THE FINE STRUCTURE OF THE VENTRAL INTERSEGMENTAL ABDOMINAL MUSCLES OF THE INSECT *RHODNIUS PROLIXUS* DURING THE MOLTING CYCLE

II. Muscle Changes in Preparation for Molting

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ABSTRACT

The development of the ventral intersegmental abdominal muscles of *Rhodnius prolixus* is triggered by feeding. The early muscle (1 day after feeding) contains essentially nonstriated fibrils. However, in cross-sections, areas indicating early I bands, Z lines, and A bands can be recognized. Interdigitating thick and thin myofilaments do not assemble into a precise lattice until sometime between 4 and 5 days after feeding. As development continues, the number of fibrils increases, the region corresponding to the Z line increases in density, and the fibrils contain more recognizable striations. The newly formed fibrils broaden as myofilaments are added peripherally. At all stages throughout development, the ratio of thin to thick myofilaments is always 6:1. The formation of fibrils in the abdominal muscles of *Rhodnius* is different from that in chick embryo skeletal muscle. The major differences are that at all stages in *Rhodnius* there are (1) a constant ratio of thin to thick myofilaments, and (2) detectable Z-line material. Other findings in *Rhodnius* suggest (1) that fusion of mononucleated cells with the multinucleated muscle cell occurs, (2) that microtubules develop in the tendon cell concomitantly with development of myofibrils in the associated muscle cell, and (3) that filaments 55A in diameter aggregate into microtubules.

INTRODUCTION

Using the light microscope, Wigglesworth (25) described the formation of the ventral intersegmental abdominal muscles of the insect, *Rhodnius prolixus*, for each molting cycle. He showed that these muscles involuted after each molt. After involution, only the sarcolemma, some cytoplasm, and the nuclei remained in the muscle. Development was triggered by a blood meal. The cell volume gradually increased, the number of nuclei increased, some fibrils appeared, and, at a later time, striations were visible. The muscle gradually broadened until it was fully developed at molting. This system is ideal for studying ultrastructural details in the development of muscle. Similar changes in other insect abdominal muscles have been studied by numerous workers using the light microscope. Crossley (4) gives an excellent review of the literature. The main object of this paper is to describe the ultrastructure of the development of these muscles in preparation for molting.
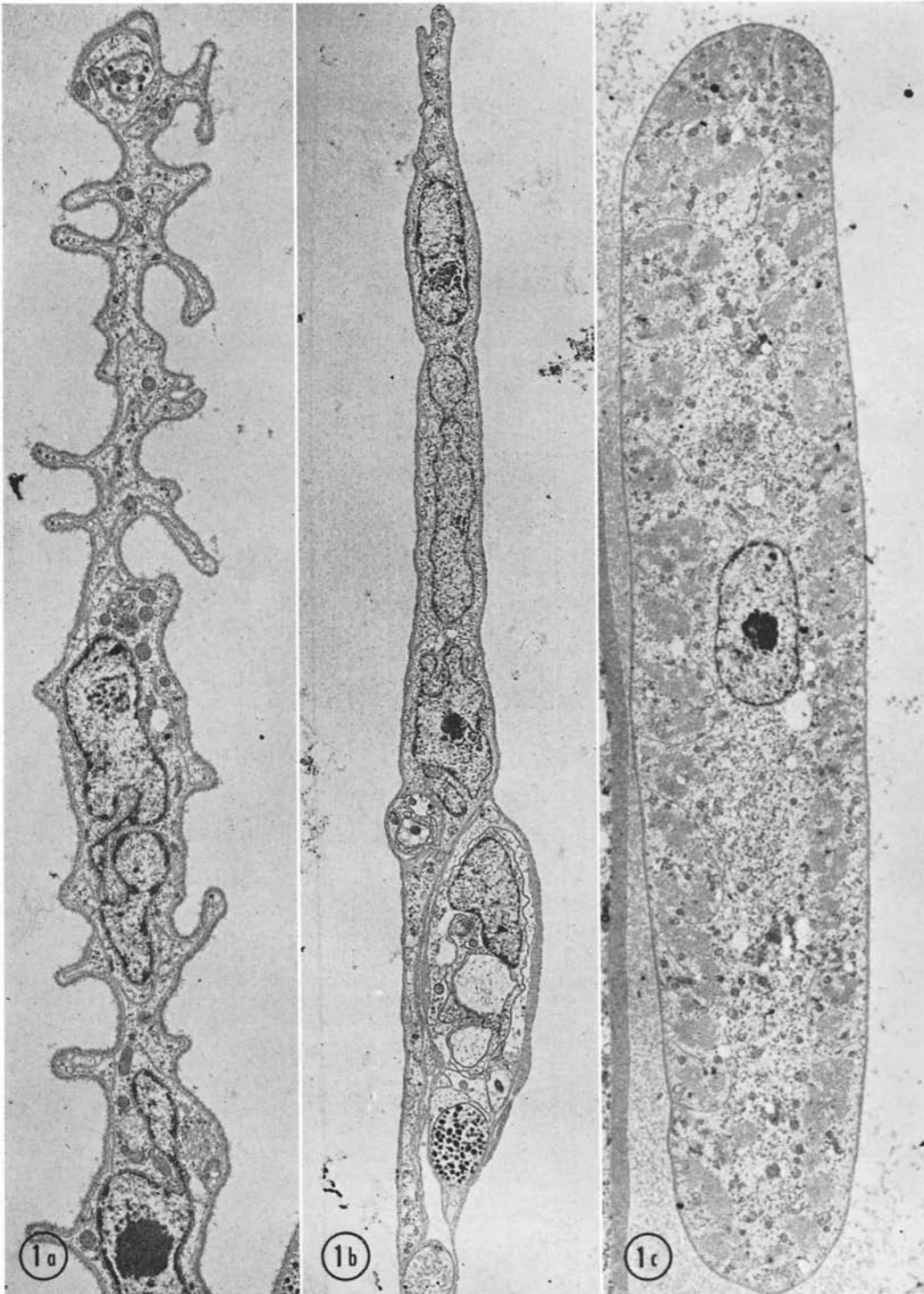
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FIGURE 1 Transverse sections through the ventral intersegmental abdominal muscles: *a*, resting muscle, × 8,000; *b*, 1 day after feeding, × 6,250; *c*, 5 days after feeding, × 5,250.

Two theories have been proposed for the increase in number of nuclei in developing muscle cells: (1) that muscle cells become multinucleated by amitoses (24, 17, 7), and (2) that multinucleated muscle cells result from fusion of myoblasts (14, 11, 13, 3, 20, 15). Evidence is presented which suggests that fusion of mononucleated cells is responsible for the increase in nuclei of the developing ventral intersegmental abdominal muscles of Rhodnius prolixus.

The ultrastructure of the ventral nerve cord and the neuromuscular junction of the ventral intersegmental abdominal muscle in molting fourth stage larvae of Rhodnius prolixus have been described (21). With the light microscope, the histology of the nerve supply to these muscles was described (26), and a study was made of the nerve endings during the molting cycle (25). The nerve supply has little effect on the developing muscles since: (1) muscle growth occurs normally even after section of the nerves (25), and (2) the nerve supply persists apparently unchanged throughout the cycle of involution (25). In this investigation, no changes in the ultrastructure of the neuromuscular junction are observed during the developmental cycle. The Schwann cells surrounding nerve axons going to developing ventral intersegmental abdominal muscles are interesting in their ultrastructure, in that their cytoplasm contains clusters of longitudinally aligned microtubules. Filaments, 55 A in diameter, lie parallel and next to the clusters of microtubules. Evidence suggesting the aggregation of these filaments into microtubules is presented.

MATERIALS AND METHODS

Fourth stage larvae (of the insects, Rhodnius prolixus) were starved at least 30 days prior to feeding. Feeding triggered development of the ventral intersegmental abdominal muscle. Some insects were sacrificed before feeding (resting muscle), while others were fed and then sacrificed at 24-hr intervals for 8 days. The ventral intersegmental abdominal muscles were fixed, embedded, and sectioned as described in the accompanying paper (21). All sections were stained with phosphotungstic acid, uranyl acetate, and lead citrate (18), in that order.

RESULTS

Muscle Changes in Preparation for Molting

Throughout development, the ventral intersegmental abdominal muscles of Rhodnius are

always multinucleated and surrounded with an extracellular amorphous coat. The muscles have a flat shape (Fig. 1 a- c). They always have fiber clefts and T-system clefts (as defined in accompanying paper [21]), Golgi apparatus, free ribosomes, polyribosomes, sarcoplasmic reticulum, triads and diads, and neuromuscular junctions. Mitochondria and microtubules are always longitudinally aligned along the long axis of the muscle. Hemidesmosomes are common throughout development; desmosomes are also present, but they are seen less frequently.

The early muscle cell contains poorly formed fibrils which are aligned along the long axis of the cell. Although these fibrils are nonstriated, a slight density probably representing early Z-line material appears at intervals along the fibrils, a finding indicating the presence of poorly formed sarcomeres. In cross-sections (Figs. 7 and 9), areas indicating early I bands, Z lines, and A bands can be recognized: they appear as patches of loosely packed thin myofilaments (I-band area), densely packed thin myofilaments in an amorphous matrix (Z-line area), and patches of loosely arranged interdigitating thick and thin myofilaments (A-band area). In longitudinal sections, it can be seen that at every stage in development at which they are observed, the thick myofilaments, along some part of their length, interdigitate with thin myofilaments. Interdigitating thick and thin myofilament are not seen to assemble into a precise lattice until 5 days after feeding (Fig. 10). At this time, the fibrils contain more recognizable stria-

TABLE I

Ratio of Thin to Thick Myofilaments in the Developing Ventral Intersegmental Muscle

Developmental stage	No. thin filaments counted	No. thick filaments counted	Ratio of thin to thick filaments
2 Days after Feeding	1,714	291	5.9:1
4 Days after feeding	1,575	259	6.0:1
5 Days after feeding	1,856	305	6.0:1
15 Days after feeding (at molting)*	5,069	841	6.0:1

* This data was obtained from material presented in the accompanying paper (21).

tions in longitudinal sections. As development continues (8 days), the striations become better developed (Fig. 11). These striations, however, are not yet as clear as those seen in the muscle at molting (21). In general, the early fibrils are peripherally located in the muscle (Fig. 1 c), and the ribosomes are heavily concentrated in the center of the muscle.

