ULTRASTRUCTURE OF DYADS IN MUSCLE FIBERS OF *ASCARIS LUMBRICOIDES*

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ABSTRACT

The dyads of *Ascaris* body muscle cells consist of flattened intracellular cisternae applied to the sarcolemma at the cell surface and along the length of T-tubules. In specimens prepared by conventional methods (glutaraldehyde fixation, osmium tetroxide postfixation, double staining of sections with uranyl acetate and lead hydroxide), both the sarcolemma and the limiting membrane of the cisterna exhibit unit membrane structure and the space between them is occupied by a layer of peg-shaped densities which is referred to as the subsarcolemmal lamina. The lumen of the cisterna contains a serrated layer of dense material referred to as the intracisternal lamina. In specimens fixed in glutaraldehyde, dehydrated, and then postfixed in phosphotungstic acid, with no exposure to osmium tetroxide or heavy metal stains, the membranous components of the dyads appear only as negative images, but the subsarcolemmal and intracisternal laminae still appear dense. Except for the lack of density in membranes and in glycogen deposits, the picture produced by the latter method is very much like that of tissue prepared by conventional methods.

INTRODUCTION

Several reports on cross-striated muscle have noted that the junctions between terminal cisternae of the sarcoplasmic reticulum and T-tubule membranes are morphologically specialized. At such "dyads" or "triads" periodically disposed densities occupy the cleft between the central and lateral elements and apparently bridge the gap between the respective limiting membranes (4, 8, 13, 14). The significance of this specialization is unknown but since dyads and triads probably serve to "couple" the sarcoplasmic reticulum (SR) and T systems together the detailed structure of these junctions is of special interest. Furthermore, the mechanisms that operate at the dyads of muscle cells may also operate at the morphologically similar "subsurface cisterns" that occur in nerve cells (9) and may even bear on the phenomenon of intercellular coupling, which, it now seems, is widespread (15).

Dyads are known to occur in obliquely striated muscles, and in an earlier study of *Ascaris* body muscle their general configuration was shown to be equivalent to that in invertebrate cross-striated muscles (10). Some indication of membrane-associated specializations at the dyads was also shown in these osmium tetroxide-fixed specimens (Fig. 12 ref. 10) but was not commented on in detail. The present report describes the ultrastructure of *Ascaris* dyads as revealed by two other methods of preparation.

METHODS

Ascaris body wall muscle was fixed for approximately 1 to 3 hr in 2.8% glutaraldehyde (biological grade) in 0.1 M phosphate buffer (pH 7.4) as described previously (11). One group of specimens was then rinsed in 0.9% NaCl and postfixed with 1% OsO_4 in 0.1 M phosphate buffer (pH 7.4). Dehydration was carried

