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

I. Muscle Structure at Molting

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ABSTRACT

Rhodnius prolixus, a South American insect, molts five times in its development to an adult after emerging from the egg. Each molting cycle is triggered with a blood-meal. The ventral intersegmental abdominal muscles of *Rhodnius* develop during each molting cycle and are functional at molting. The fine structure of these fully developed muscles from fourth stage larval insects is studied. They have the characteristic structure of slow muscles. They have multiple motor nerve endings, and the myofibrils are poorly defined in cross-section. Longitudinal sections show long sarcomeres (8–10 μ), irregular Z-lines, and no apparent H zones. No M line is seen. Transverse sections through the A-band region show that each hexagonally arranged thick filament is surrounded by 12 thin filaments. Two thin filaments are shared by two neighboring thick filaments. The ratio of thin to thick filaments is 6:1. This structure is related to that found in vertebrate skeletal muscle and insect flight muscle.

INTRODUCTION

Most insects molt at least three or four times in their development to adults after emerging from the egg. In some insects, 30 or more molts occur during normal development. *Rhodnius prolixus*, a South American blood-sucking insect, molts five times during the developmental phase. Each molting cycle is initiated with a blood-meal and is controlled by at least three hormones (38). The ventral intersegmental abdominal muscles of *Rhodnius* develop during each molting cycle and are functional at molting. At the time of molting, these muscles are flat bands (approximately 35 μ thick, 100 μ wide, and 1 mm long) which extend from the anterior margin of one segment to the anterior margin of the next segment. There are nine muscle-bands on each half of every ventral abdominal segment (36).

Although the ultrastructure of the ventral intersegmental abdominal muscles of *Rhodinus prolixus* is the major part of this investigation, observations have also been made on the myotendonal, tendon-cuticle, and neuromuscular junctions. The ultrastructure of the abdominal muscle myofibrils at molting is compared to that of vetebrate skeletal and insect flight muscle. Vertebrate skeletal muscles have thick and thin filaments arranged in a double hexagonal array in the A bands (13, 19). The thin filaments are located in the trigonal posi-

tion between three thick filaments. Thick and thin filaments of insect flight muscles are also arranged in a double hexagonal array (18). In this case, the thin filaments are located midway between adjacent thick filaments. In both cases, each thick filament is surrounded by six thin filaments. In the ventral intersegmental abdominal muscles of *Rhodnius prolixus*, each thick filament is surrounded by 12 thin filaments.

MATERIALS AND METHODS

The insects (*Rhodnius prolixus*[1]) were placed in glass jars topped with screened lids and kept in an incubator at 26°C. A high relative humidity was maintained by placing the jars in a water bath. At feeding time, the jars were removed from the incubator and held inverted upon the ear of a rabbit sitting in a basket. The blood-meal lasted until the insects stopped feeding. This generally took about 30 min. All five larval stages were cultured, but observations were made only on the fourth stage. At 26°C, the interval between feeding and molting is 15 days for the fourth stage larvae. Only bugs caught in the process of molting were sacrificed.

Glutaraldehyde, obtained from Union Carbide Corporation in 25% solution, was diluted to 4.3% with 0.1 M Veronal buffer, pH 7.4. The insect was immersed in the 4.3% glutaraldehyde solution and dissected under a Bausch and Lomb dissecting microscope at room temperature. The ventral abdominal wall was isolated by the method of cutting along the margins of the abdomen with iridectomy scissors. The abdominal wall was then transferred to a fresh glutaraldehyde solution and carefully cut into small fragments containing the attached muscle. The ventral abdominal wall remained in glutaraldehyde for a total of 75 min before being washed for 10 min in 0.1 M Veronal buffer, pH 7.4. The tissue was postfixed in a solution of 1% OsO_4 in 0.1 M Veronal buffer, pH 7.4, for 45 min. It was washed briefly in the Veronal buffer, dehydrated in a graded series of ethanol solutions starting with 70% ethanol, and embedded in Araldite. Sections were cut with a Porter-Blum microtome. Ribbons of sections showing gray, silver, or slightly gold interference colors were picked up on uncoated 300-mesh grids. Electron micrographs were obtained from sections which were stained with either (1) Phosphotungstic acid (PTA) followed by uranyl acetate (UrAc), (2) Uranyl acetate followed by lead citrate (25), or (3) PTA, UrAc, and lead citrate, in that order. All micrographs were obtained with a Siemens Elmiskop I.

[1] I am indebted to Dr. Jack Jones of the University of Maryland, Department of Entomology, for a colony of *Rhodnius prolixus* and for advice in maintaining the colony.

RESULTS

The ventral intersegmental abdominal muscles of *Rhodnius prolixus* are fully developed and maximal in size at molting. Cross-sections of these muscles at low magnification indicate that each muscle has a flat shape. In Fig. 1, the muscle appears to be composed of more than one myofiber separated by an extracellular space. However, these fibers have large areas of cytoplasmic continuity at different levels in the muscle. The muscle is always covered with an extracellular amorphous coat. This coat has two layers (Fig. 1). One layer forms an outer casing for the entire muscle (Fig. 1, long arrows). The other layer adheres to the surface plasma membrane (Fig. 1, short arrows). Tracheoles and nerves are generally found within the space between the two amorphous layers (Figs. 1 and 7). This space, which includes the tracheoles and nerves as well as the amorphous coat lining the surface plasma membrane, will henceforth be referred to as a fiber cleft.

