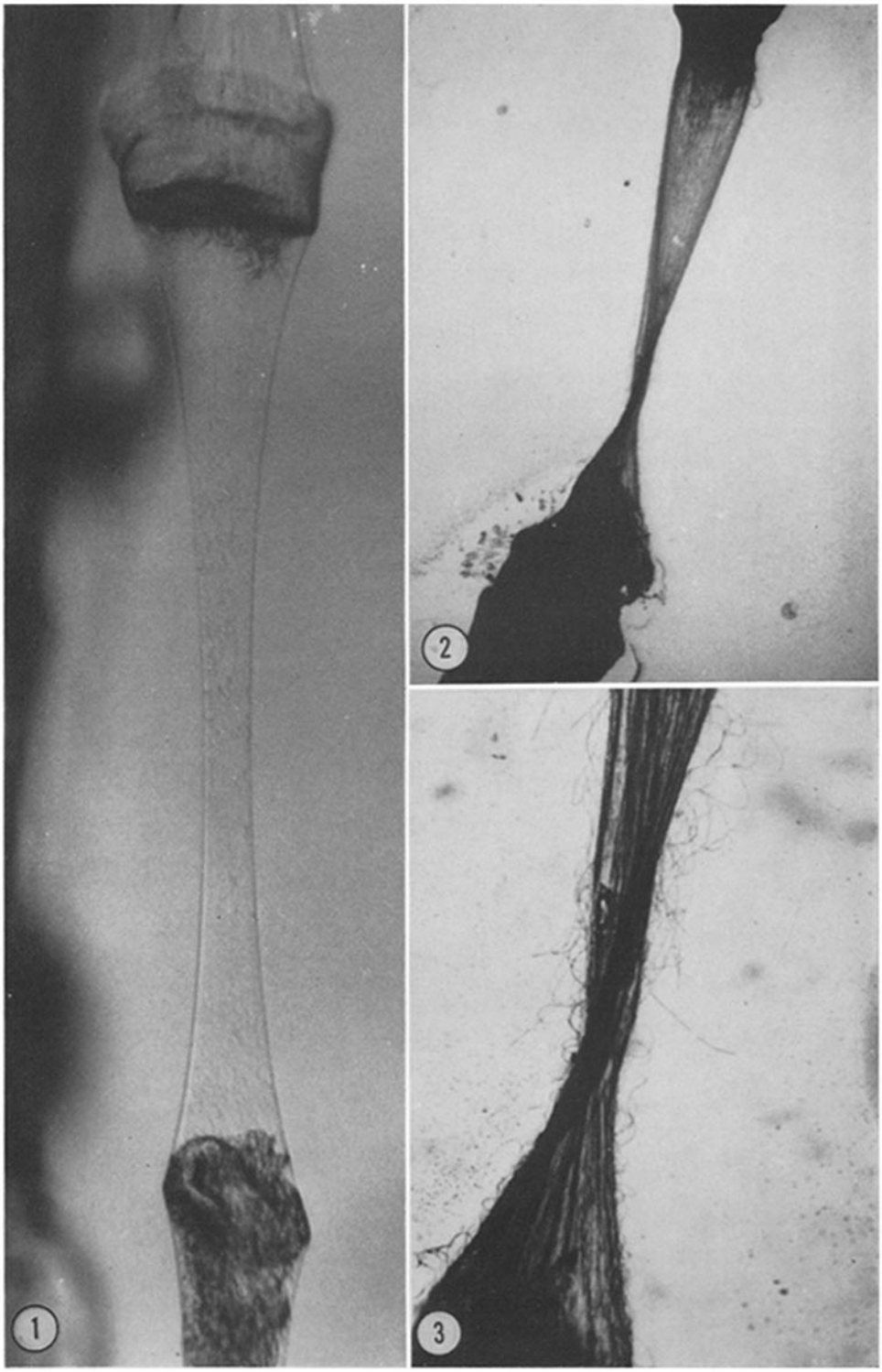
"I allude to a tubular membranaceous sheath of the most exquisite delicacy, investing every fasciculus from end to end, and isolating its fibrillae from all the surrounding structures." (474)

". . . I have been in the habit of styling it Sarcolemma, a term descriptive of its nature." (475)

It is precisely the various levels of this structure, the "sarcolemma," that will be the concern of this paper.