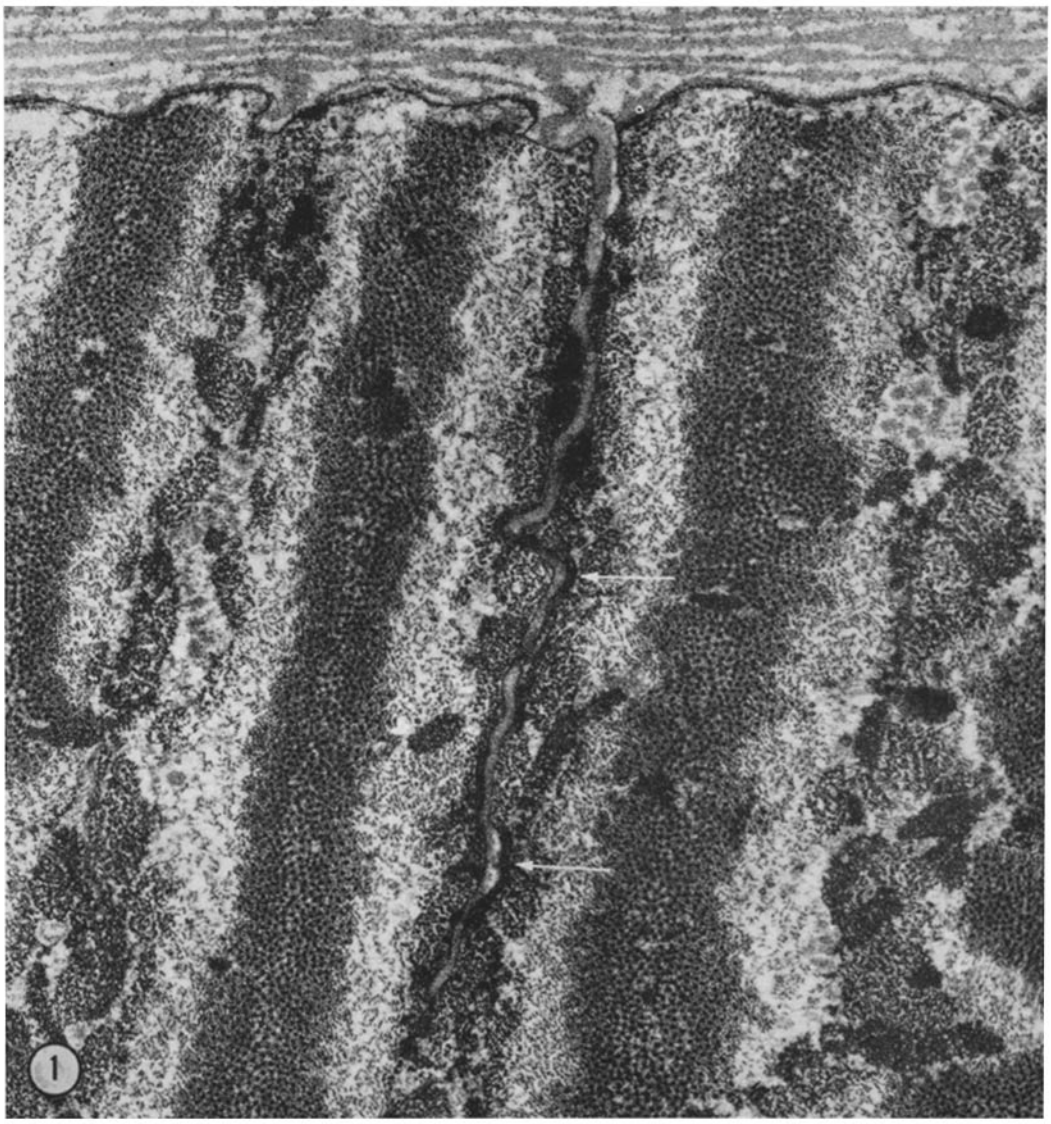


FIGURE 1 Transverse section through an *Ascaris* muscle fiber postfixed with osmium tetroxide. A T-tubule extends into the fiber. Sacs of sarcoplasmic reticulum, two of which are shown by arrows, form dyads with the T-tubule membrane. The components of the dyads are not resolved at this magnification. × 33,000.

out in a graded series of methanol solutions followed by propylene oxide, and then the tissue was embedded in Araldite. Thin sections were mounted on either bare or carbon-coated grids, and stained with uranyl acetate followed by lead hydroxide. A second group of specimens was dehydrated after glutaraldehyde fixation without exposure to OsO_4. Following dehydration these specimens were soaked in a freshly prepared 1% solution of phosphotungstic acid (PTA) in methanol for 1 hr (1), infiltrated with a 75% solution of the embedding medium in methanol, and then embedded flat in the Araldite mixture. 1 μ sections were cut and stained with toluidine blue for survey purposes and the blocks then trimmed for thin sectioning. After 1 to 3 additional days at 65°C, thin sections were cut and picked up on carbon–Formvar–