Rhodnius has two distinct systems of membrane-limited components (Fig. 2 *a*). One system is similar to the T system of vertebrate skeletal muscle (6, 23), in that it is confluent with the extracellular space. The other is similar to the sarcoplasmic reticulum. In *Rhodnius*, the T system consists of (1) parallel plasma membrane sheets longitudinally oriented and separated by an approximately 200-A space (henceforth called T system clefts) (35), and (2) T tubules which branch from the T system clefts and run both transversely and longitudinally between the myofibrils (Fig. 2 *a*). The T system clefts are distinct from the fiber clefts, in that the separation between the membranes of the T system clefts is constant and the membranes never have an amorphous coat on the external surface. The sarcoplasmic reticulum in *Rhodinus* is seen as part of the triads or diads. Triads and diads are sites of intimate association between the sarcoplasmic reticulum and the T system clefts. Triads and diads of this muscle are not present at regular intervals with respect to the myofibrils. Fig. 2 *a* shows triads and diads in cross-sections; Fig. 2 *b* shows a diad in longitudinal section.

Desmosomes can be seen in this muscle (Fig. 3 *a*). They consist of parallel plasma membranes separated by an intercellular space approximately 240 A wide. This space is bisected by a slender line.

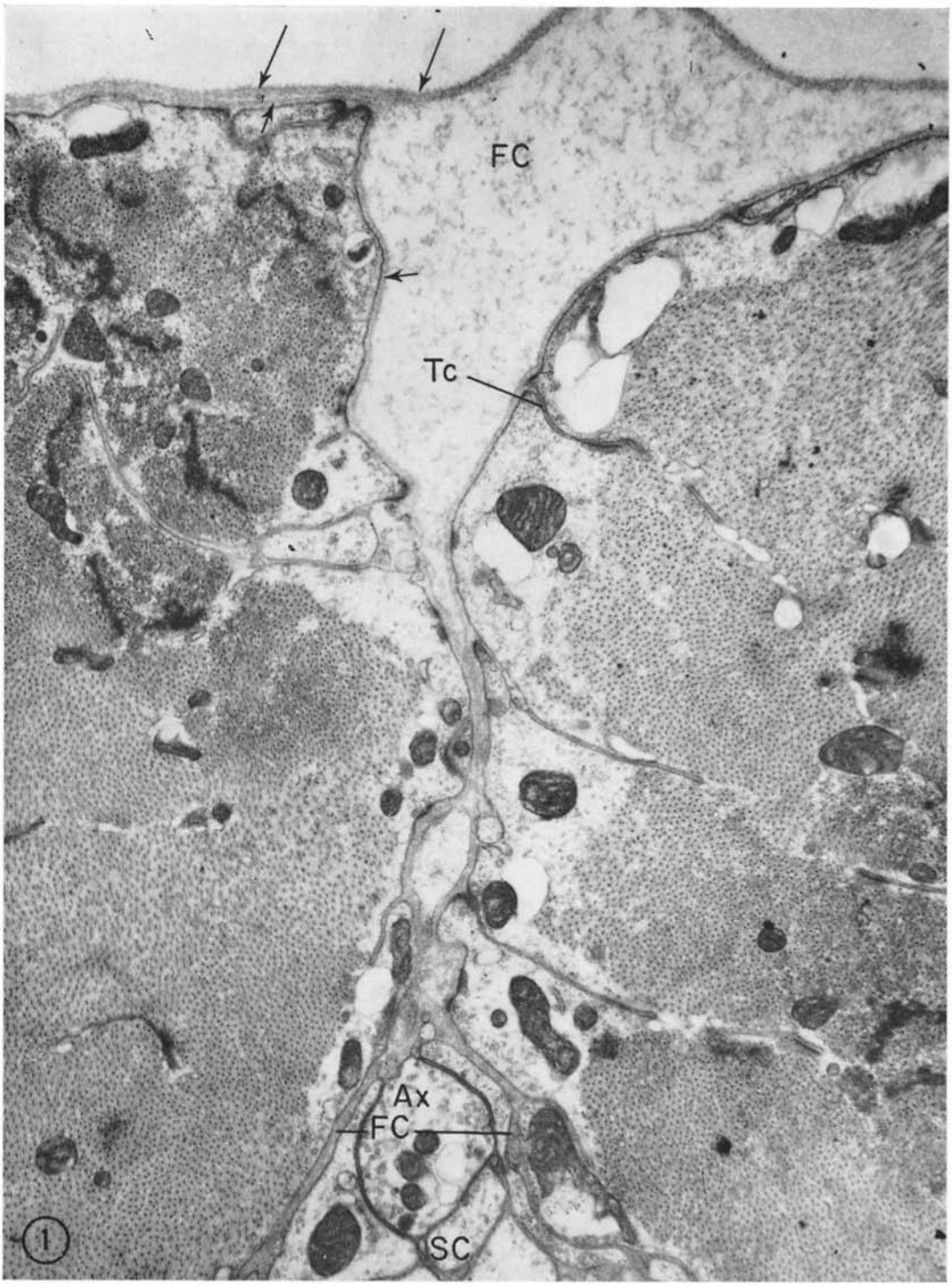

FIGURE 1 Transverse section through a fully developed ventral intersegmental abdominal muscle. One layer of the extracellular amorphous coat (long arrows) forms an outer casing for the entire muscle; the other layer (short arrows) adheres to the surface plasma membrane. Axons (*Ax*) and Schwann cells (*SC*) lie in the fiber cleft (*FC*). The membrane of the T-system cleft (*Tc*) is continuous with the surface plasma membrane. Stained with PTA and UrAc. × 20,000.