Examination of such tubes as in Fig. 1 with various microscopic techniques, namely, ordinary and phase optics, leaves the observer with the impression of a "structureless" entity. Indeed, this is just the impression expressed by Schwann and Bowman. By resorting to silver stains, however, as done by Bairati (4) in recent years, a fine network of fibrils can be seen around the periphery as in Fig. 2 with a glassy or smooth matrix as the continuous phase of the tube's wall (Fig. 3). Although the nature of this matrix remains unknown, the fact that a characteristic color is seen in the periphery of muscle cells stained with periodic-acid Schiff (PAS) stain permits the inference that a mucopolysaccharide is associated with the tube. The observables thus described exhaust the possibilities of new evidence at this level of analysis. With the advent of the electron microscope, the work of Reed and Rudall (5) in 1948 provided the first clear evidence of the ultra-

[1] Bowman in his paper (1) acknowledges a previous description of this structure by Schwann (*cf.* footnote p. 475).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structure of the tube from the outer aspect. The fine fibrils as revealed by the silver stains are shown in their electron micrographs of replicas to have striations suggesting collagen filaments.[2] Thus the fibrils as seen in the silver preparations are most likely several of the individual filaments grouped in a "cable." The matrix is seen only as background between these filaments.

Proceeding to thin sections of methacrylate-embedded material prepared in the conventional manner, we see in Fig. 4 a view of the peripheral region of the muscle fiber. In the outermost region of the cell, from without inward, four distinct components may be observed. The first component is a mesh of extremely delicate filaments which measure approximately 100 A units in diameter. These filaments were first described by Robertson (7), and it can be seen in Fig. 5 that they lack a distinct periodicity, *e.g.*, striations, but, in some cases, they do appear to have a faintly beaded structure. The second component is another filamentous layer, composed of collagen filaments, with characteristic striations, which measure approximately 300 A in diameter. These filaments would appear to be identical with the collagen filaments of similar diameter seen in the Reed and Rudall (5) replicas of the surface of the sarcolemma and in the micrographs of fragmented muscle by Draper and Hodge (6). The morphology of the individual filaments in the two filamentous layers is quite constant. However, both layers display wide fluctuations in the density of filament population along the length of the muscle cell. In some regions the collagen filaments appear concentrated, suggesting several layers in braid-like weave, while in other regions they are sparse. It may be of interest to note that staining with phosphotungstic acid usually emphasizes the collagen filaments in a markedly selective manner.

The third component is a uniform layer measuring 300 to 500 A in width, having a structureless or faintly granular appearance, and appearing more distinct in sections stained with the "lead hydroxide" solution of Watson. This layer or region has been referred to by recent authors as the "amorphous layer" (6), "ground substance" (8), "basement membrane" (7), or "cuticular layer" (9). Presumably, as mentioned above, this is the region stained by the positive periodic-acid Schiff reaction for mucopolysaccharides (8). It should be noted that Draper and Hodge (6) have pointed out the presence of "a network of very fine fibrils (*ca.* 100 A)," appearing without

[2] By means of electron micrographs of fragmented muscle, Draper and Hodge (6) confirmed the presence of collagen filaments. However, the reader is cautioned that the surmisal by the authors of an outer structureless membrane overlying the collagen filaments is probably due to an error in interpretation of their data.