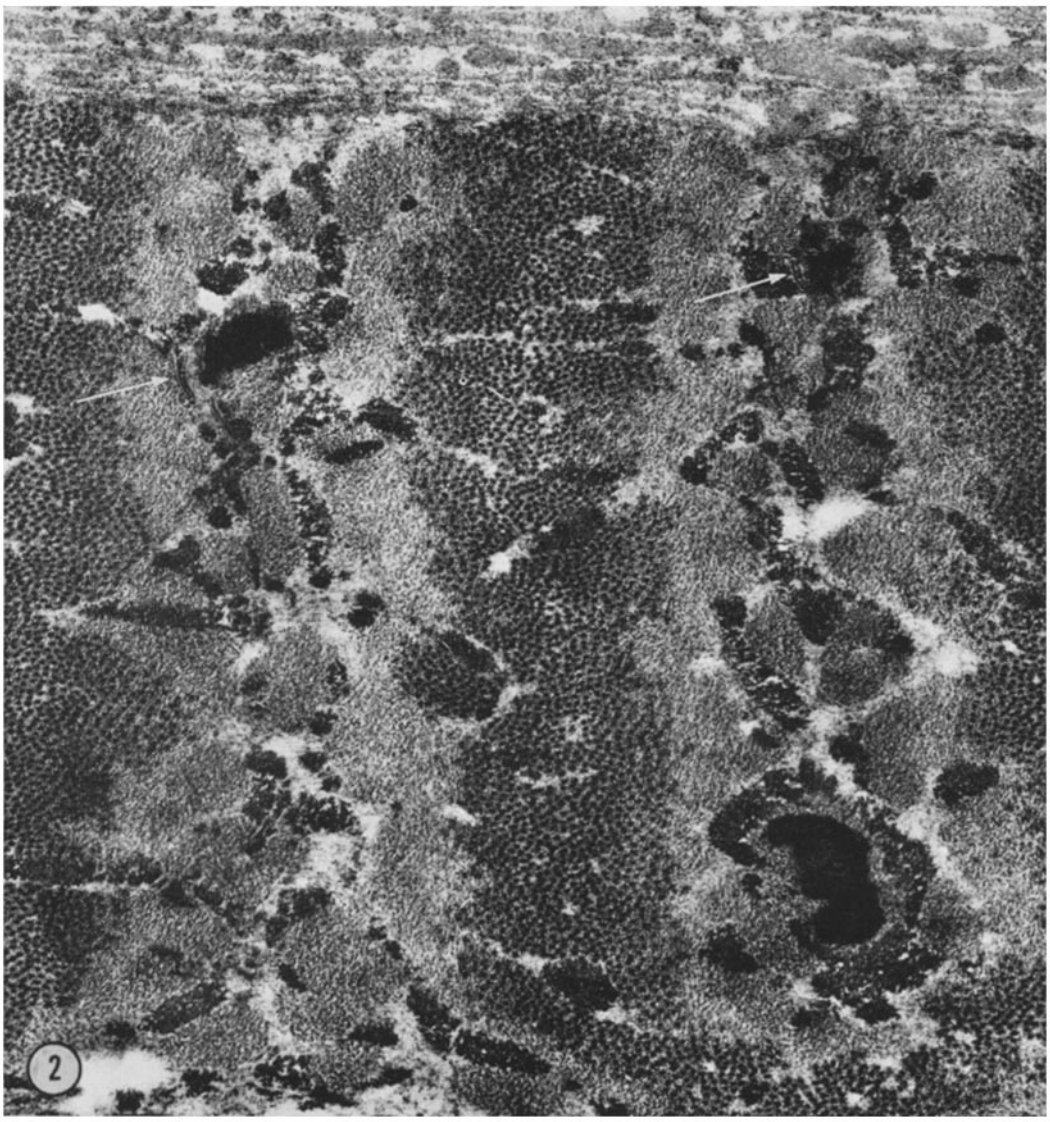


FIGURE 2 Muscle fiber postfixed with phosphotungstic acid instead of osmium tetroxide. Cytoplasmic and extracellular components are comparable in appearance to those in Fig. 1. The locations of two dyads are marked by arrows. Neither the plasma membrane nor cisternal membranes are stained by this method. White patches represent glycogen deposits which are also unstained. × 33,000.

coated grids and examined by electron microscopy (60 kv) without further treatment.

RESULTS

The T system of *Ascaris* muscle consists of deep tubular invaginations of the plasma membrane into the contractile cortex of the muscle fiber (Fig. 1). Unlike the T-tubules of many cross-straited muscles, those of *Ascaris* muscle are relatively broad ($\sim$0.1 μ) and contain strands of the amorphous connective tissue that surrounds the fibers. The sarcoplasmic reticulum consists almost exclusively of flattened membranous cisternae which form dyads with the plasma membrane either at the surface of the fiber or along T-tubules (Fig. 3). Frequently, a cisterna is wrapped around

a T-tubule in such a way that, when the tubule is cut across, a large portion of its perimeter is covered by the cisternal profile (Fig. 4 *b*). A line drawn radially from the center of such a complex thus intersects three membranes: the sarcolemma lining the T-tubule, the superficial membrane of the SR cisterna, and the deep membrane of the SR cisterna. These membranes will be referred to as membrane # 1, # 2, and # 3, respectively, in the descriptions that follow.