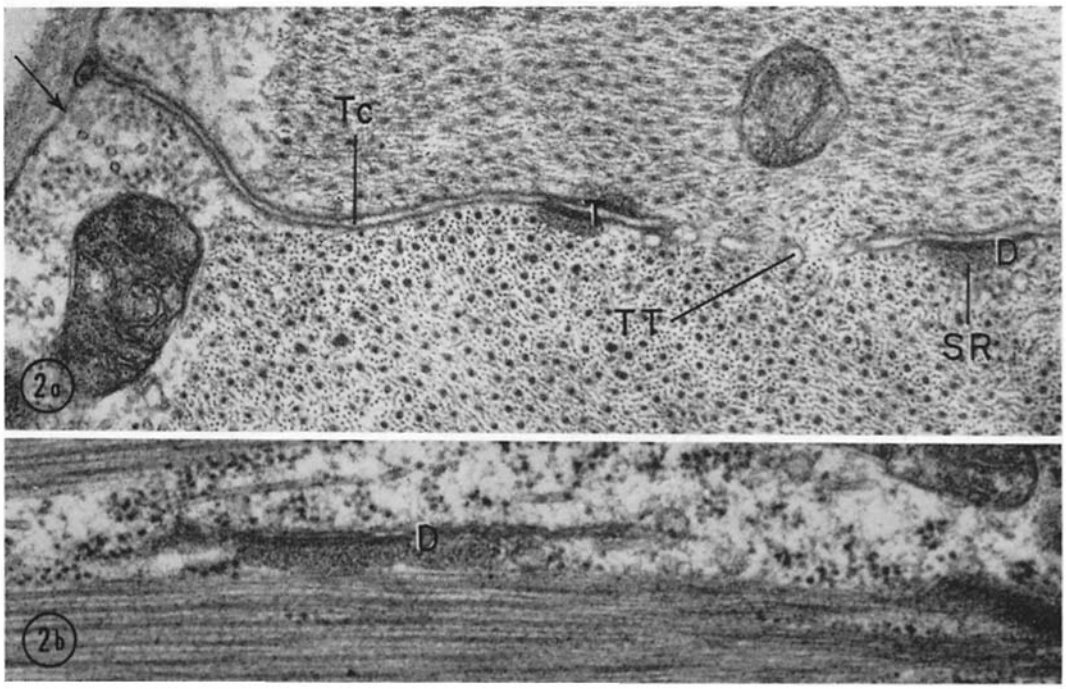

FIGURE 2 *a* Transverse section through a fully developed ventral intersegmental abdominal muscle. The membranes of the T-system clefts (*Tc*) are continuous with the surface plasma membrane (arrow). T tubules (*TT*) branch from the T-system clefts. Triads (*T*) and diads (*D*) are sites of intimate association between the sarcoplasmic reticulum and the T-system clefts. Stained with PTA and UrAc. × 50,000.

FIGURE 2 *b* Longitudinal section showing a diad (*D*). Stained with PTA and UrAc. × 55,000.

A dense homogeneous layer is closely applied to the cytoplasmic side of each plasma membrane. Desmosomes sectioned in a plane transverse to the muscle fiber were found near the opening of T system clefts to the surface plasma membrane (Fig. 3 *b*). Hemidesmosomes are also present. In the hemidesmosome, only one plasma membrane is present with a dense homogeneous layer closely applied to the cytoplasmic side. Hemidesmosomes are found along the surface plasma membrane (Fig. 3 *b*).

Two types of filaments are found attached to the dense homogeneous layer of the desmosomes and hemidesmosomes (Fig. 4): (*a*) 75-A-thin myofilaments (to be described below), and (*b*) 55-A desmosomal tonofilaments. It was difficult to see desmosomal tonofilaments in longitudinal sections, and they were observed best in transverse sections through the desmosomes. Thin myofilaments (75 A in diameter) of the myofibrils attach to the dense homogeneous layer of the desmosomes and hemidesmosomes and extend into the nearest A band.

The myofibrils at the ends of the muscle at the myotendonal junction also insert onto desmosomes (Fig. 5). On the muscle side of the myotendonal junction, longitudinally aligned thin myofilaments attach to the dense homogenoeus layer of the desmosome. On the side of the tendon cell, densely packed, longitudinally aligned microtubules attach to the dense layer of the desmosome. At the tendon-cuticle junction (Fig. 6), the longitudinally aligned microtubules of the tendon cell attach to a dense homogeneous layer on the cytoplasmic side of the cell membrane (hemidesmosome).

Longitudinal sections of the muscle present at molting show the characteristic structure of slow muscles (Fig. 7). They have long sarcomeres (8–10 μ), irregular Z lines, short I bands, and no M lines. A higher magnification of the I-band regions can be seen in Fig. 9. It is difficult to observe the details in the fine structure of the Z line. Thick