At all stages of development, the ratio of thin to thick myofilaments is 6:1 (Table I). The total mumber of myofilaments, T-system clefts, diads, and triads increases during development. Some ribosomes are sometimes seen on the membranes of the sarcoplasmic reticulum component of the diads (Fig. 7). Occasionally, the sarcoplasmic reticulum component extends a considerable distance away from the T-system clefts (Fig. 9).

Myofilaments are not randomly dispersed in the cytoplasm of this muscle. Filments can be seen associated with desmosomes, hemidesmosomes, microtubules, or easily identifiable early myofibrils. Only rarely can nonaligned, isolated filaments be seen in the center of the muscle among the ribosomes. The six types of filaments found in the developing ventral intersegmental abdominal muscles are: (1) Desmosomal tonofilaments (55 A in diameter) which represent one of the two types of filaments (Fig. 2 a) seen attached to the dense homogeneous layer of the desmosomes and hemidesmosomes. The tonofilaments seen in the developing muscles have the same diameter as those seen at molting (21). (2) Filaments, approximately 55 A in diameter, which are always clearly asso-
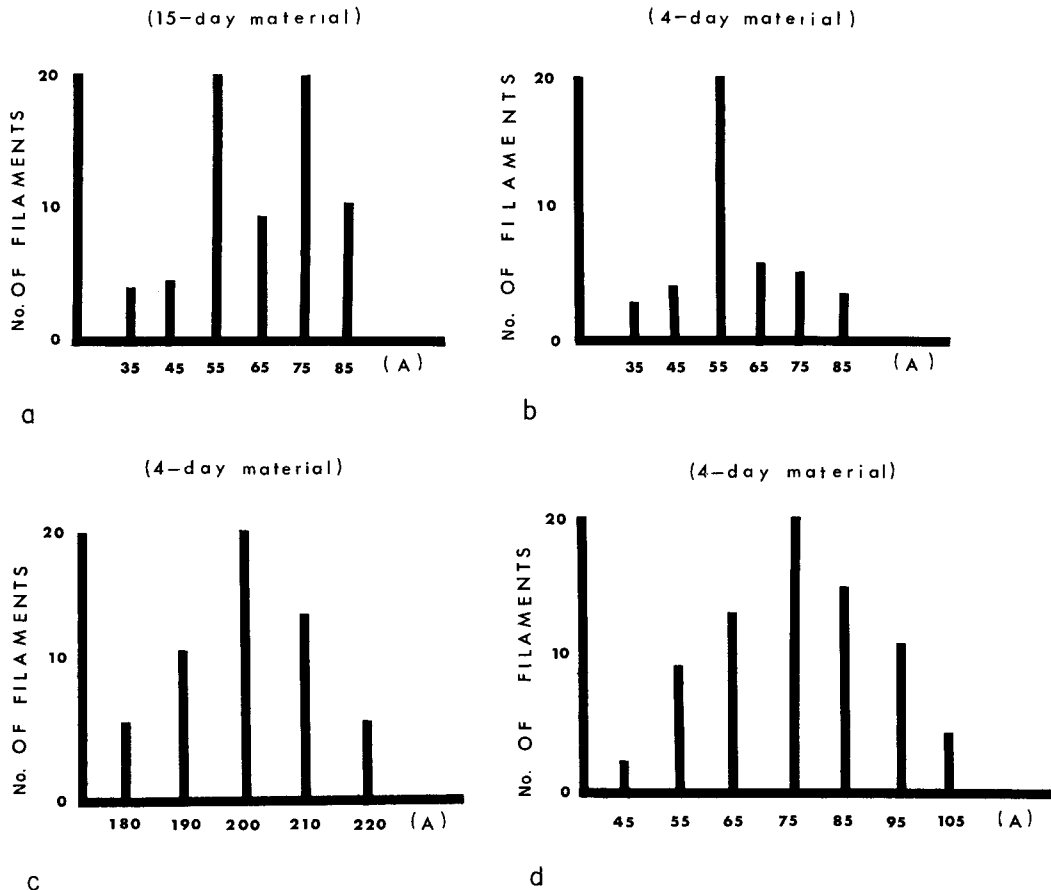

FIGURE 2 Size distribution (diameter) of filaments seen in the developing ventral intersegmental abdominal muscles. a, Diameter of filaments in desmosomes and hemidesmosomes. b, Diameter of filaments intermingling with microtubules. c, Diameter of thick myofilaments. d, Diameter of thin myofilaments. The data in Fig. 2 a was obtained from material presented in the accompanying paper (21).

ciated with microtubules and are oriented parallel to them. These show a very narrow size distribution around 55 A (Fig. 2 b). It is difficult to see these filaments in longitudinal sections through the developing muscles, and they are never observed in the muscles at molting (21). They can be clearly identified as filaments by following them in sequential cross-sections through the muscle for at least 3 or 4 μ (Fig. 3). 55-A filaments are also seen lying parallel to clusters of microtubules in the cytoplasm of the tendon (Fig. 17) and Schwann (Figs 21 and 22) cells. (3) Thick myofilaments (Fig. 9) which are approximately 200 A in diameter. These show a distribution in size from approximately 180 to 220 A (Fig. 2 c). These filaments are identified as part of the clearly recognizable early fibril. Thick myofilaments seen in developing muscle cells have the same diameter as those seen at molting (21). (4) Thin myofilaments (Fig. 9) which are approximately 75 A in diameter. They vary from approximately 45 to 105 A in diameter, with a maximum number of them at 75 A (Fig 2 d). These are always found interdigitating with the thick myofilaments of the fibrils and, therefore, are recognizable as thin myofilaments. At the end of a fibril, the thin myofilaments attach to the dense homogeneous layer of the desmosomes and hemidesmosomes along with the tonofilaments. (5) Filaments approximately 55 A in diameter which are seen only in the resting muscle and are oriented parallel to the long axis of the muscle (Fig. 5). (6) Filaments approximately 45 A in diameter which are occasionally seen in dividing mononucleated cells (presumptive myoblasts) (Fig. 15).

STARVED INSECT (RESTING MUSCLE): The resting muscle has a highly convoluted plasma membrane which sometimes acquires the profile of a nearby nucleus (Fig. 4). Nuclei are highly lobulated. Warren and Porter (23) described bundles of filaments (50–70 A in diameter) in the resting muscle. Bundles of filaments (approximately 55 A in diameter) similar to those described by Warren and Porter (23) as 50–70 A in diameter are seen (Fig. 5). Tonofilaments (55 A in diameter) are seen associated with desmosomes and hemidesmosomes; they are most easily seen at the myotendonal junction (Fig. 17). Thin and thick myofilaments have never been observed in the resting muscle.

1 DAY AFTER FEEDING: The plasma mem-

brane of the muscle cell is less convoluted. As described by Warren and Porter (23), the bundles of filaments (50–70 A in diameter) seen in the resting muscle are not seen 1 day after feeding; however, thick and thin myofilaments are seen. Fig. 6 is a cross-section of the muscle 1 day after feeding. Both the thick (200 A in diameter) and thin (75 A in diameter) myofilaments are aligned parallel to the long axis of the muscle. In cross-sections, the thick and thin myofilaments are seen to be grouped together, but are more or less randomly arranged within the groups. At this stage, few microtubules or T-system clefts are present in the cytoplasm. Mitochondria are much smaller than those seen in the muscle at molting (21) and are always aligned parallel to the long axis of the muscle.

2 DAYS AFTER FEEDING: The plasma membrane of the muscle cell is no longer convoluted. The number of mitochondria, microtubules, and myofilaments has increased. Longitudinally aligned thick and thin myofilaments interdigitate and are still randomly arranged in groups as seen in cross-section (Fig. 7). Fig. 7 also shows early fibrils consisting of areas representing early I bands, A bands, and Z lines. Areas representing early Z line can also be seen in the longitudinal section in Fig. 8. Only rarely, nonaligned isolated filaments (arrow, Fig. 8) can be seen in the center of the muscle among the ribosomes.

4 DAYS AFTER FEEDING: The multinucleated muscle has increased in size by 4 days after feeding. The cytoplasm is now heavily packed with early fibrils. Mitochondria have increased in number and length. Most nuclei are no longer lobulated. In cross-sections of 4-day material (Fig. 9), the bundles of thick and thin myofilaments still do not show a definite pattern of aggregation.