In specimens postfixed with osmium tetroxide all of these membranes exhibit a trilaminate or "unit membrane" structure the thickness of which is ~75 A in the case of membrane # 1 and ~50 A in the case of membranes # 2 and # 3 (Fig. 4 *d*). Membrane # 1 is separated from membrane # 2 by a gap ~120 A wide; however, most of that distance is bridged by a layer of peg-shaped densities which extend radially from membrane # 2 towards membrane # 1 (Figs. 4 *a* and 4 *b*). This layer will be referred to as the "subsarcolemmal lamina." Each component peg is ~200 A in breadth and the center-to-center distance between pegs is ~300 A. The bases of the pegs are contiguous with membrane # 2 but their rounded apices are not contiguous with membrane # 1. Membranes # 2 and # 3 are ~140 A apart, this distance corresponds to the lumen of the SR cisterna. However, this space, too, is partially filled with a layer of dense material which is applied to membrane # 3 and extends towards membrane # 2 (Figs. 3 inset, 4 *c* and 4 *d*). This layer, which will be referred to as the "intracisternal lamina," may also exhibit a periodic structure consisting of saw-tooth-shaped projections, the points of which appear to touch membrane # 2. The center-to-center distance between these teeth is ~100A, and their disposition bears no fixed relationship to that of the pegs between membranes # 2 and # 1.

At low magnification, specimens postfixed with phosphotungstic acid (Fig. 2) closely resemble those postfixed with osmium tetroxide. Indeed, Figs. 1 and 2 are difficult to tell apart at a glance. In both cases the densest structures seen are the "dense bodies" and nearby aggregates of thin filaments. The A zones contain clearly defined thick filaments and the I zones contain discrete, thin myofilaments. The matrices of mitochondria and lysosomes in the sarcoplasm are very dense (not shown) and the amorphous strands of connective tissue outside the fibers are moderately dense. The only obvious distinguishing characteristic of the PTA-treated specimens in comparison with the conventionally prepared tissue is the apparent absence of glycogen and membranes. Plasma membranes, T-tubule membranes, and the SR membranes are seen only negatively.

At high magnification (Fig. 5), details in the PTA-treated material are not as sharp as those in conventionally prepared specimens. Nevertheless, dyads are readily recognizable as pairs of parallel linear densities. One member of the pair can be identified immediately as the subsarcolemmal lamina by its characteristic dentate configuration (Figs. 5 *a* and 5 *c*). The other member, which undoubtedly corresponds to the intracisternal lamina, shows some evidence of serration (Fig. 5 *c*) and often exhibits a bulbous expansion at one edge which presumably represents the margin of the terminal cisterna (Fig. 5 *c*, bottom).

The membranes of the T-tubule and SR cisterna, although not stained, are clearly outlined by the neighboring dense structures. Membrane # 1 is bordered on one side by the connective tissue elements within the T-tubule and on the other side by the subsarcolemmal lamina. Membrane # 2 is bordered by the subsarcolemmal lamina and the intracisternal lamina. Membrane # 3 is bordered on one side by the intracisternal lamina and on the other side by myofilaments, dense bodies, or other cytoplasmic structures. The PTA-stained material delineates these membranes sharply not only at the dyads but also at the junctions of dense bodies with the plasma membrane (Fig. 5 *b*) where the latter appears as an uninterrupted white line. A dense subsarcolemmal lamina has also been identified at dyads of several smooth and cross-striated invertebrate muscles following PTA postfixation.

Bloom and Aghajanian (1) have emphasized the selectivity of PTA postfixation, under certain conditions, in demonstrating synaptic complexes in the central nervous system. In *Ascaris* muscle, however, as well as in the other smooth and striated muscles that have been examined, it is the *non*-selectivity of the method which is striking. This apparent discrepancy probably depends in part on differences in preparative procedure. In addition, it probably also reflects the uniqueness of neuropil in having an abundance of cell membranes, which do not bind PTA, but a paucity of cytoplasmic organelles and a virtual absence of formed extracellular elements. Muscle, in contrast, possesses a