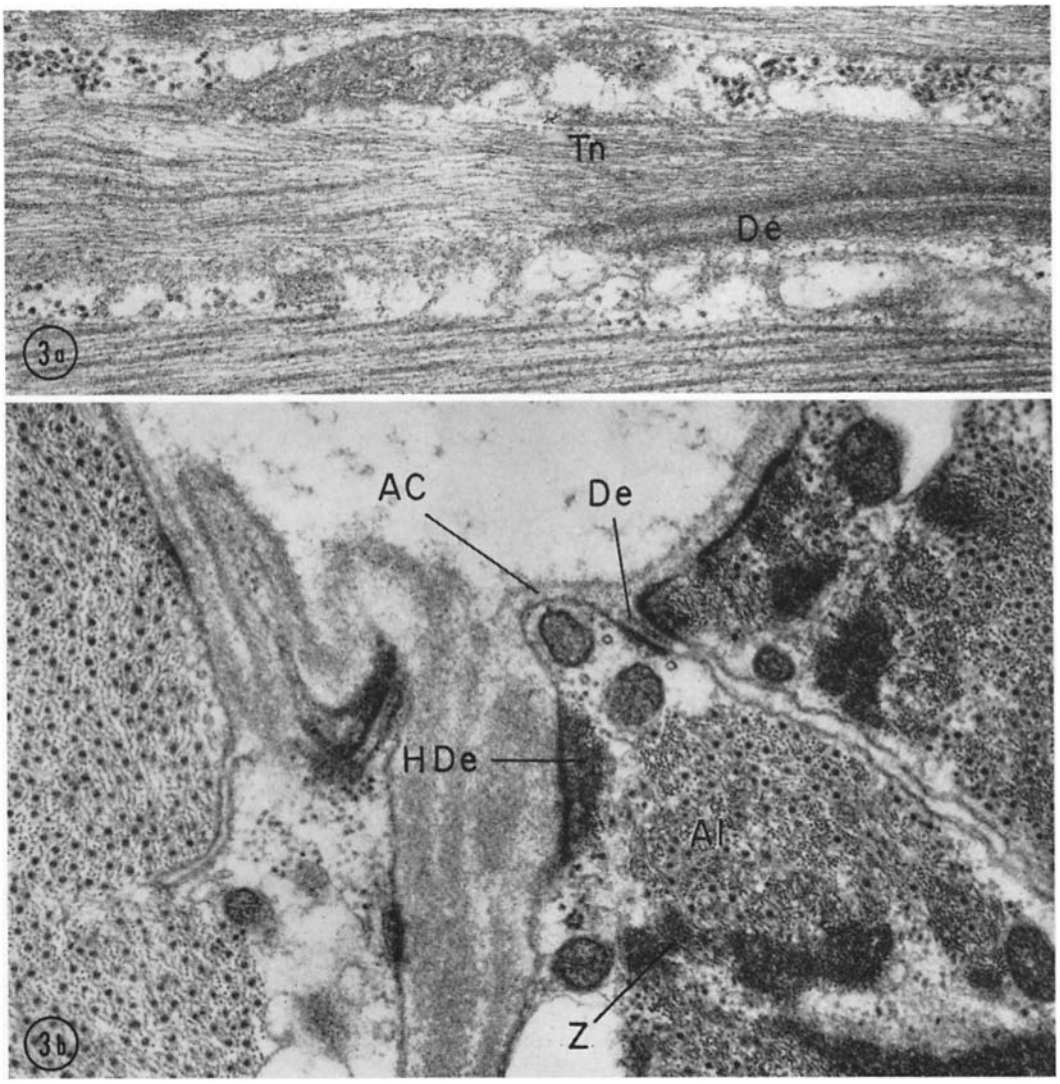

FIGURE 3 *a* Longitudinal section through a fully developed ventral intersegmental abdominal muscle. Thin myofilaments (*Tn*) attach to the dense homogeneous region of the desmosome (*De*) and extend into the A band. Stained with PTA and UrAc. × 55,000.

FIGURE 3 *b* Transverse section through the fully developed ventral intersegmental abdominal muscle showing a hemidesmosome (*HDe*). Transverse sections through the Z line (*Z*) and A-I junction (*AI*) can also be seen. A desmosome (*De*) is seen at the opening of the T-system cleft. Amorphous coat layer adhering to the plasma membrane (*AC*). Stained with PTA and UrAc. × 52,500.

and thin myofilaments interdigitate in the A band. They are, respectively, 200 (Fig. 8 *a*) and 75 A (Fig. 8 *b*) in diameter. Thin filaments are never larger than 75 A in diameter (Fig. 8 *b*). An approximate 420-A cross-bridge periodicity can be seen in parts of the A band (Fig. 10). The H zone (area of no overlap of thin and thick filaments in the center of the A band) is not apparent in longitudinal sections; however, transverse sections through the muscle do suggest the existence of an H zone. Fig. 11 is a cross-section through the center of the A band. The number of thin fila-

ments surrounding each thick filament decreases from right to left. Thick filaments with fewer surrounding thin filaments represent the H-zone area sectioned transversely. Fig. 12 is a transverse section through the more lateral region of the A band.

Thick filaments are in a hexagonal array. The center-to-center spacing of the thick filaments is about 560 A. Each thick filament is surrounded by 12 thin filaments. In a cross-sectional area of the A band, 5,069 thin and 841 thick filaments were counted. This gives a ratio of 6.0 thin filaments to every thick filament.

Other components of the muscle cell include long mitochondria, microtubules, and an occasional Golgi apparatus. Mitochondria and microtubules are aligned parallel with the fibrils. Microtubules are 250 A in diameter. The muscle cell is multinucleated; most nuclei are elongated and centrally located along the long axis of the muscle.

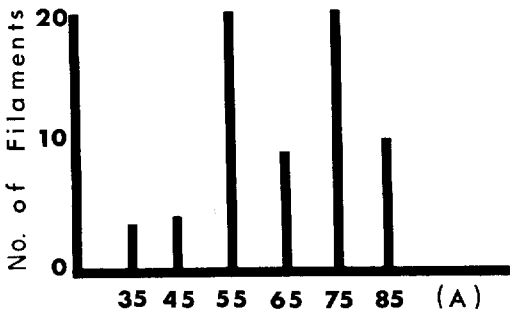

FIGURE 4 Diameter of filaments in desmosomes and hemidesmosomes.

INNERVATION OF THE VENTRAL ABDOMINAL MUSCLE

The ventral nerve cord in *Rhodnius* branches as it proceeds caudally from the subesophageal ganglia. Muscular branches innervate visceral and segmental musculature, while sensory branches innervate integumental sense organs (37).

The ventral nerve cord is enclosed in an extracellular sheath (amorphous coat), the neural lamella. The sheath encloses tracheole cells, Schwann cells (neurilemma cells) and axons. Fig. 13 is a cross-section through the ventral nerve cord. The extracellular sheath contains 150-A filaments which were reported by Smith and Wigglesworth (31) to be collagen. A Schwann cell containing a contorted nucleus and a prominent nucleolus is seen in the center of Fig. 13. The cytoplasm of the Schwann cell is filled with longitudinally oriented microtubules and an occasional mitochondria. Axons range from 0.2 to 5 μ in diameter. They have mitochondria, some longitudinally oriented microtubules (neuro- or axo-tubules), and axofilaments (neurofilaments) 100 A in diameter (arrow, Fig. 13, insert). Some axons have vesicles (*V*) (approximately 0.1 μ in diameter) containing dense material.