5 DAYS AFTER FEEDING: The muscle further increases in size with the appearance of more myofilaments, microtubules, and mitochondria. Lobulated nuclei are never seen at this stage. Cross-sections of 5-day material show thick and thin myofilaments associated for the first time in a regular lattice (compare Fig. 10 with Fig. 9). This lattice is identical to that seen in the muscle at molting (21). Longitudinally aligned microtubules occur in the cytoplasm and are sometimes seen in spaces occurring within the fibrils in cross-section; they are never found to substitute for a thick myofilament in the lattice. Although a lattice is present
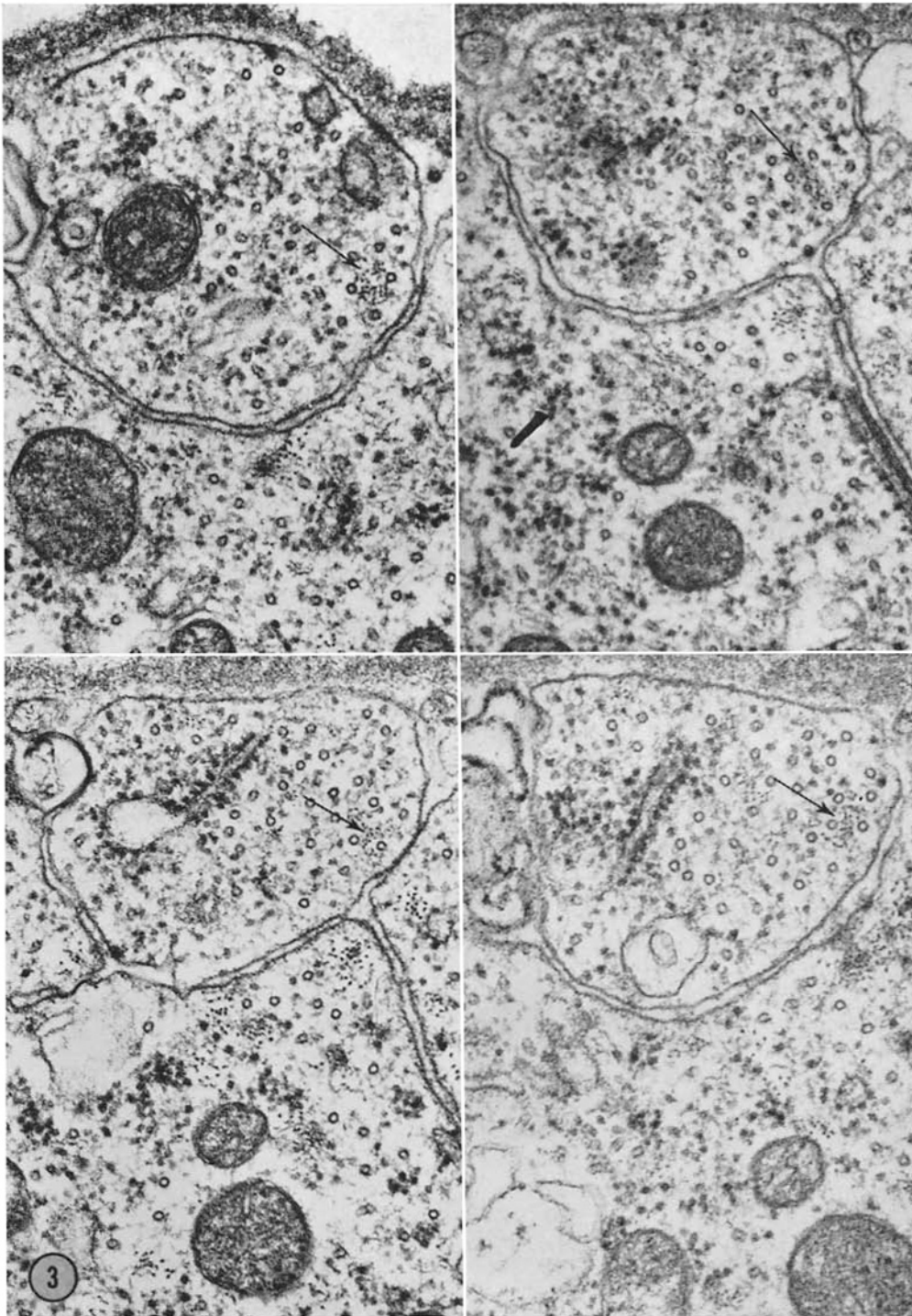
FIGURE 3 Sequential transverse sections through the ventral intersegmental abdominal muscle 4 days after feeding showing that the 55-A dots (arrows) associated with microtubules are actually filaments. The filaments lie parallel to the clusters of microtubules. × 60,000.
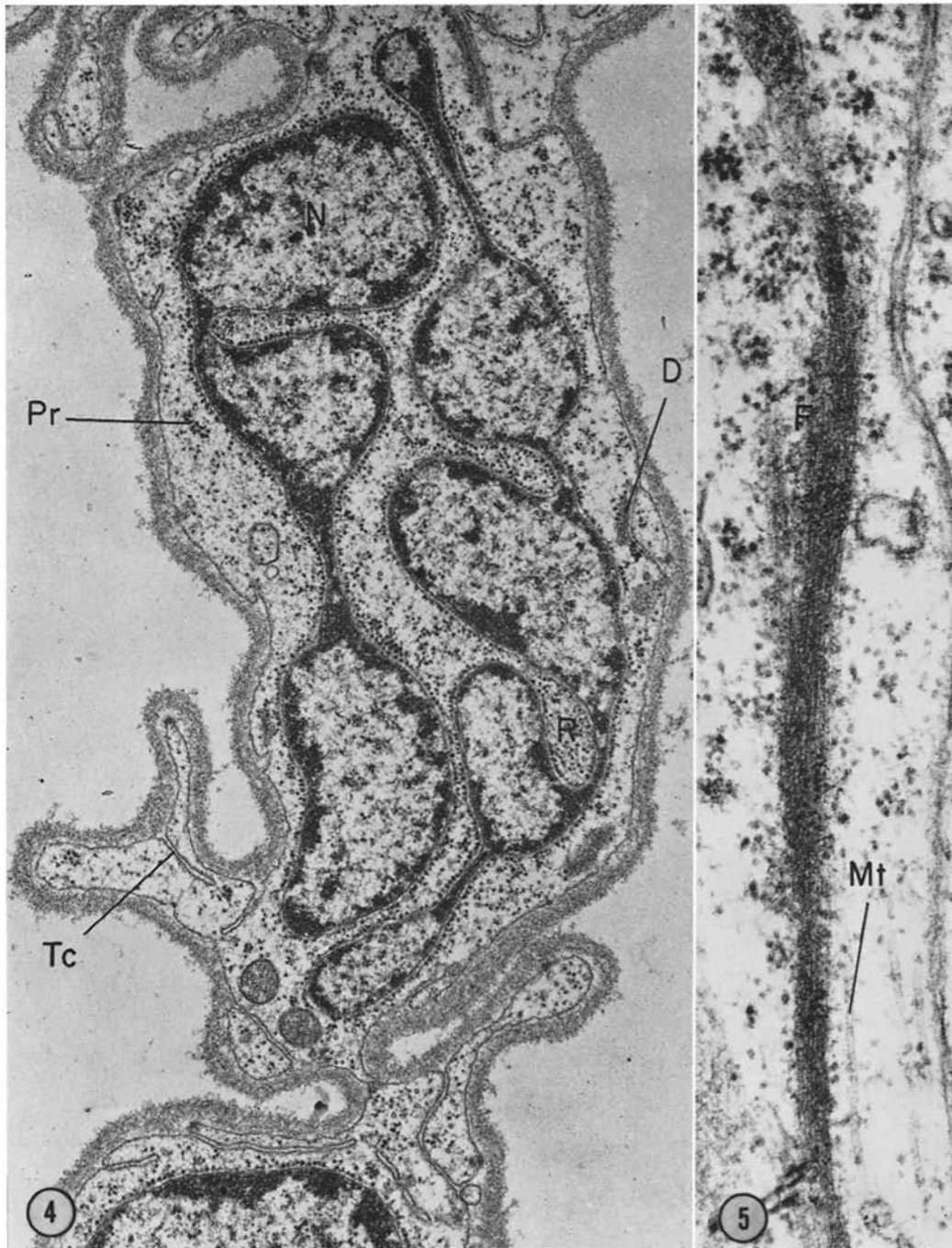
FIGURE 4 Transverse section through the resting ventral intersegmental abdominal muscle. T-system clefts (*Tc*), diads (*D*), ribosomes (*R*), polyribosomes (*Pr*), and lobulated nuclei (*N*) are seen. Myofilaments have not been observed. × 25,000.

FIGURE 5 Longitudinal section through the resting ventral intersegmental abdominal muscle showing longitudinally aligned microtubles (*Mt*) and filaments approximately 55 A in diameter (*F*). × 41,000.
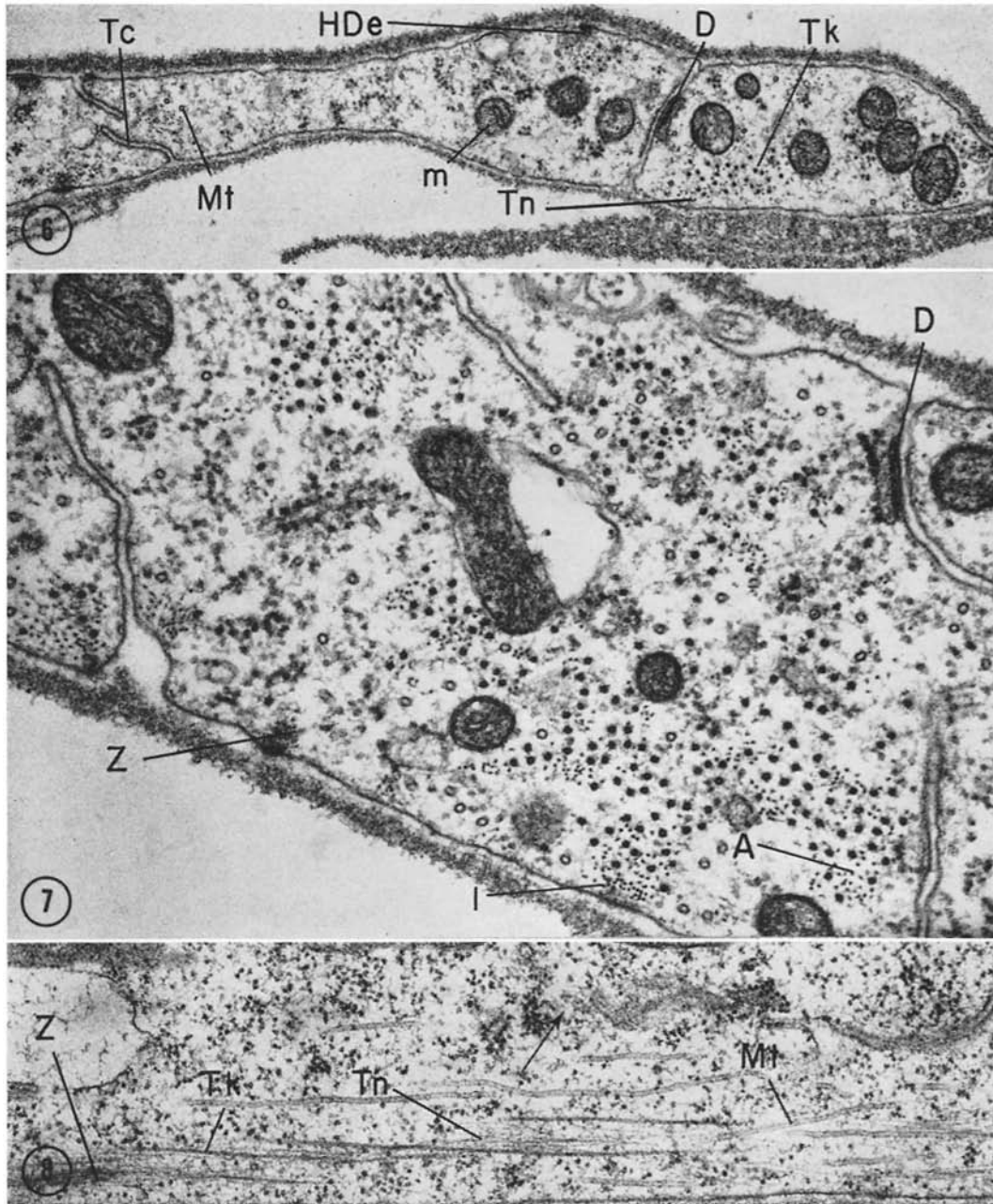
FIGURE 6   Transverse section through the ventral intersegmental abdominal muscle 1 day after feeding. Thin (Tn) and thick (Tk) myofilaments are longitudinally aligned and lie in clusters. Mitochondria (m) and microbubules (Mt) are also longitudinally aligned. Hemidesmosome (HDe); diad (D); and T-system cleft (Tc). $\times$ 26,000.