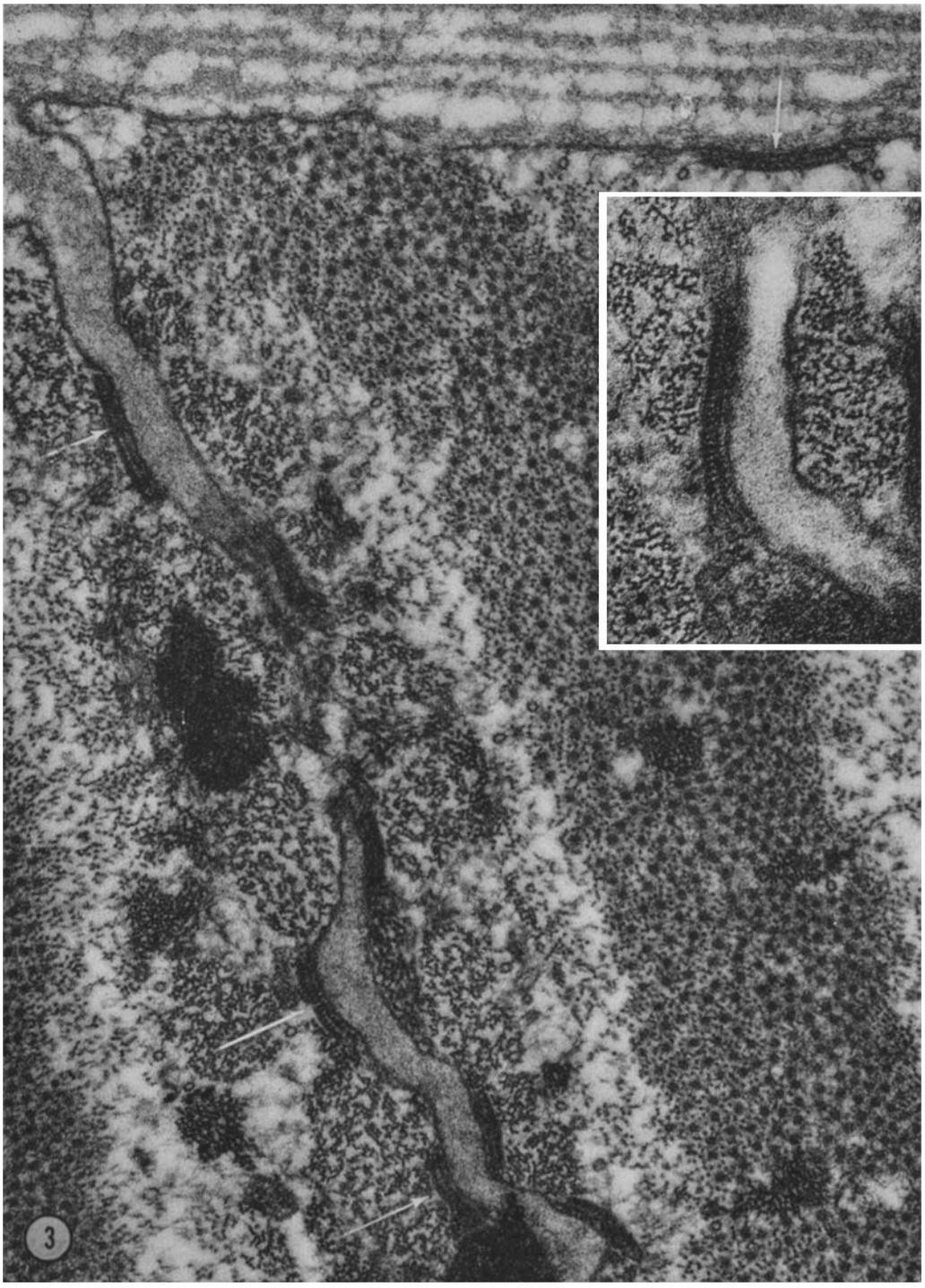


FIGURE 3 Muscle postfixed with osmium tetroxide. Arrows point to three cisternae forming dyads along a T-tubule and to a fourth cisterna forming a dyad at the surface of the fiber. Periodic densities are barely discernible within all four cisternae and also between the fourth cisterna and the sarcolemma. *Inset.* Dyad at higher magnification showing intracisternal serrated densities. × 73,000. *Inset* × 107,000.

distinct connective tissue sheath around the cells and a large complement of filaments within them, and these components, as well as most other constituents of cells, seem to bind PTA non-specifically resulting in a general enhancement of contrast much like that caused by osmium tetroxide.

The picture produced by PTA postfixation is complementary to that produced by permanganate fixation. The latter method brings out membranes only; the former brings out almost everything except membranes. Thus, PTA postfixation may prove to be generally useful as an alternative to osmium tetroxide postfixation as well as in situations where it is desirable to eliminate the staining of membranes in order to emphasize membrane-associated structures. The principal limitation of the method is that it enhances contrast everywhere in the tissue; therefore, structures throughout the thickness of a section appear superimposed, and it is presumably for this reason that details appear less sharp than in conventionally prepared specimens, in which only one surface is stained.