FIGURE 1

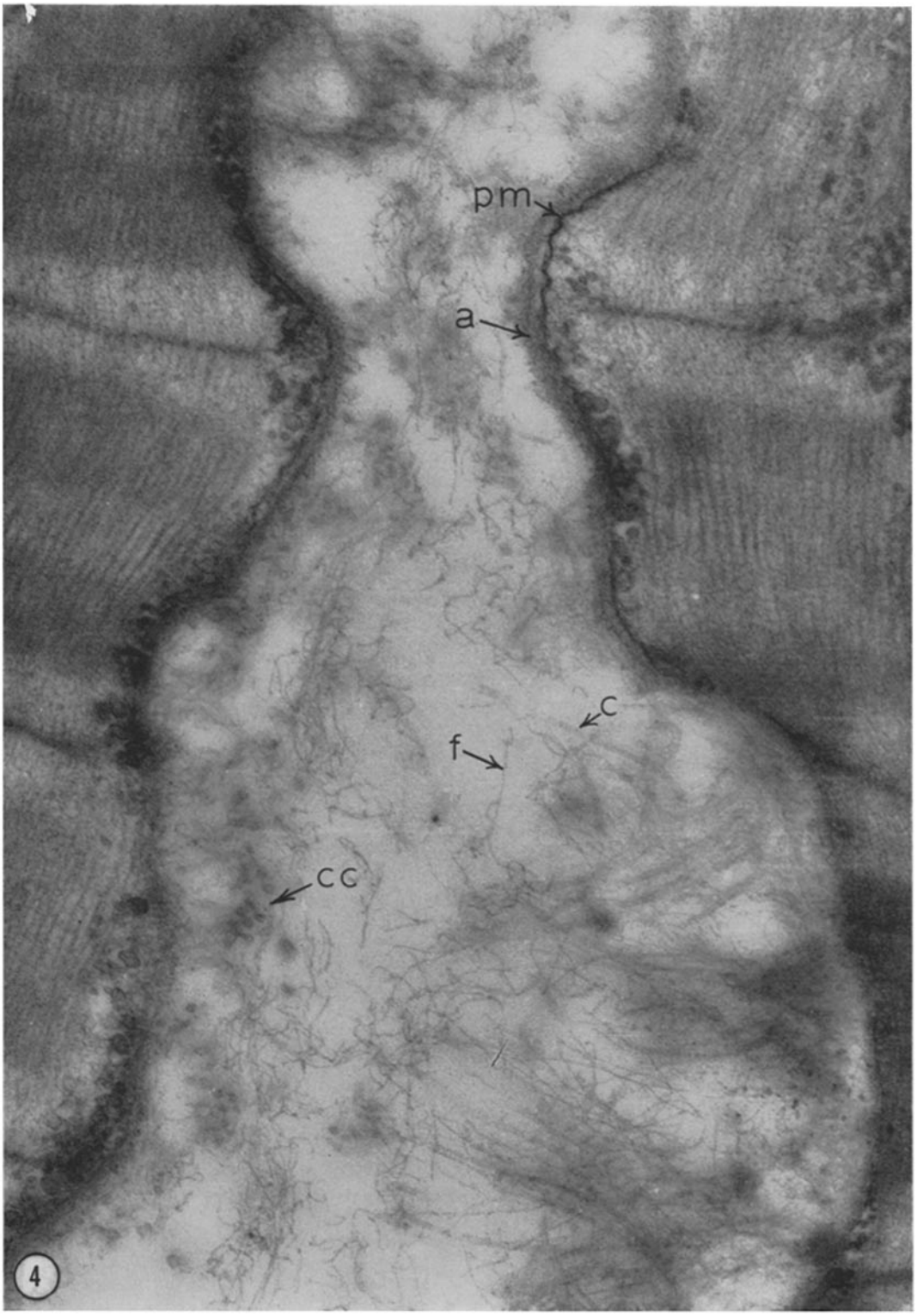
Photomicrograph by a combination of reflected and transmitted light illustrating a length of sarcolemma. The specimen is a fresh single muscle cell from the tibialis anticus of the frog, and has been dissected free of endomysial connective tissue. A few minutes before taking this photograph the cell was gently pinched with fine forceps, resulting in the "retraction area" depicted here, which is composed entirely of the empty tube-like sarcolemma described by Bowman (1). The retracted, "clotted" ends of damaged myoplasm are seen at the upper and lower margins of the illustration. Except for a faint and irregular granularity, the sarcolemma appears structureless under these conditions and the argyrophilic fibrils seen in silver stained preparations are not visible. × 340.

FIGURE 2

Photomicrograph of a retraction area, similar to that seen in Fig. 1, stained by Laidlaw's silver impregnation method for connective tissue (LCT). The empty tube of sarcolemma has become twisted in the process of preparation. Whole mount in gelatin. × 320.

FIGURE 3

Oil immersion photomicrograph of central portion of Fig. 2 illustrating the fibrils associated with the sarcolemma. × 1,200.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characteristic striations, "embedded" in the amorphous layer referred to further as consisting of "matrix material." It is not unlikely that the faintly granular appearance in our micrographs of this layer in cross-section can be attributed in part to a fine fibrillar component.

The fourth component, intimately related to the inner surface of the amorphous layer and, like it, enhanced by lead hydroxide stain, is interperted in the customary manner as the plasma membrane of the muscle cell. The thickness of this component is approximately 100 A. With regard to the plasma membrane, it may be of interest to note the presence of randomly disposed "vesicles" or "oval bodies" located immediately adjacent to the inner surface of the plasma membrane and, in some cases, apparently "breaking out of" or "pinching off" from the plasma membrane itself. Such "vesicles" or "bodies"[3] are invariably seen on the cytoplasmic side of the plasma membrane. In this connection, it is pertinent to point out that Porter and Palade (10) discussed the possible relationship of these structures with the system of the endoplasmic reticulum. While it is suggestive that such elements could be shuttling back and forth between the plasma membrane and the reticulum, the electron micrographic evidence to date is quite ambiguous with regard to this point.