FIGURE 7   Transverse section through the ventral intersegmental abdominal muscle 2 days after feeding, showing early fibrils. The fibrils consist of areas representing early I bands (I), Z lines (Z), and A bands (A). Diad (D). $\times$ 60,000.

FIGURE 8   Longitudinal section of ventral intersegmental abdominal muscle 2 days after feeding. Occasionally nonaligned, isolated filaments (arrow) are seen in the center of the muscle among the ribosomes. Area indicating early Z line (Z). Thick myofilament (Tk); thin myofilament (Tn); microtubule (Mt). $\times$ 32,500.
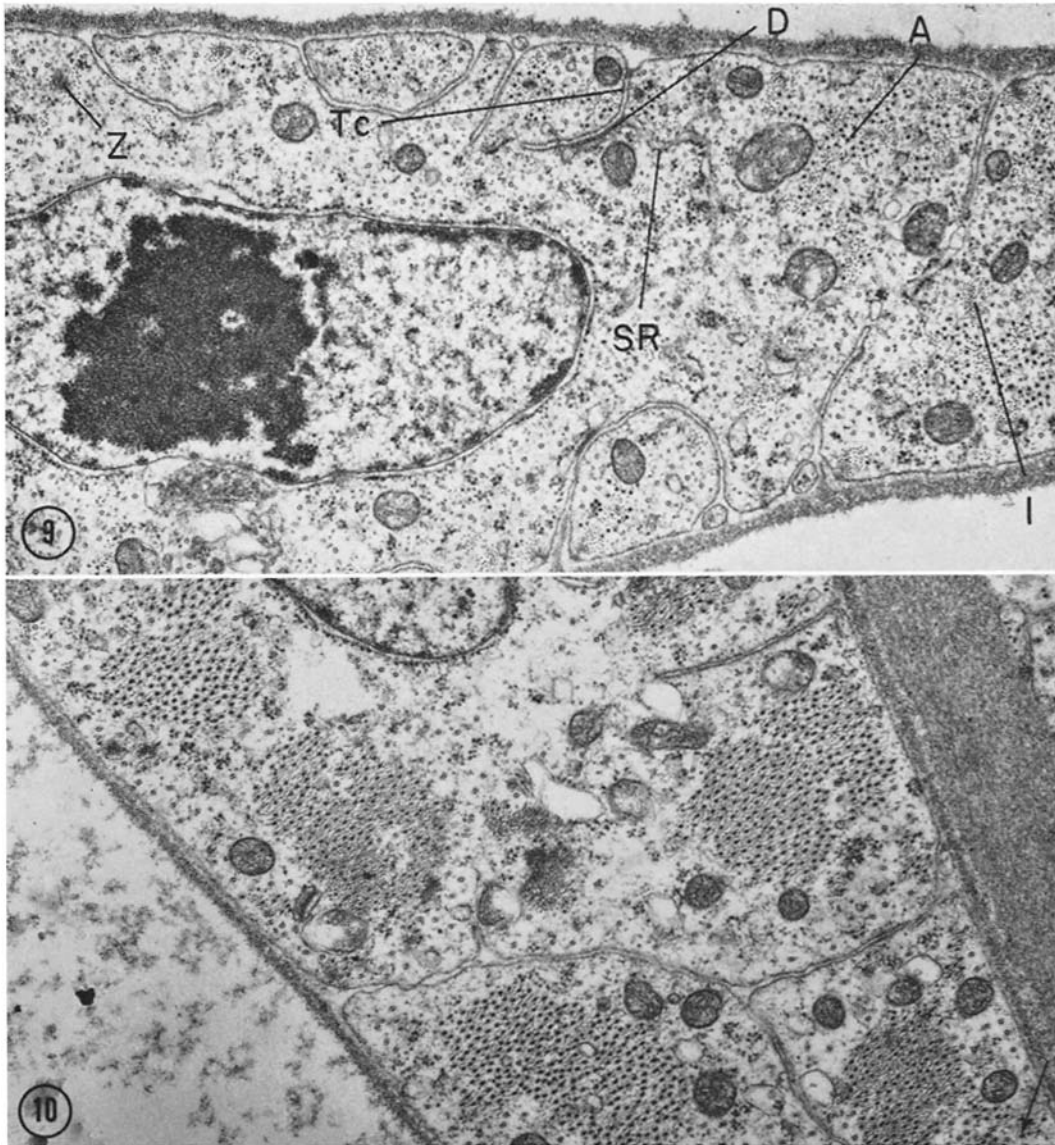
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FIGURE 9 Transverse section through the ventral intersegmental abdominal muscle 4 days after feeding. Areas representing early I bands (*I*), A bands (*A*), and Z lines (*Z*) are seen. Occasionally, the sarcoplasmic reticulum (*SR*) component of the diad (*D*) extends some distance from the T-system clefts (*Tc*). × 23,750.

FIGURE 10 Transverse section through the ventral intersegmental abdominal muscle 5 days after feeding. Thin and thick myofilaments aggregate to form a lattice structure. × 24,000.

in cross-sections, the cross-striated pattern in longitudinal sections is still not as definite as in the adult.

8 DAYS AFTER FEEDING: All structures seen in previous stages are seen at this stage. The alignment of myofilaments to give a striated fibril has improved (Fig. 11), but striations are not yet as clear as in the muscle at molting (21). There is an increased density of the developing Z line in the center of the I-band area.
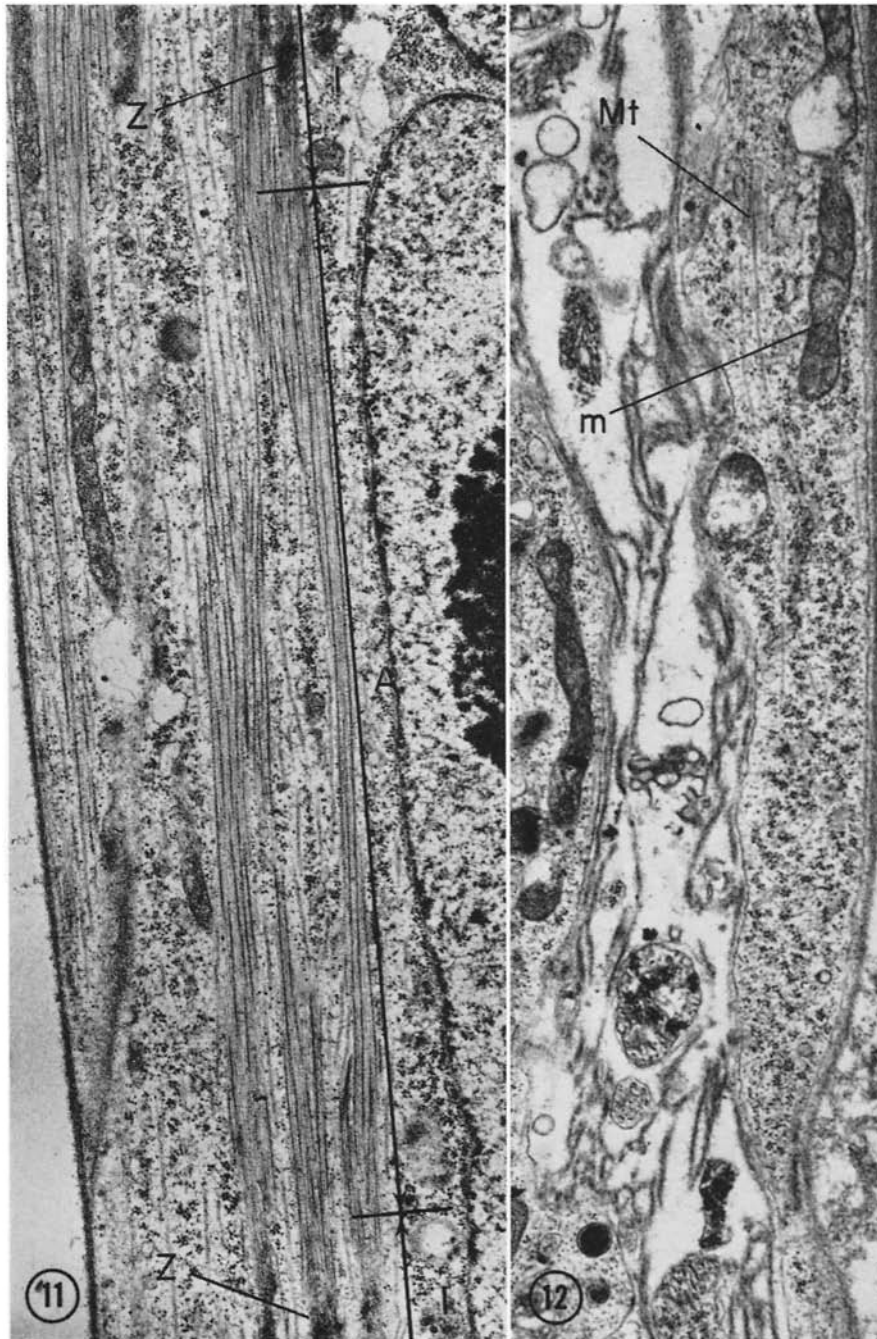
FIGURE 11   Longitudinal section through the ventral intersegmental abdominal muscle 8 days after feeding. The visibility of the striations has improved, but they are not yet as clear as in the mature muscle (15 days after feeding). A band (A); I band (I); Z line (Z). × 16,500.