Schwann cells surround the axons of muscular nerves which branch from the ventral nerve cord (34) as well as axons lying within fiber clefts (Fig. 1). Fig. 14 is a longitudinal section of the ventral intersegmental abdominal muscle showing a neuromuscular junction. One axon makes several neuromuscular synapses with the muscle. Synaptic vesicles (*Sv*), 400–800 A in diameter, can be seen in the axon clustering in the vicinity of the synaptic terminal. Septate junctions (arrows, Fig. 14 and 15) occur at the synaptic terminal. Adjacent plasma membranes of the septate junction are approximately 350 A apart and are joined by septa approximately 65 A thick. The septa repeat every 65 A. Septate junctions are also observed connecting adjacent epithelial, tracheole, Schwann, and tendon cells in *Rhodnius*.

DISCUSSION

The ventral intersegmental abdominal muscles present in *Rhodnius* at the time of molting may be classified as the slow muscle type. They do not have end-plates; they have multiple motor endings (34, 36, 37), and the myofibrils are poorly defined in cross-section. These slow muscles have long sarcomeres (8–10 μ), irregular Z lines, and short I bands. No M line is seen. Each sarcomere is composed of thick and thin myofilaments. Thin filaments extend from an irregular Z line and interdigitate with thick filaments in the A band. The H zone (in the center of the A band where there are no thin filaments) is not apparent in longitudinal sections (Fig. 7); but in transverse sections there are indications of the existence of an H zone (Fig. 11). Transverse sections in the middle of the A band reveal a gradual transition from thick filaments surrounded by a maximum of 12 thin filaments to decreasing numbers of thin filaments. If the thin filaments are of constant length and extend from an irregular Z line, the observations in transverse sections would be expected. Also, a clear H zone in longitudinal sections would

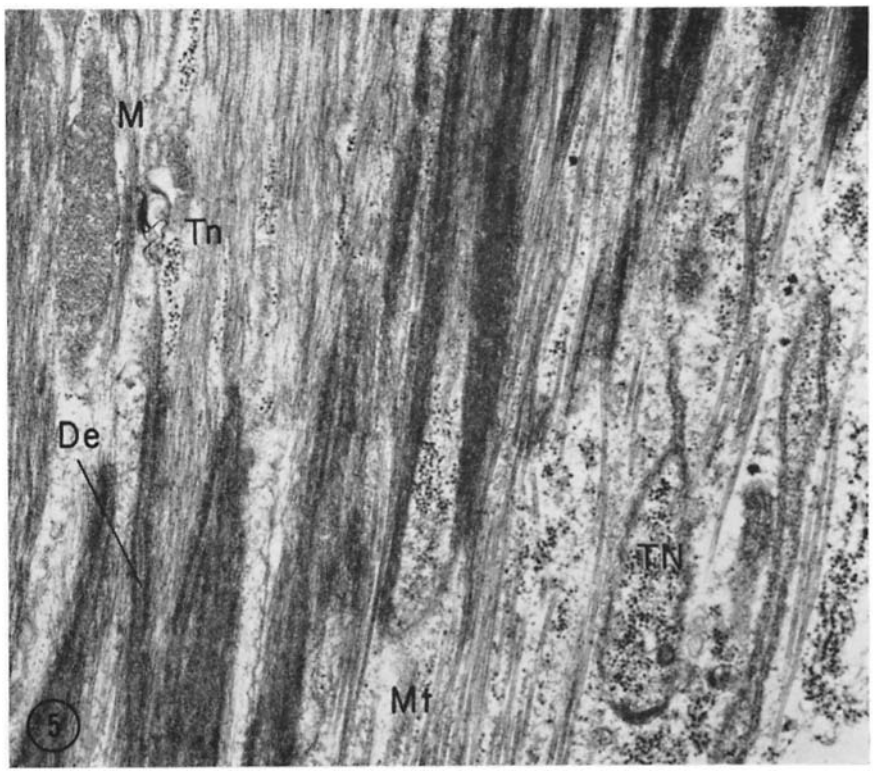

FIGURE 5 Longitudinal section through the myotendonal junction of the fully developed ventral intersegmental abdominal muscle. A desmosome (*De*) is seen at the contact between the tendon cell (*TN*) and the muscle (*M*) forming the myotendonal junction. On the muscle side of the junction, longitudinally aligned thin myofilaments (*Tn*) attach to the dense homogeneous layer of the desmosome. On the side of the tendon cell, longitudinally aligned microtubules (*Mt*) attach to the dense layer of the desmosome. Stained with PTA, UrAc, and lead citrate. × 27,500.

be seen with difficulty. Hagopian (8) observed similar sarcomeres in the leg muscles of the cockroach and saw clear H zones after stretching the myofiber. Cross-sections near the A–I junction (Fig. 3 *b*) show many thin filaments and an occasional thick one. Cross-sections through the rest of the A band show 12 thin filaments around each thick filament.

Desmosomes are structural specializations of the plasma membrane. They were first observed on adjoining epithelial cells at the sites where "terminal bars" were demonstrated by classical staining techniques (5). Desmosomes are occasionally seen in the fully developed ventral intersegmental muscles of *Rhodnius prolixus*. The thin myofilaments of the I band attach to the dense homogeneous layer. In this way, the fibrils are anchored to the plasma membrane. The desmosomes in this muscle may be functionally similar to the intercalated discs of cardiac muscle (5). Desmosomes and hemidesmosomes at the myotendonal and tendon-cuticle junctions serve to anchor the muscle at its origin and insertion. Desmosomes are also seen at the place where the T-system clefts open to the surface plasma membrane in *Rhodinus*. Here they may serve to maintain the relationship of the membranes of the T-system clefts. In their absence, the parallel membranes might be easily separated.