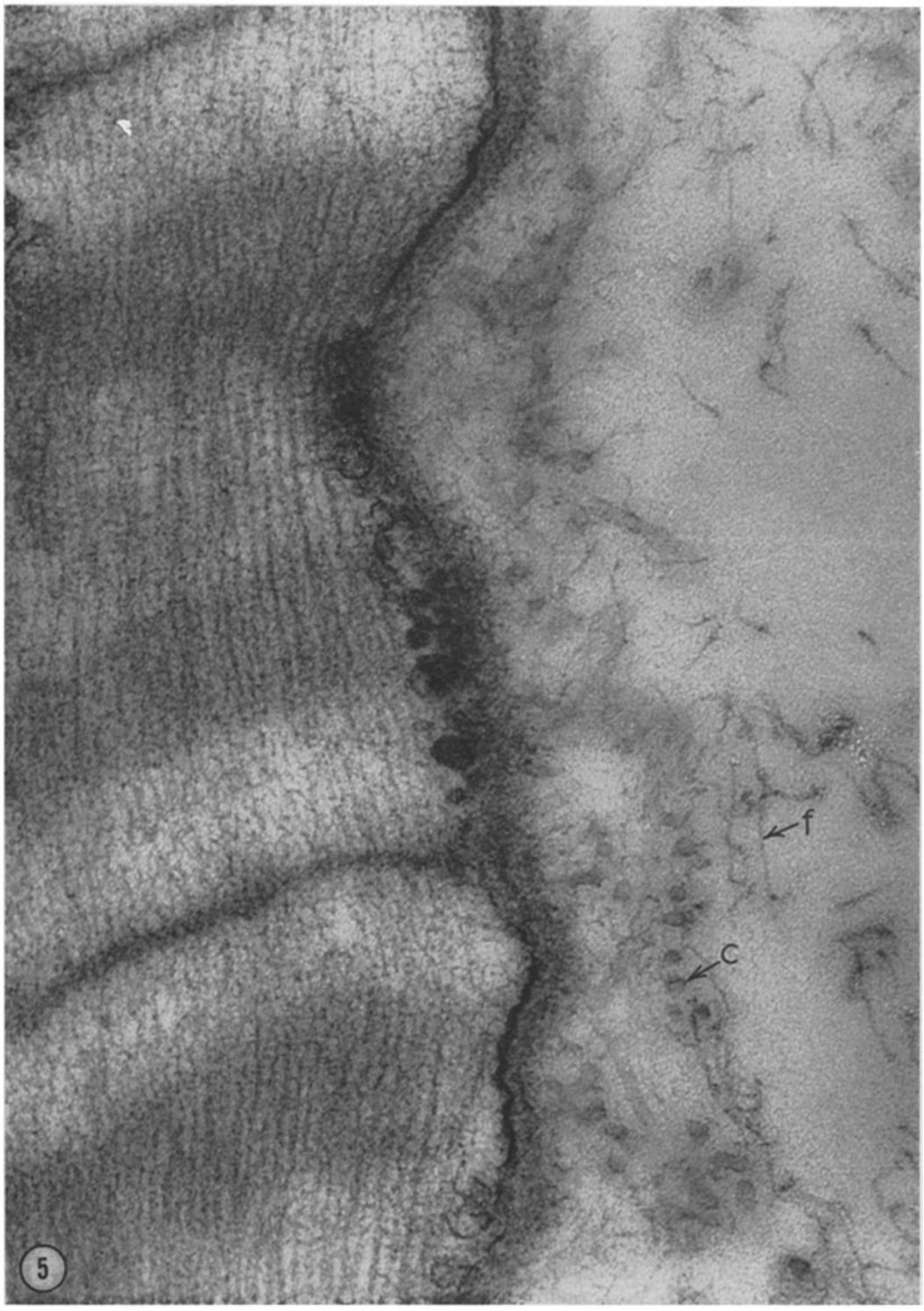
It should be emphasized that the plasma membrane and the "ground substance" or "basement membrane" are two *distinct* structures. It is true that in general they are intimately related but under certain conditions they can be visualized as separable entities. This is seen most dramatically in preparations of atrophied fibers resulting from denervation (Birks, Katz, Miledi (11)). In this connection it should be noted that examination of the empty sarcolemma-tube in electron micrographs of methacrylate-embedded material reveals that in general the plasma membrane and the amorphous layer are still closely associated, the plasma membrane appearing either superficially "intact" as in Fig. 7 or markedly disintegrated as in Fig. 6. Although not presented in this paper, some micrographs have revealed preparations with only the amorphous layer present, the plasma membrane having been distinctly pulled away during the formation of the retraction clot. With regard to the possibility of physiological activity being associated with the tube of sarcolemma, at least as evidenced by electromotive force and electrotonic response, our repeated attempts with microelectrode techniques have thus far always demonstrated complete absence of such signs of activity and thus force the conclusion that such preparations are purely passive. This, of course, is clearly consistent with the customary expectations concerning a damaged plasma membrane. It is interesting to note that mechanical studies (12, 13) on the sarcolemma have demonstrated the remarkable tensile strength of this structure. While it seems reasonable to attribute this property to the braid of collagen filaments which constitutes an important component of the sarcolemma, the relative importance of the other component, the basement membrane, remains to be established.