FIGURE 12   Longitudinal section through the ventral intersegmental abdominal muscle 4 days after feeding showing the elongated portion of a mononucleated cell. Mitochondria (m) and microtubule clusters (Mt) are longitudinally aligned. × 20,000.

Multinucleation

An attempt was made to obtain information concerning the mechanism by which the number of nuclei increases during the development of the ventral intersegmental abdominal muscle. Material obtained 4 days after feeding was used.

Mononucleated cells are seen at the edges of the muscles (Fig. 12 and 14). They lie within fiber clefts and are identified as mononucleated cells by examining sequential sections through the muscle. Close apposition of the mononucleated cells with the muscle occurs, excluding the extracellular amorphous coat in the areas of contact (Fig. 14). Some mononucleated cells are spindle shaped and

the elongated portion of the cell is oriented parallel to the long axis of the muscle (Fig. 12). The micro-tubules and mitochondria are aligned along the long axis of the elongated mononucleated cells. In addition, mononucleated cells have many free ribosomes, rough endoplasmic reticulum, and centrioles. No myofilaments, diads and triads, and T-system clefts are ever seen. Fig. 13 is a cross-section through two mononucleated cells which appear to have fused (arrows). Sequential sections show that the cross-sectional area of the cyto-plasmic continuity between the cells in Fig. 13 is small. Fig. 14 shows a mononucleated cell in mitosis. This cell is in close contact with and al-
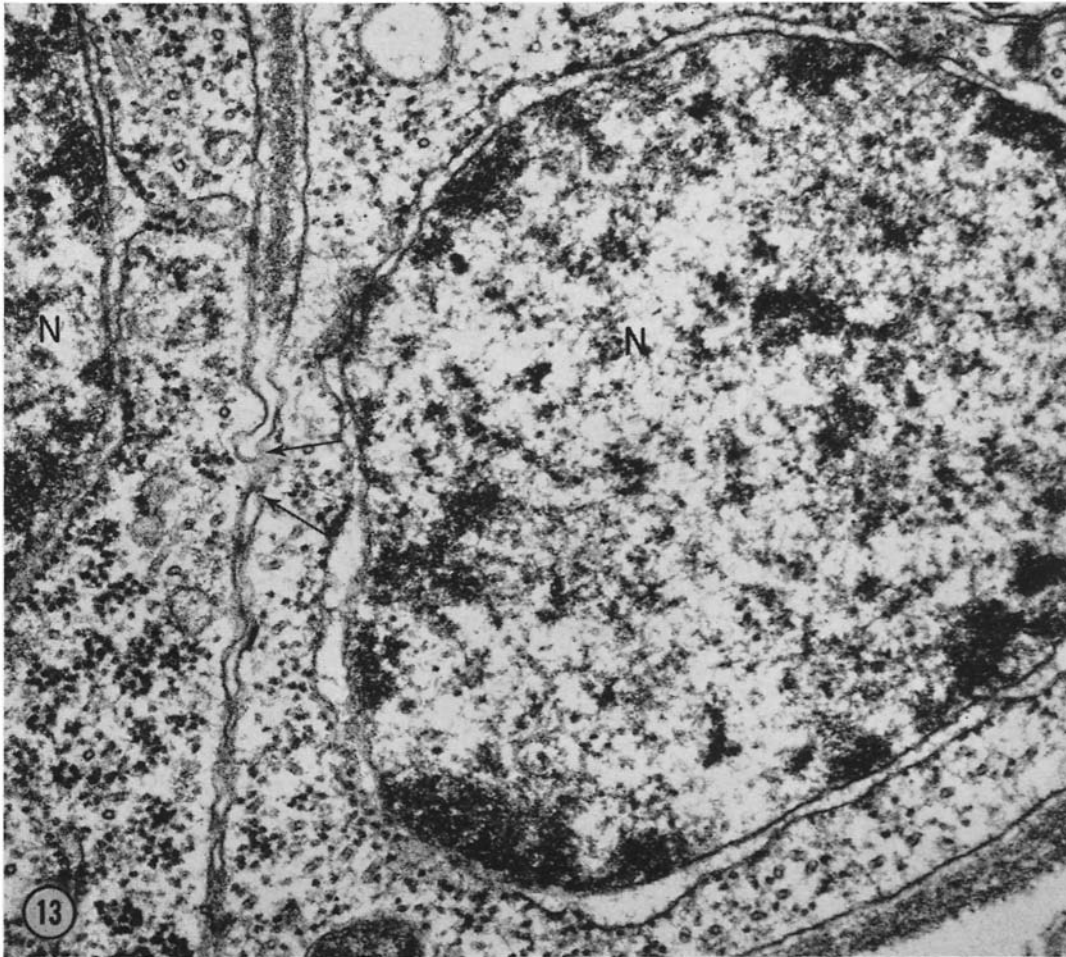

FIGURE 13 Transverse section through the ventral intersegmental abdominal muscle 4 days after feeding showing two mononucleated cells. Their plasma membranes have fused (arrows). Nucleus (N). × 35,000.
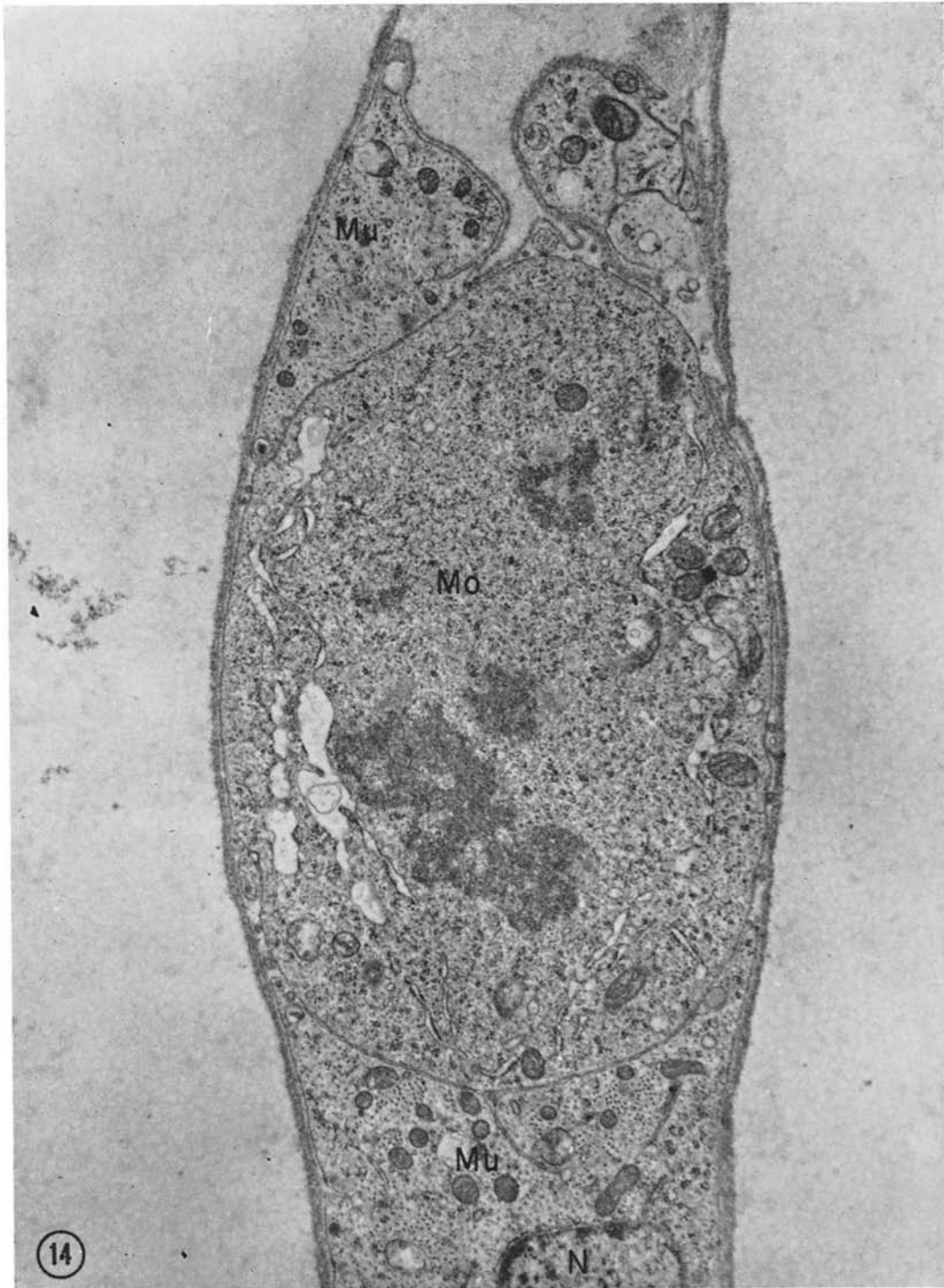
FIGURE 14 Transverse section through the ventral intersegmental abdominal muscle 4 days after feeding showing a mononucleated cell (*Mo*) in mitosis. The mitotic cell is in close contact with and almost completely surrounded by the multinucleated muscle cell (*Mu*). Nucleus (*N*). × 16,000.

most completely surrounded by the multinucleated muscle cell. Both cells are enclosed by the extracellular amorphous coat. The mitotic cell contains free ribosomes, rough endoplasmic reticulum, Golgi apparatus, and unaligned mitochondria. Filaments, approximately 45 A in diameter, are occasionally seen (Fig. 15). No myofilaments, diads and triads, and T-system clefts can be seen in this cell.