Desmosomes have been seen in other muscles. Recently, Smith et al. (30) reported desmosomes connecting two muscles fibers in the seminal vesicle of the stick-insect, *Carausius morosus*. Thin myofilaments of the I-band were attached to the desmosome. Nakao (21) saw desmosomes in the skeletal muscles of Ammocetes. In this case, thin

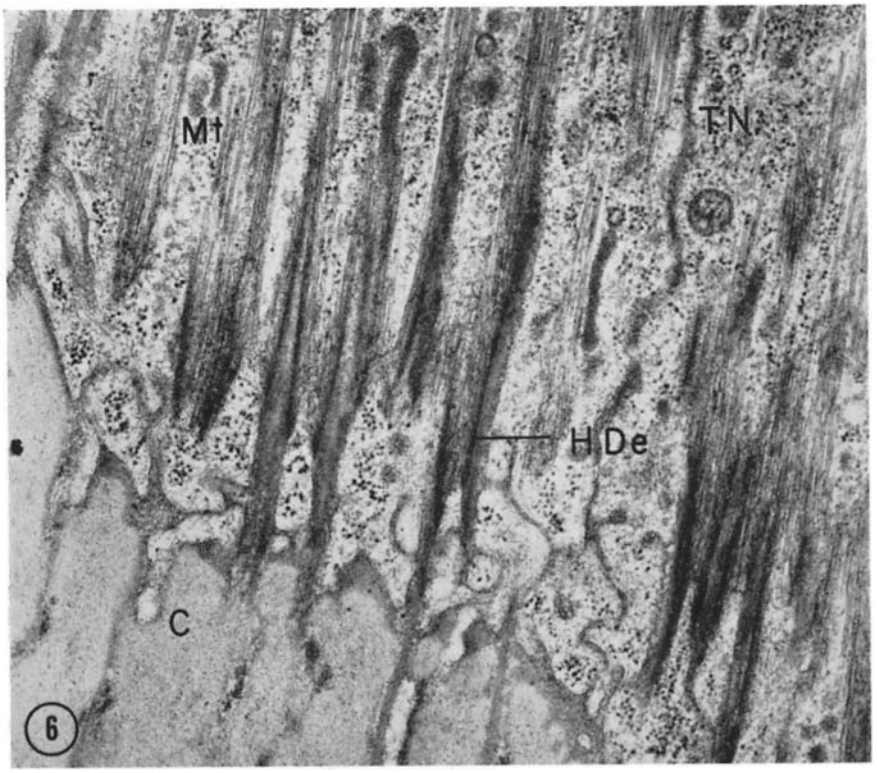

FIGURE 6 Longitudinal section through the tendon-cuticle junction of the fully developed ventral intersegmental abdominal muscle. Longitudinally aligned microtubules (*Mt*) of the tendon cell (*TN*) attach to the dense layer on the cytoplasmic side of the cell membrane. Hemidesmosome (*HDe*); cuticle (*C*). Stained with PTA, UrAc, and lead citrate. × 27,500.

myofilaments were not associated with the desmosome.

Hemidesmosomes are frequently seen in the fully developed ventral intersegmental abdominal muscles of *Rhodnius prolixus*. They occur on the surface plasma membrane of the muscle. Here they form anchoring points for attaching the myofibrils to the plasma membrane.

Vertebrate skeletal muscles have been shown to have (1) a longitudinally oriented system of cisternae (sarcoplasmic reticulum), and (2) a transversely oriented tubular system (T system) which is confluent with the extracellular space (22, 23, 6). The possible physiological role of the T system in muscular contraction has been described. In brief, Hill (12) showed in frog sartorious muscle that, after peripheral excitation, it took less than 40 msec for the onset of contraction to occur in the center of the fiber. He stated that it would take much longer than this for a contraction triggering substance (activating substance) to diffuse from the periphery to the center of the fiber. A. F. Huxley and coworkers (14–16), using microdepolarization techniques, discovered a transverse conduction of excitation at the level of the triad. Peachey and Porter (24), Smith (28), Franzini-Armstrong, and Porter (6), and Peachey (23) suggested that the T system may act as a pathway along which surface excitation is conducted into the fiber. The T system reduced the distance across which the contraction triggering substance would have to diffuse. H. E. Huxley (17) and Page (22), using markers (ferritin), showed that the T tubules are confluent with the extracellular space. Ferritin penetrated the T tubules, but was never seen in the intracellular sarcoplasmic reticulum. Morphological evidence for the continuity of the T-system clefts with the surface plasma membrane is easily seen in *Rhodnius prolixus* (Figs. 1 and 2 *a*).