By way of summary, the "sarcolemma" as

[3] Reed and Rudall in their replica micrographs of the inner surface of the sarcolemma noted the presence of "discrete corpuscles," approximately 300 A in diameter. It is interesting to speculate whether these are the "vesicles" seen in ultrathin sections.

FIGURE 4

Electron micrograph of two muscle fibers especially chosen to bring out two views of the collagen filaments associated with the amorphous layer. The filaments (*c*) associated with the fiber to the left can be seen sectioned transversely. Note also some filaments appearing in groups to form "cables" (*cc*). Aggregates of this kind, and larger, are probably the fibrils seen in the silver preparations (see Fig. 3). To the lower right, collagen filaments can be seen sectioned longitudinally with faint striations characteristic of collagen. The fine filaments (*f*), displaying no significant structure, can be seen dispersed throughout. The amorphous layer (*a*) in close association with the plasma membrane (*pm*), which can be seen to be much darker in contrast, is clearly visible for both fibers. The "vesicles" are seen dispersed along the cytoplasmic surface of the plasma membrane and, like the latter, darkly stained. Preparation stained for 30 minutes with lead hydroxide solution. × 54,000.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originally defined by William Bowman consists of four components:

1. An outer layer, variable in thickness, of unidentified fine filaments, randomly oriented, measuring less than 100 A in diameter.

2. A braid-like layer, variable in thickness, of collagen filaments, measuring approximately 300 A in diameter.

3. An amorphous layer, resembling basement membrane matrix, measuring 300 to 500 A in thickness. According to Draper and Hodge (6), fine filaments (*ca.* 100 A) are embedded in this layer.

4. A layer interpreted as the plasma membrane of the muscle cell. Vesicles are randomly disposed along the cytoplasmic surface of the plasma membrane and, occasionally, continuous with it.

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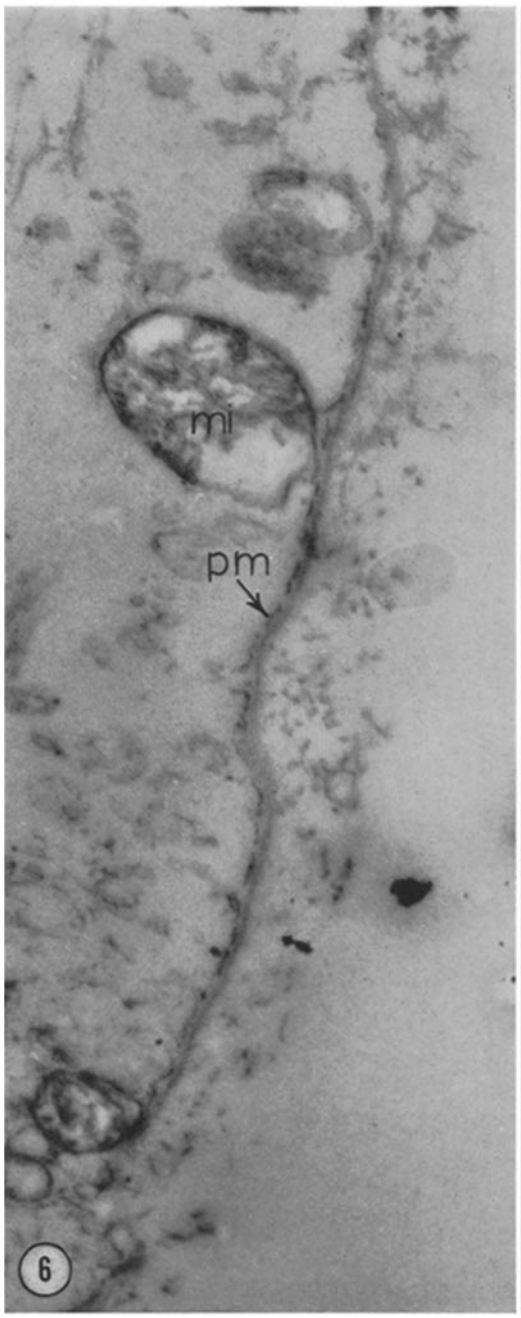
FIGURE 5