Tergosternal Muscle During the Molting Cycle

The abdomen of *Rhodnius prolixus* consists of 10 or 11 segments. A typical segment consists of a tergum or dorsal plate, a sternum or ventral plate, and a lateral area of membrane connecting the tergum and sternum. The various segments of the abdomen are connected by a series of muscular bands which maintain body form. In most abdominal segments in *Rhodnius*, the tergite and sternite of the same segment are connected by perpendicular tergosternal muscles. The tergosternal muscles

do not degenerate after molting. The muscle at 2 days after feeding is unchanged in shape and size from the muscle of the unfed insect. Cross-sections of 2-day material (Fig. 16) show that the muscle is packed with well organized myofilaments.

Tendon Cells During the Molting Cycle

TENDON CELL OF THE VENTRAL INTERSEGMENTAL MUSCLE: Fig. 17 is a cross-section through the myotendonal junction of the resting ventral intersegmental abdominal muscle. The bottom portion of the micrograph is muscle. The structure of the muscle at this stage of development has been described above. The top portion of the micrograph is the tendon cell. The tendon cell contains a lobulated nucleus, ribosomes, and clusters of microtubules. Filaments, 55 A in diameter, lie among and parallel to the microtubules. Desmosomes are present at the junction of muscle and tendon cell. Desmosomal tonofilaments 55 A in diameter (Fig. 17, *Dt*) are present in both cells.
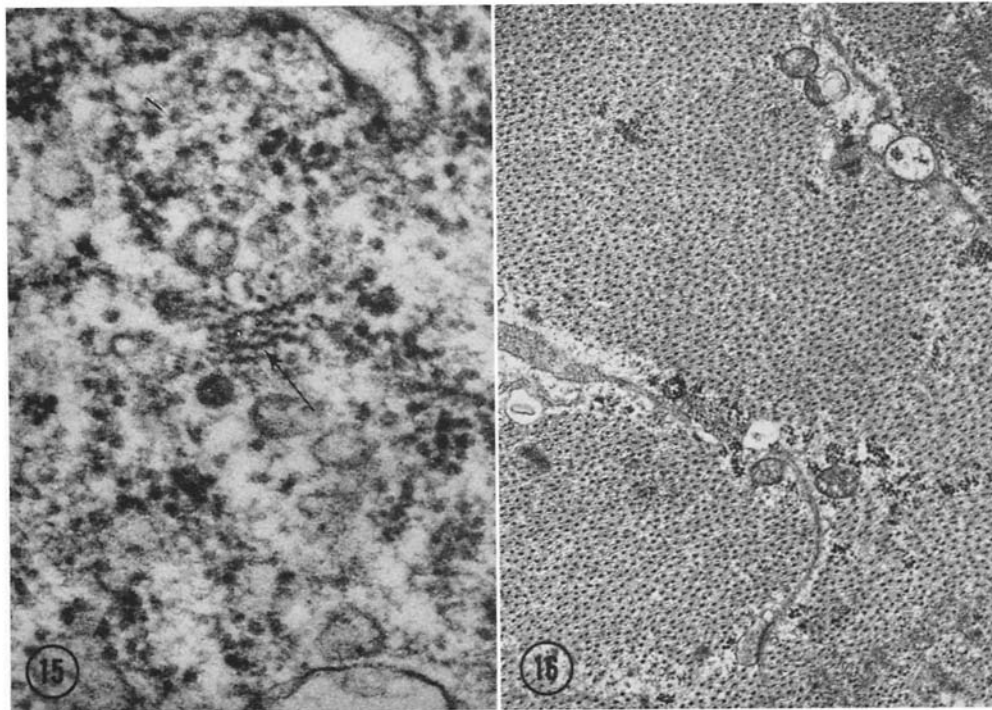

FIGURE 15   Transverse section through the mitotic cell seen in Fig. 14 showing filaments (arrow) approximately 45 A in diameter. × 97,500.

FIGURE 16   Transverse section through the tergosternal muscle 2 days after feeding. Note that the myofilaments are organized into a lattice. × 21,000.
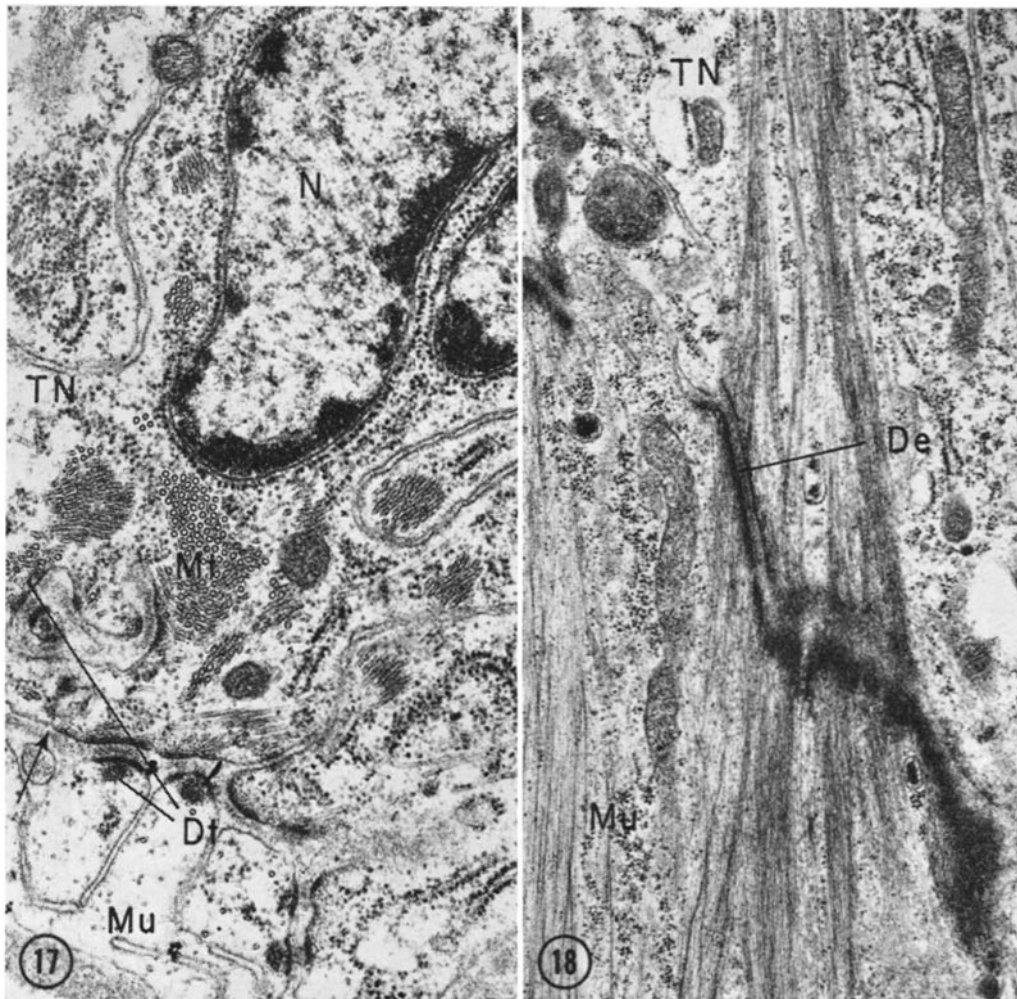
FIGURE 17 Transverse section through the myotendonal junction of the resting ventral intersegmental abdominal muscle. Desmosomal tonofilaments (*Dt*) (55 A in diameter) are seen in the cytoplasm of the muscle (*Mu*) and of the tendon cell (*TN*). An extracellular amorphous material (arrow) is seen between the muscle and tendon cell. Note the clusters of microtubules (*Mt*) in the tendon cell. Nucleus (*N*). × 31,000.

FIGURE 18 Longitudinal section through the myotendonal junction of the ventral intersegmental abdominal muscle 8 days after feeding. Note the desmosome at the junction of tendon (*TN*) and muscle (*Mu*) cell. *De*, desmosome. × 23,000.

Fig. 18 is a longitudinal section of the myotendonal junction of the ventral intersegmental abdominal muscle 8 days after feeding. The bottom portion of the micrograph is muscle; the top portion of the micrograph is the tendon cell. A desmosome is seen at the myotendonal junction. The gap between the parallel plasma membranes is bisected by a dense line. On the muscle side, longitudinally aligned thin myofilaments attach to the dense homogeneous layer of the desmosome and extend into the A band. On the side of the tendon cell, longitudinally aligned microtubules attach to the dense homogeneous layer of the desmosome.

TENDON CELL OF THE TERGOSTERNAL MUSCLE: As described earlier, the tergosternal muscles are

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densely packed with myofilaments during the intermolt period. A parallel observation is that the tendon cell of this muscle is densely packed with microtubules during this period. Fig 19 is a cross-section of the myotendonal junction of the tergosternal muscle 2 days after feeding. Fig. 20 is a cross-section of the tendon-cuticle junction of the tergosternal muscle 2 days after feeding. Longitudinally aligned microtubules attach to the dense homogeneous material of the hemidesmosome on the cytoplasmic side of the finger-like invaginations of the plasma membrane. Cuticular material lies within the finger-like invaginations. This tendon cell has a nucleus, few ribosomes, and many microtubules.