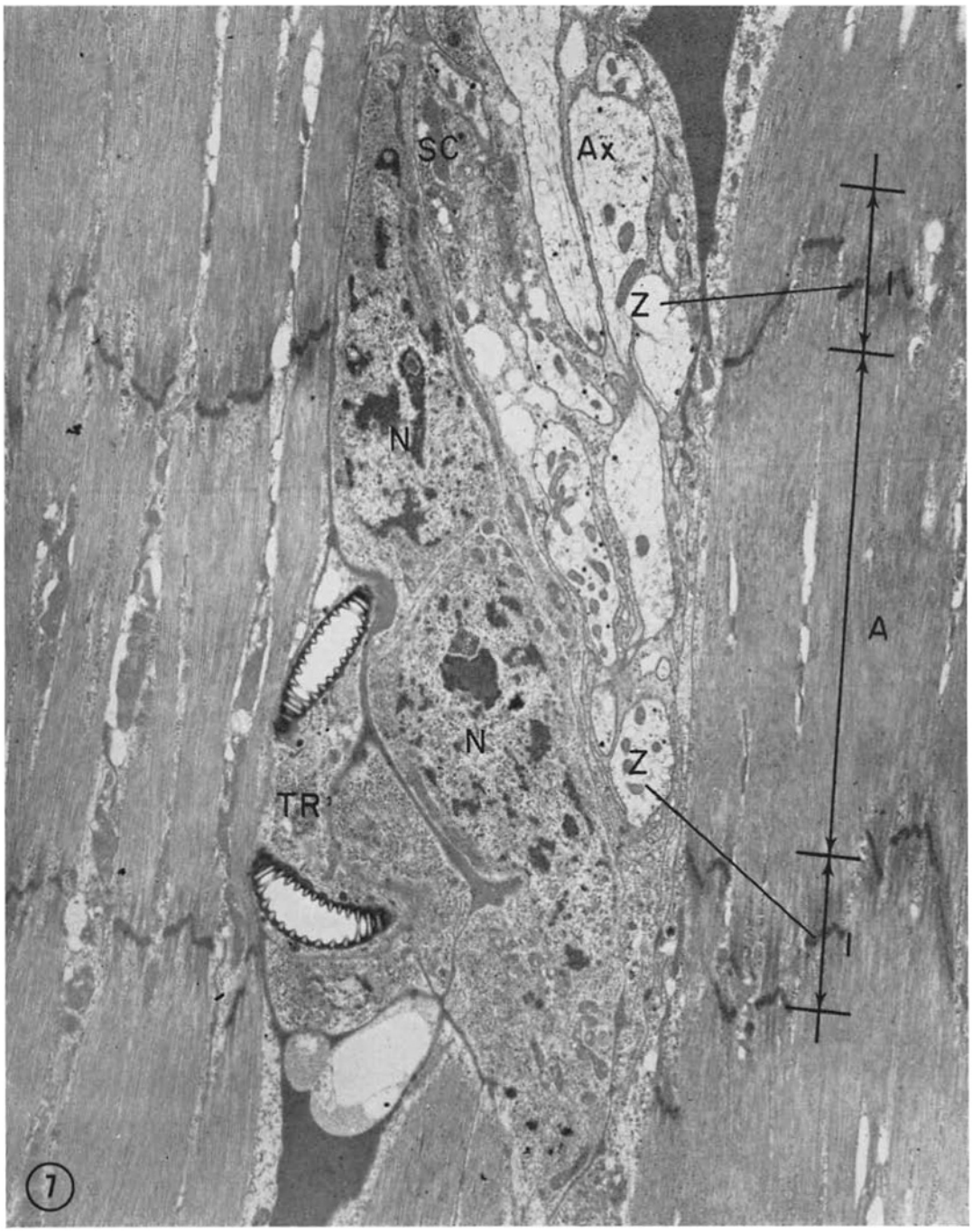

FIGURE 7 Longitudinal section through a fully developed ventral intersegmental abdominal muscle. Sarcomeres have irregular Z lines (*Z*), short I bands (*I*), long A bands (*A*), and no apparent H zone. No M line is seen. Tracheole cells (*TR*) and their nuclei (*N*), Schwann cells (*SC*), and axons (*Ax*) are also seen lying in a fiber cleft. Stained with PTA and UrAc. Approximately × 12,000.

In all striated muscles observed, thick and thin filaments overlap in the A band. The relative positioning of myofilaments is different in the various muscle types. In vertebrate skeletal muscle, for example, there is a double hexagonal array of thick and thin filaments (Fig. 16, diagram *a*). The ratio of thin to thick filaments is 2:1. Each thin filament is shared by three thick filaments (13, 19). Insect flight muscle also consists of a double hexagonal array of thin and thick filaments (Fig. 16, diagram *b*). The ratio of thin to thick filaments is 3:1. Each thin filament is shared by two thick filaments (18). There is a hexagonal array of thick filaments in *Rhodnius*; however, each thick filament is surrounded by 12 thin filaments. Two possibilities for the arrangement of 12 thin filaments around each thick filament are shown in Fig. 16, diagram *c* and *d*. In diagram *c*, six of the 12 thin filaments are located midway between adjacent thick filaments, and each of the remaining six thin filaments is located in a trigonal position with respect to three adjacent thick filaments. In diagram *d*, each of six groups of two thin filaments is located between two adjacent thick filaments. In both of these cases, the thick and thin filaments will all be interconnected either directly or indirectly by cross-bridges. The ratio of thin to thick filaments in the former case is 5 to 1; in the latter case, the ratio is 6:1. By counting a large number of myofilaments in the A band, and obtaining the ratio of thin to thick filaments in *Rhodnius*, we can differentiate between these two possibilities. Since a ratio of six thin filaments to each thick filament was obtained, it was concluded that the myofilament array in diagram *d* is present in *Rhodnius*. In this case, two thin filaments are shared by two neighboring thick filaments. This organization can be related to that found in vertebrate skeletal muscle. In diagram *d* of Fig. 16, if all the neighboring thick filaments are joined by lines, groups of three thin filaments will be found in the triangles formed. If each group of three thin filaments is replaced with one thin filament, the array becomes that of vertebrate skeletal muscle. The array seen in insect flight muscle (Fig. 16, diagram *b*) can be obtained by replacing the two thin filaments shared between two thick filaments by one thin filament.