Electron micrograph of the left hand fiber, in another field, at higher magnification. The relative size of collagen filaments and finer filaments is more clearly seen. A loose braid-like configuration of several layers of collagen filaments is seen along the surface of the amorphous layer.

Preparation stained for 30 minutes with lead hydroxide solution. × 88,000.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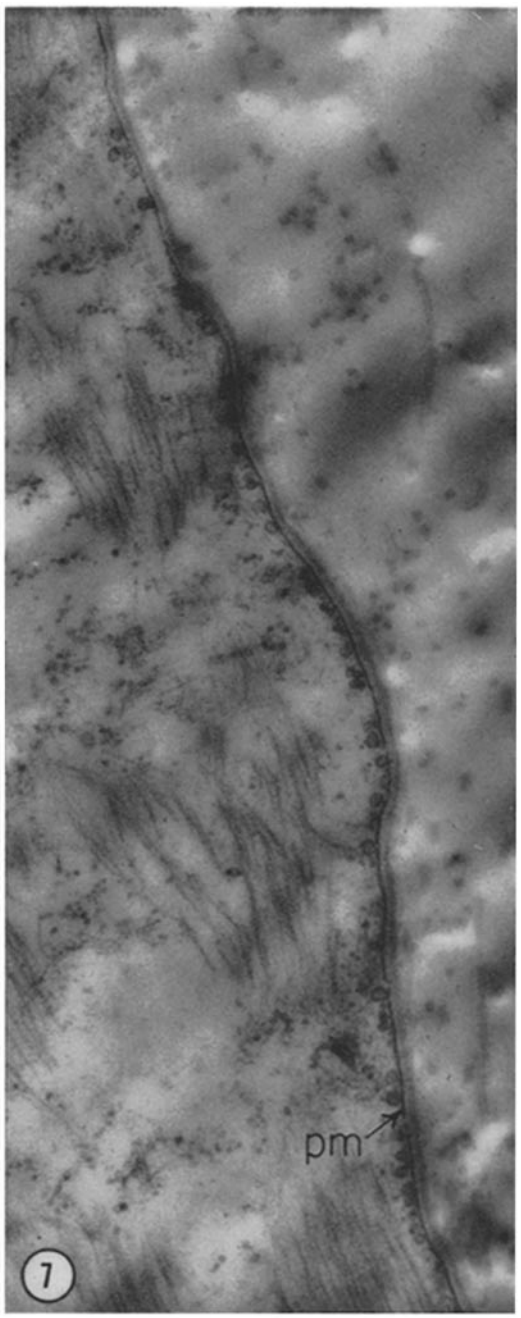
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FIGURE 6

Electron micrograph of a muscle fiber in the region of a retraction clot such as seen in the lower right hand field in Fig. 1. Upward, the figure extends to the empty tube of sarcolemma, downward to the intact myoplasm. Collagen filaments are seen in cross-section distributed along the outer surface of the amorphous layer. Remnants of the plasma membrane (*pm*) can be seen in close association with the latter.

The fiber was dissected in Ringer's solution and the retraction clot formation was induced and allowed to form for several minutes. The conventional fixation procedure followed. The extracted mitochondria (*mi*) are some of the signs of damaged tissue which must be accepted as an inevitable consequence of the clot formation.

Preparation stained for 20 minutes with lead hydroxide solution. × 32,000.

FIGURE 7

Electron micrograph of another preparation in the region of the empty tube of sarcolemma. The plasma membrane (*pm*) in this instance appears to be more "intact" than in the previous micrograph. Note also the marked presence of "vesicles" on the cytoplasmic side of the plasma membrane. Remnants of the myoplasm are seen by the appearance of fragmented myofilaments. The amorphous layer and collagen filaments are also seen on the outer surface.

Preparation stained for 30 minutes with lead hydroxide solution. Epon embedding. × 33,000.