DISCUSSION

The findings may be summarized as follows:

1. The dyads of *Ascaris* muscle exhibit two morphological specializations. One, the subsarcolemmal lamina, consists of a layer of peg-shaped densities between the plasma membrane and the terminal cisterna. This structure corresponds to that occurring at the dyads of cross-striated muscles in two other phyla. The second specialization is the intracisternal lamina, which occupies the flattened lumen of the terminal cisterna. It is a continuous layer which, however, also displays a periodicity at its surface.

2. After postfixation with PTA instead of OsO_4 both of these laminae appear dense despite the fact that the membranous components of the dyads appear only as negative images.

3. In the muscles that have been examined, PTA postfixation produces a nonselective enhancement in the density of cytoplasmic, nuclear, and extracellular components, but not in that of plasma membranes, intracellular membranes, or glycogen.

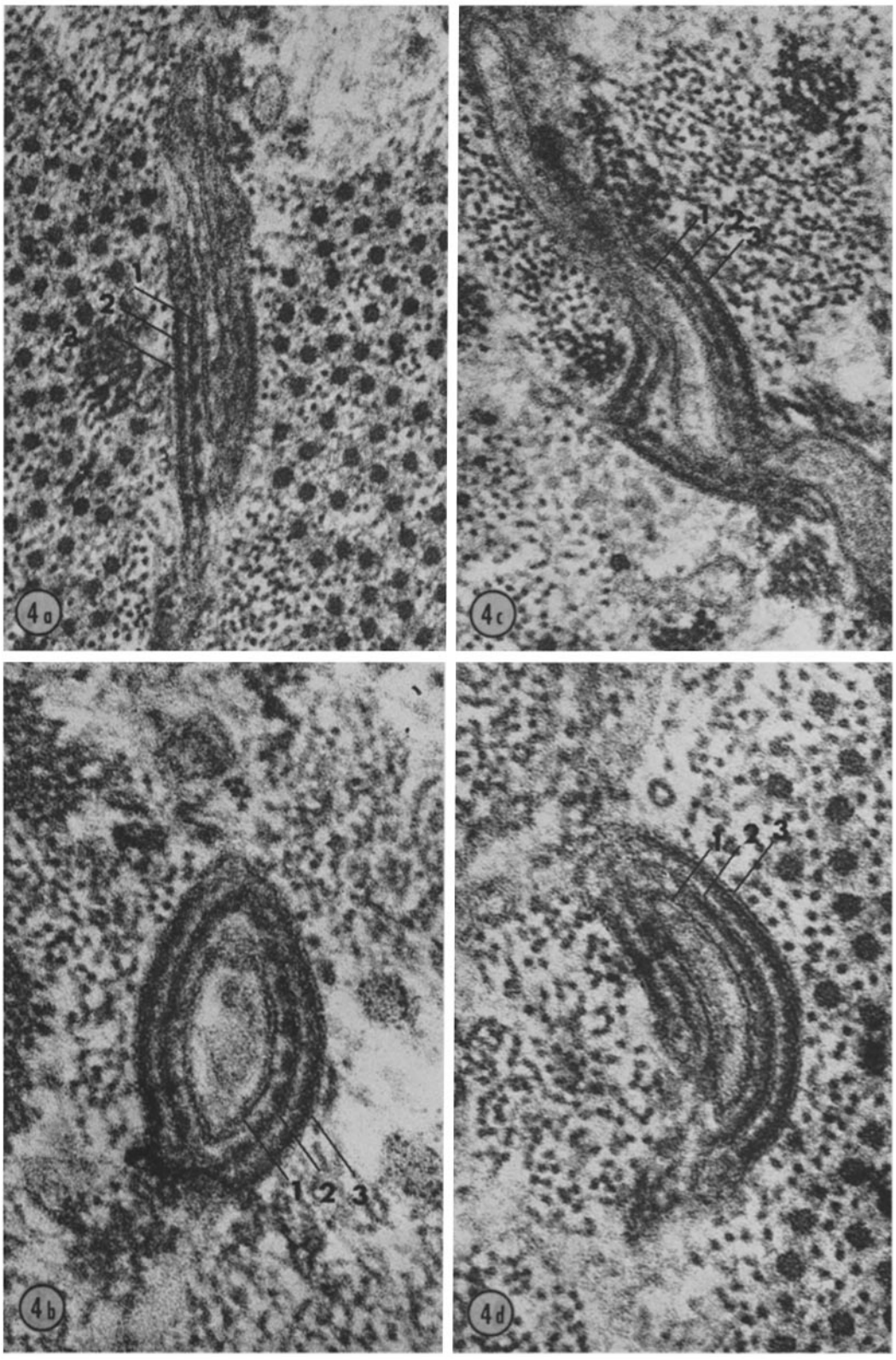
Structure and Significance of Dyads

The triad as an entity was first described by Porter and Palade (7) in vertebrate cross-striated muscle, and its counterpart, the dyad, was subsequently found in invertebrate cross-striated muscle as well (e.g., ref. 13). Similar cisternal structures in close apposition to the plasma membrane and T-tubules were later identified as dyads in obliquely striated muscles (10, 12), and in the present paper it is shown that in *Ascaris* fibers these complexes do, indeed, exhibit a structural specialization equivalent to that present in cross-striated muscles.