Peripheral Nervous System

As was described in the accompanying paper (21), the ventral nerve cord sends muscular nerve branches to the segmentally arranged abdominal muscles. Transverse sections of muscular nerve branches lying adjacent to developing ventral intersegmental abdominal muscles at 1, 2, and 4 days after feeding were studied. In all three of the developmental stages studied, the muscular nerve branches are covered with an amorphous coat. The Schwann cell cytoplasm contains mitochondria, some rough endoplasmic reticulum, ribosomes, and clusters of longitudinally oriented microtubules. In addition, 55-A filaments can be seen intermingled with the microtubules (Figs. 21 and 22). Sequential sections are used to identify the filaments. These filaments have the same diameter as the wall of the microtubule (Fig. 22).

The axons (Figs. 21 and 23) have mitochondria, microtubules (neuro-, or axotubules), occasional axofilaments (neurofilaments) 100 A in diameter, and vesicles containing dense material. A higher magnification of one of these axons is shown in Fig. 23. The 55-A wall of the microtubules is disorganized in some cases and can be seen to consist of 55-A filaments. 55-A filaments on rare occasions can be seen in the center of the microtubules (see asterisk).

DISCUSSION

Muscle Changes in Preparation for Molting

Various investigators have studied the appearance of striations in early forming fibrils (6, 8, 24, 22, 10, 12, 19, 1). The evidence suggested that the early fibril may: (1) develop striations from an early nonstriated fibril, or (2) show striations in the first fibrils seen. The early fibrils in the developing ventral intersegmental abdominal muscles are nonstriated and are longitudinally aligned in the muscle cell. Although longitudinal sections of these early fibrils are nonstriated, there are indications of poorly formed I bands, Z lines, and A bands. In cross-sections, the early fibrils appear as patches of loosely packed thin myofilaments (I-band area), densely packed thin myofilaments in an amorphous matrix (Z-line area), and patches of loosely arranged interdigitating thick and thin myofilaments (A-band area) (Figs. 7 and 9). As development continues, the number of myofilaments increases, the region corresponding to the Z line increases in density, and the fibrils contain more recognizable striations. The newly formed fibrils broaden as myofilaments are added peripherally. Fibrils are attached to the hemidesmosomes at the level corresponding to the Z-line area. Attachment of fibrils to the plasma membrane in this way probably accounts for peripheral location of the fibrils in the developing muscle.

The development of fibrils in the ventral intersegmental abdominal muscles of *Rhodnius* is different from that in embryonic chick muscle. Thin myofilaments are present in great excess in developing chick muscle cells before the first appearance of thick myofilaments, and polyribosomes appear simultaneously with the thick myofilaments (1). In *Rhodnius*, both filament types are always present in the same ratio throughout development (Table I) and polyribosomes are always seen. Although polyribosomes are present in the starved insect (resting muscle), thick myofilaments are never seen. The polyribosomes at this stage may be inactive, and activation may be caused by the hormones released as a result of feeding. Throughout development in the chick, the thick myofilaments are aggregated with thin myofilaments in a lattice structure (1). In *Rhodnius*, groups of myofilaments were observed with random organization within the groups until 5 days after feeding when a precise lattice is formed. The very clear change in the organization of the myofilaments within bundles which occurs between 4 and 5 days after feeding (Fig. 9 and 10) suggest that an abrupt change has occurred in the interactions between the myofilaments. At present, no clues are available to explain the reason for this change. In the chick, the Z material appears only at a particular stage after the nonstriated fibril is formed (1),
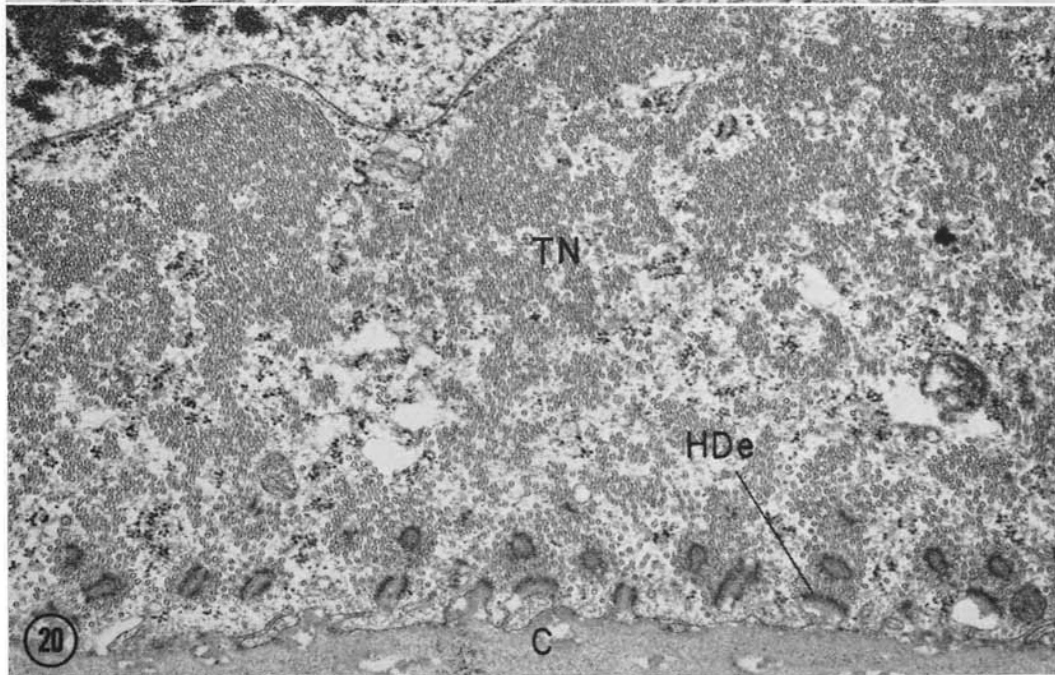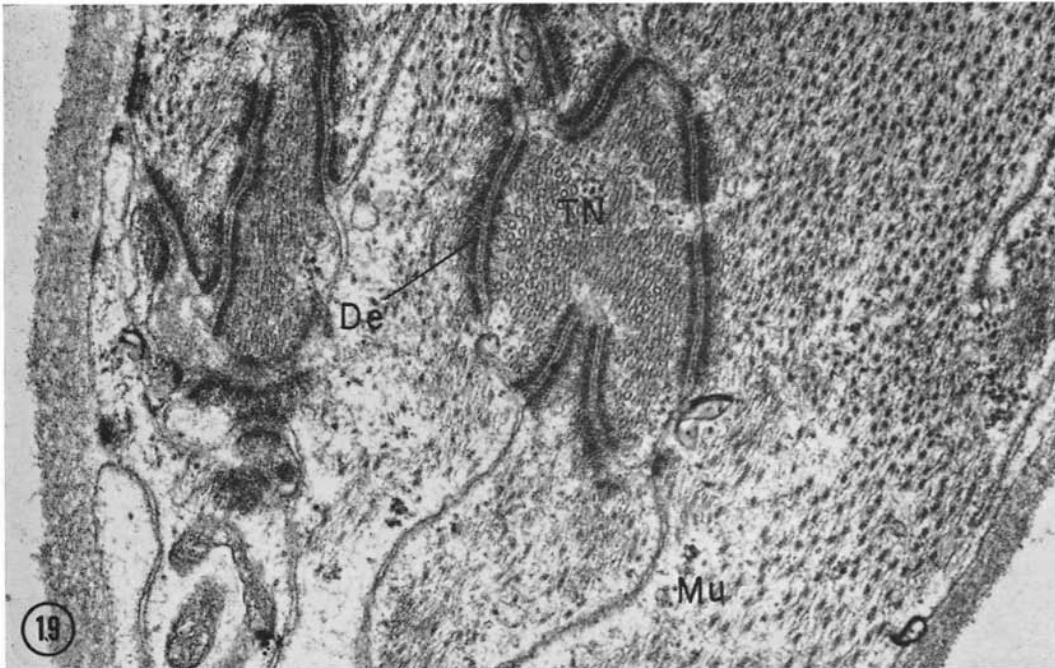
FIGURE 19 Transverse section through the myotendonal junction of the tergosternal muscle 2 days after feeding. Tendon cell (*TN*); muscle (*Mu*); desmosome (*De*). × 37,500.

FIGURE 20 Transverse section through the tendon-cuticle junction of the tergosternal muscle 2 days after feeding. The tendon cell (*TN*) is packed with microtubules. Hemidesmosome (*HDe*); cuticle (*C*). × 25,000.

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where as in *Rhodnius* the Z material is present throughout development. This may be related in some way to the differences in the ratio of thin to thick myofilaments throughout development in the two muscles. Allen and Pepe (1) suggested that the excess of thin myofilaments in chick muscle resulted in maximum involvement of interacting sites which led to parallel alignment of thin and thick myofilaments in a precise relationship within bundles. Such a mechanism is not possible in *Rhodnius* since a constant ratio of thin to thick myofilaments is present throughout development. In this case, the presence of Z-line material may play some role in the aligment of filaments.

Filaments with various diameters are seen. A particular filament cannot be identified on the basis of diameter alone. For instance: (*1*) Desmosomal tonofilaments and microtubule filaments have the same diameter (55 A). However, they are related to entirely different structures, suggesting that they may be composed of entirely different proteins. Both types of filaments are seen in many cells. (*2*) The diameter of thin myofilaments changes during development. Some of the thin myofilaments of the early myofibril measure up to approximately 105 A in diameter (Fig. 2 *d*, 4-day material). At molting (15-day material), they are never larger than 75 A in diameter (21). This suggests that there may be a redistribution of material between the filaments, leading to a decrease in diameter of the thin myofilaments during development. Recent evidence obtained by Pepe[1] suggested that a similar change occurs in chick embryonic muscle. He found that in material fixed in glutaraldehyde and followed by osmium tetroxide fixation, the background thin myofilaments were made up of a dense central core 70 A in diameter and a less dense surrounding material, to give a 100-A filament. On aggregation into the early fibrils, the myofilament diameter was always approximately 70 A. In material fixed in osmium tetroxide alone, both the background myofilaments and those in the early fibril had a diameter of 70 A although the background myofilaments showed some material attached at irregular intervals along their length (1). Heuson-Stiennon (9) suggested that during development of rat skeletal muscle the background myofilaments increase in diameter to form thick myofilaments. This is in contradiction to the results of Allen and Pepe (1) who showed, using negative staining of homogenized chick

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[1] F. A. Pepe. Unpublished data.

somites, that the first thick filaments observed were of the same diameter as adult thick filaments. (*3*) Other filaments are observed in *Rhodnius*. In dividing presumptive myoblasts, bundles of filaments 45 A in diameter are seen (Fig. 15); and in the resting muscle, bundles of filaments 55 A in diameter are seen (Fig. 5). It is difficult to identify these other than on the basis of size since they are not related to a specific structure.