Dyads and triads are probably sites at which terminal cisternae of the sarcoplasmic reticulum are "coupled" to the plasma membrane. Signals from the plasma membrane presumably trigger the release of calcium ions from either the terminal cisternae themselves or from the whole SR into the sarcoplasm, and the consequent increase in calcium concentration within the myofibrils in turn brings about contraction (6). *Ascaris* muscle is distinctive in that the sarcoplasmic reticulum is virtually nonexistent except for the very components that form the dyads. However, this striking paucity of SR undoubtedly reflects only the slow speed of *Ascaris* muscle and does not necessarily imply any qualitative difference in the role of the SR of this muscle.

Structural specialization is visible in two places at the dyads of *Ascaris* muscle: between the cisternal membrane and plasma membrane and between the two cisternal membranes. In both instances the specialization appears to consist of a dense material

FIGURE 4 Dyads postfixed with osmium tetroxide. In all cases the T-tubule membrane is labeled *1*; the immediately adjacent cisternal membrane is labeled *2*, and the deeper cisternal membrane is labeled *3*. *a*. Peg-like densities extend from membrane #2 towards membrane #1. Membrane #3 appears thickened. × 140,000. *b*. Peg-like densities occupy most but not all of the width of the gap between membranes #2 and #1. Membrane #3 appears trilaminate and has a layer of dense material applied to its luminal surface. × 210,000. *c*. The dense material on the luminal surface of membrane #3 is serrated. × 140,000. *d*. Unit membrane structure is clearly visible in all three membranes. The dense layer on the luminal surface of membrane #3 is serrated in one region. × 210,000.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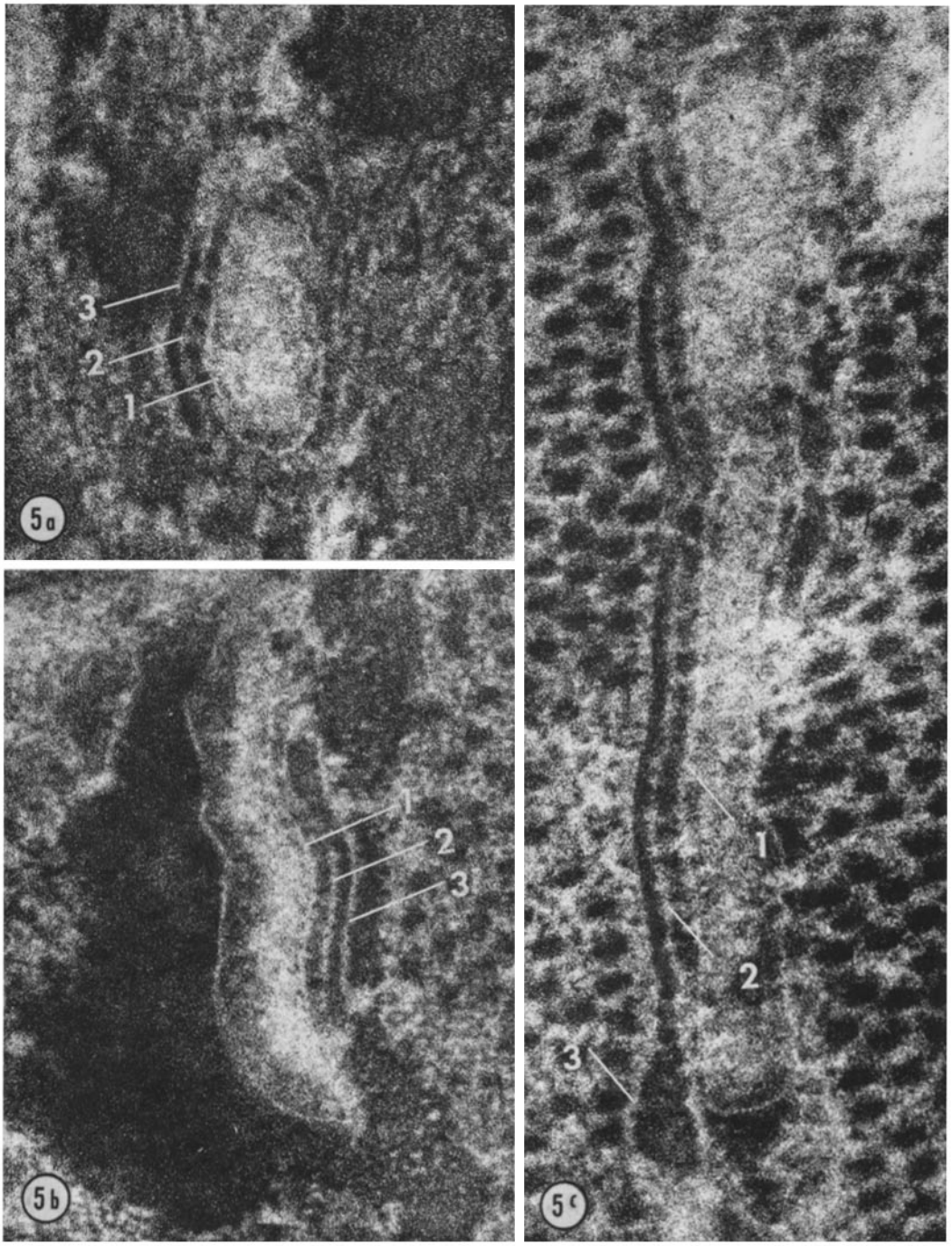