Multinucleation

Two theories of multinucleation have been proposed: (*1*) that muscle cells become multinucleated by amitosis (24, 17, 7) and (*2*) that multinucleated muscle cells form from fusion of myoblasts (14, 11, 13, 3, 20, 15). Multinucleated muscle cells are present during the intermolt period in *Rhodnius prolixus* (Fig. 1 and 4). Wigglesworth (25) states that mitotic figures are seen, from 2 to 7 days after feeding, in the ventral intersegmental muscles of fourth stage instars and that this finding implies a great increase in number of muscle nuclei. In this study, "presumptive myoblasts" (proliferating mononucleated cells which have not yet synthesized myofilaments [16]) are seen in mitosis (Fig. 14). The mitotic cells are found next to, but not incorporated into, the multinucleated muscle cell. Nuclei of the multinucleated muscle cells are never seen dividing. Crossley (4), in describing transformations of the abdominal muscles of the blowfly, observed that myoblasts (i.e., presumptive myoblasts) migrated from insect histoblastic tissue to the developing muscle. In *Rhodnius*, the presumptive myoblasts may also originate from histoblastic tissue. Presumptive myoblasts are most easily found in fiber clefts at the edges of the muscle. After mitosis, these cells become elongated and oriented parallel to the long axis of the multinucleated muscle. The cytoplasm contains many ribosomes as well as longitudinally aligned mitochondria and microtubules with their associated filaments 55 A in diameter. The 55 A filaments may aggregate to form microtubules as is discussed below ("Peripheral Nervous System"). From the observations, it is concluded that some of the daughter cells of presumptive myoblasts: (*1*) become elongated (Fig. 12), (*2*) fuse with mononucleated cells (Fig. 13), and (*3*) also fuse with the multinucleated muscle cells, resulting in an increase in the number of nuclei in the multinucleated muscle cells during development.
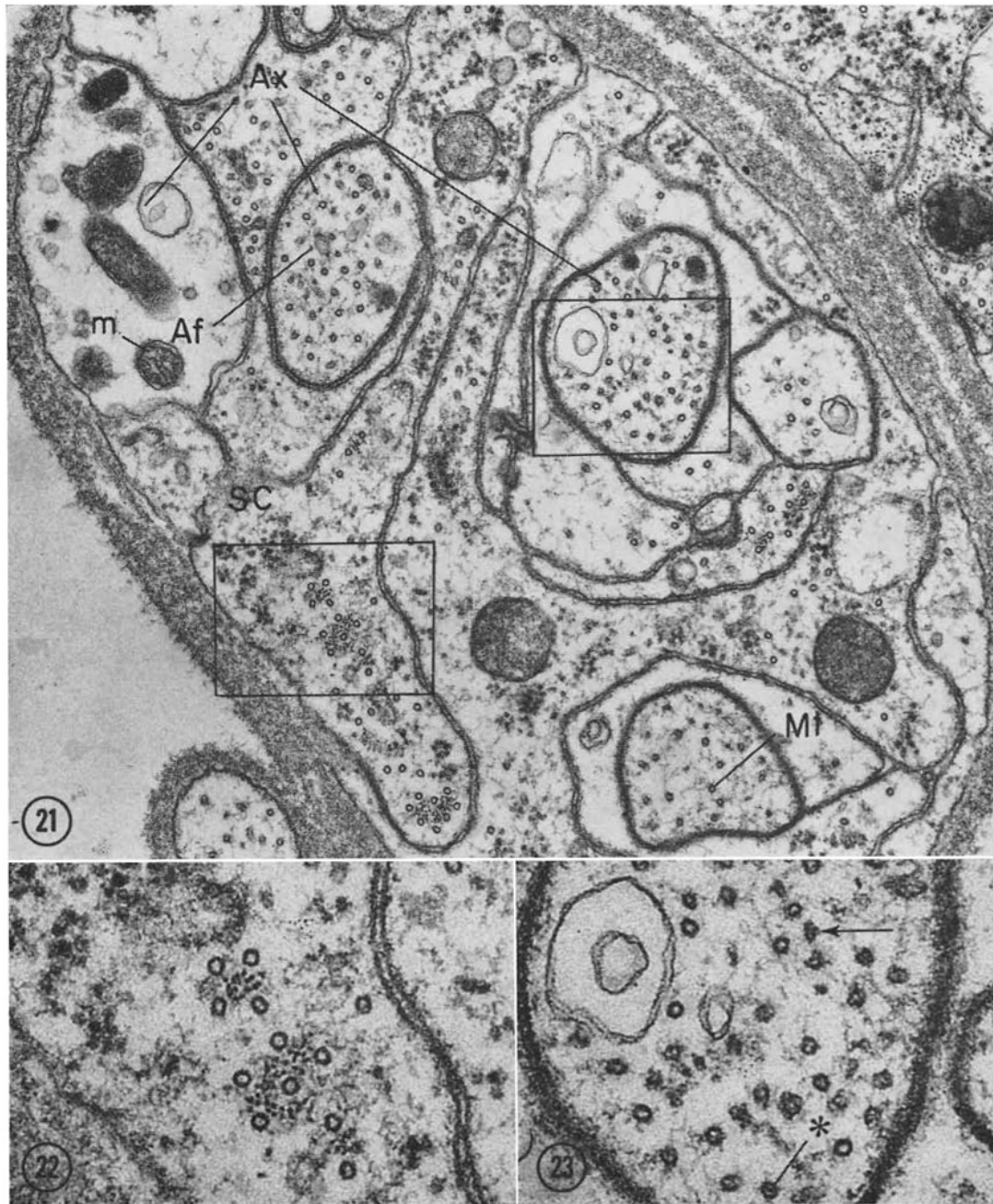
FIGURE 21 Transverse section through a nerve branch to the ventral intersegmental abdominal muscle 4 days after feeding. Axons (*Ax*) contain mitochondria (*m*), microtubules (neuro- or axo-tubules) (*Mt*), and axofilaments (*Af*) 100 A in diameter. Schwann cell (*SC*). × 42,500.

FIGURE 22 Higher magnification of the cytoplasm of the Schwann cell outlined in Fig. 21. The cytoplasm of the Schwann cell contains filaments (55 A in diameter) lying parallel and next to clusters of microtubules. The filaments have the same diameter as the wall of the microtubule. × 102,000.

FIGURE 23 Higher magnification of the axon outlined in Fig. 21. The 55-A wall of the microtubule (neuro- or axo-tubule) is disorganized (arrow). The wall is seen to consist of 55-A filaments. 55-A filaments are rarely seen in the center of the microtubules (asterisk). × 102,000.

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Tergosternal Muscle during the Molting Cycle

Tergosternal muscles do not degenerate after molting. They are packed with myofilaments even in the unfed fourth stage larvae. Cross-sections at 2 days after feeding show thin and thick myofilaments interdigitating into a well organized lattice structure (Fig. 16). From this study no evidence could be obtained concerning why the tergosternal muscles are effected minimally and the ventral intersegmental muscles are effected maximally during the molting cycle.

Tendon Cells during the Molting Cycle

The tendon cells of the mature muscles of the insects *Calliphora erythrocephala* (2) and *Rhodnius prolixus* (21) have been studied. In this investigation, changes in the tendon cells of the ventral intersegmental abdominal muscles and of the tergosternal muscles of *Rhodnius prolixus* were studied in relation to the changes in the associated muscle cells.

THE TENDON CELLS OF THE VENTRAL INTER-SEGMENTAL ABDOMINAL MUSCLE: In the starved insect, the associated muscle is in the resting state. At this time, the tendon cell contains only a few clusters of microtubules (Fig. 17). By 8 days after feeding, the muscle is partially developed. The cytoplasm of the tendon cell then contains longitudinally aligned microtubules although they are not present in very large quantities (Fig. 18). At molting, the muscle is fully developed and the associated tendon cell is densely packed with microtubules (21). From these observations it, seems that as fibrils are formed in the muscle cell, there is a concomitant formation of microtubules in the associated tendon cell.

THE TENDON CELL OF THE TERGOSTERNAL MUSCLE: At 2 days after feeding, the associated muscle cell is densely packed with myofilaments (Fig. 16) and the tendon cell is densely packed with microtubules (Fig. 20). Therefore, under conditions in which drastic changes have occurred in the ventral intersegmental abdominal muscle and its associated tendon cells, the tergosternal muscle and its associated tendon cell are unaffected. The changes occurring in the muscle cell with respect to loss or retention of myofilaments are reflected in the associated tendon cell with respect to loss or retention of microtubules.

Peripheral Nervous System

The axons and Schwann cells of the muscular nerve branches were studied. Longitudinally oriented microtubules occupy only a small amount of the cytoplasm of the Schwann cell. Clusters of 55-A filaments are aligned parallel and adjacent to the microtubules. Occasionally, the axons of these nerve branches also have 55-A filaments aggregated to form the walls of disorganized microtubules (Fig. 23). That 55-A filaments structurally resemble the substructure of the microtubule wall suggests that 55-A filaments aggregate to form microtubules.

Fawcett (5) notes that the axons of some animals have many neurofilaments and few microtubules; in other animals, the reverse is true. He suggests that axofilaments and microtubules may represent alternative modes of aggregations of the same macromolecular subunits. In *Rhodnius*, the 100-A axofilaments may represent aggregates of two or three 55-A microtubule filaments.

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