FIGURE 5 Dyads postfixed with phosphotungstic acid. The membranous components of the dyads, which are numbered the same way here as in Fig. 4, appear as white lines. *a*. The subsarcolemmal lamina between membranes #1 and #2 consists of peg-shaped densities. Compare with Fig. 4 *b*. × 190,000. *b*. A T-tubule is flanked by a dyad on the right and a dense body on the left. Another small dense body adjoins membrane #3 of the dyad and delineates it sharply. × 150,000. *c*. The intracisternal lamina is seen as a continuous dense line which is serrated along part of its length and which ends at the bottom of the figure in a bulbous expansion. × 195,000.

interposed between unit membranes which are not themselves morphologically specialized. This material is of unknown composition but its affinity for both osmium tetroxide and phosphotungstic acid plus its resistance to extraction by alcohol prior to PTA treatment are consistent with the presence in it of a substantial protein (1) and/or polysaccharide (5) component.

The significance of the dense material in either location can only be guessed at. The subsarcolemmal lamina bears a slight resemblance to some of the densities that appear intracellularly at desmosomes (3) and may therefore signify adhesion between the sarcolemma and SR sac. Depending on its properties, this material could also serve as a dielectric between the plates of a capacitor. Capacity coupling at dyads has been suggested previously (12). Its periodic disposition is also reminiscent of the structure of septate desmosomes and suggests the possibility of low resistance electrical coupling at dyads (cf. 15). With regard to the intracisternal lamina, Essner et al. (2) have reported the presence of a nucleoside phosphatase in the terminal cisternae of cardiac muscle cells; therefore, the dense material could represent the location of this or some other enzyme associated with ion transport. In view of the evidence that the SR of cross-striated muscle is capable of taking up and releasing calcium, this material could also represent a calcium-sequestering substance.

Unfortunately, there is at present no evidence to support any of these hypothesized roles; therefore the dyad must be regarded, for the time being, as a unique and undoubtedly important structure which occurs in obliquely striated as well as cross-striated muscle and whose function has not yet been elucidated. The peg-shaped densities between the cisternal and T-tubule membranes have now been described in three widely different phyla, which suggests that they are not merely incidental components of the complex. Whether the intracisternal dense material at *Ascaris* dyads also has a counterpart in the sarcoplasmic reticulum of other muscles remains to be seen.

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