Other muscles which have more than six thin filaments surrounding each thick filament have been reported in the literature. Brandt et al. (2) counted six to eight thin filaments in the leg muscle of the crayfish, while Swan (32) counted nine to 12 thin filaments also in the leg muscle of crayfish. Variations from ten up to 12 thin filaments surrounding each thick filament were reported in various invertebrate muscles (10, 1, 27, 20, 26, 8, 4). Toselli (33) reported 12 thin filaments surrounding each thick filament in the ventral intersegmental abdominal muscle of *Rhodnius prolixus*. Later, Smith et al. (30) and Smith (29) described muscles in the cockroach, moth, and stick-insect in which 12 thin filaments were also counted around each thick filament. Swan (32) concluded that each thick filament interacts with its own set of six thin filaments and that no sharing of thin filaments by neighboring thick filaments occurs. With such a situation, it would be expected that separation of the thick filaments, each with their own set of six thin filaments, would occur frequently, especially

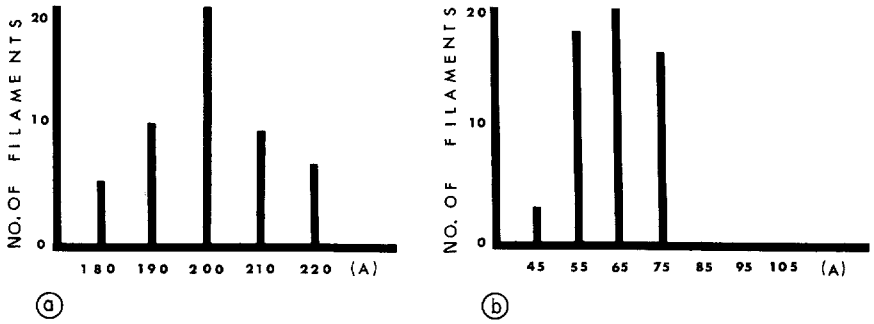

FIGURE 8 Diameter of: (*a*) thick myofilaments (interdigitating with thin myofilaments); and (*b*) thin myofilaments (interdigitating with thick myofilaments). Thin myofilaments are never larger than 75 A in diameter.

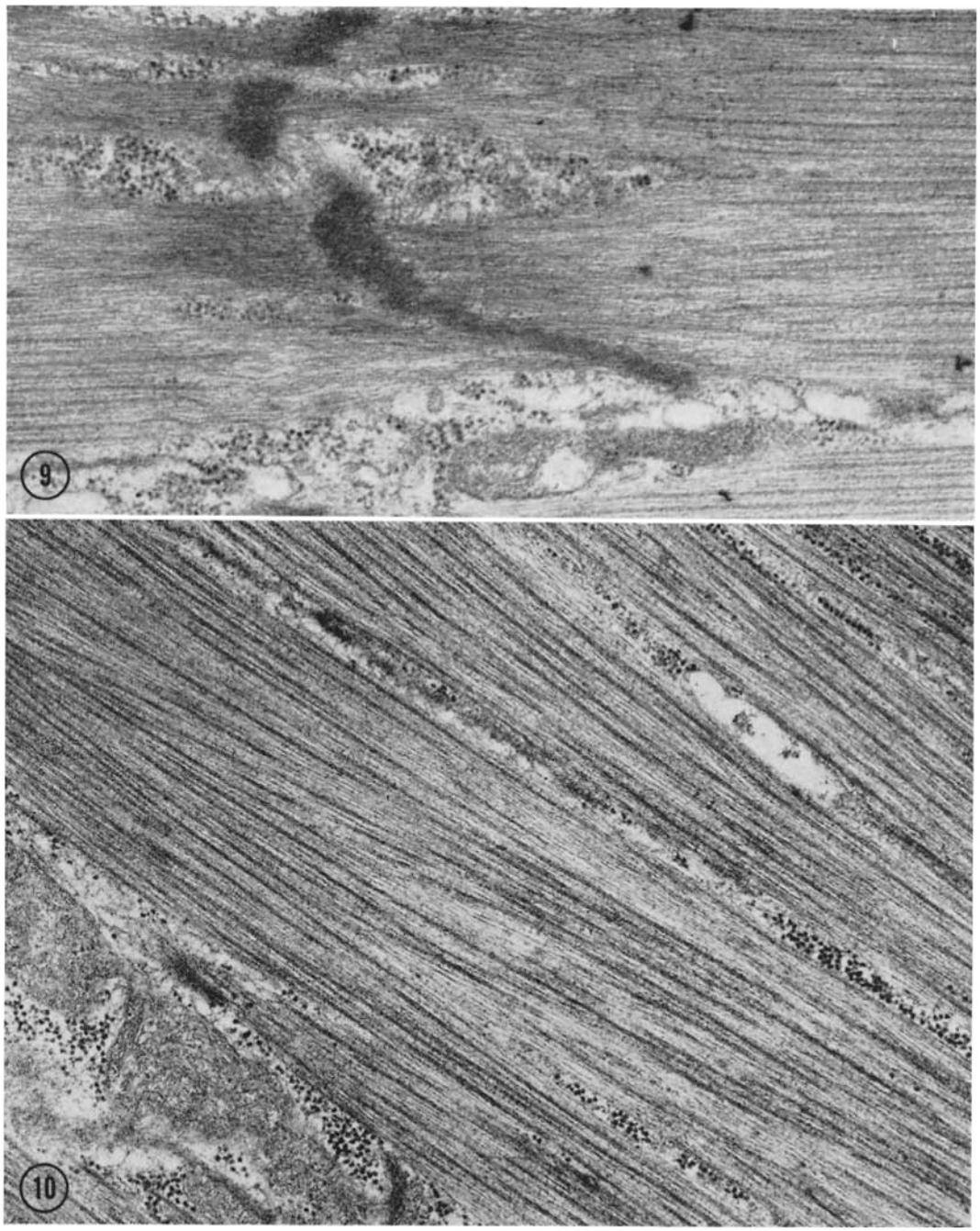

FIGURE 9 Longitudinal section through the I band of a fully developed ventral intersegmental abdominal muscle. Stained with PTA and UrAc. × 38,750.

FIGURE 10 Longitudinal section through a fully developed ventral intersegmental abdominal muscle showing an approximately 420-A cross-bridge periodicity. Stained with PTA, UrAc, and lead citrate. × 40,000.

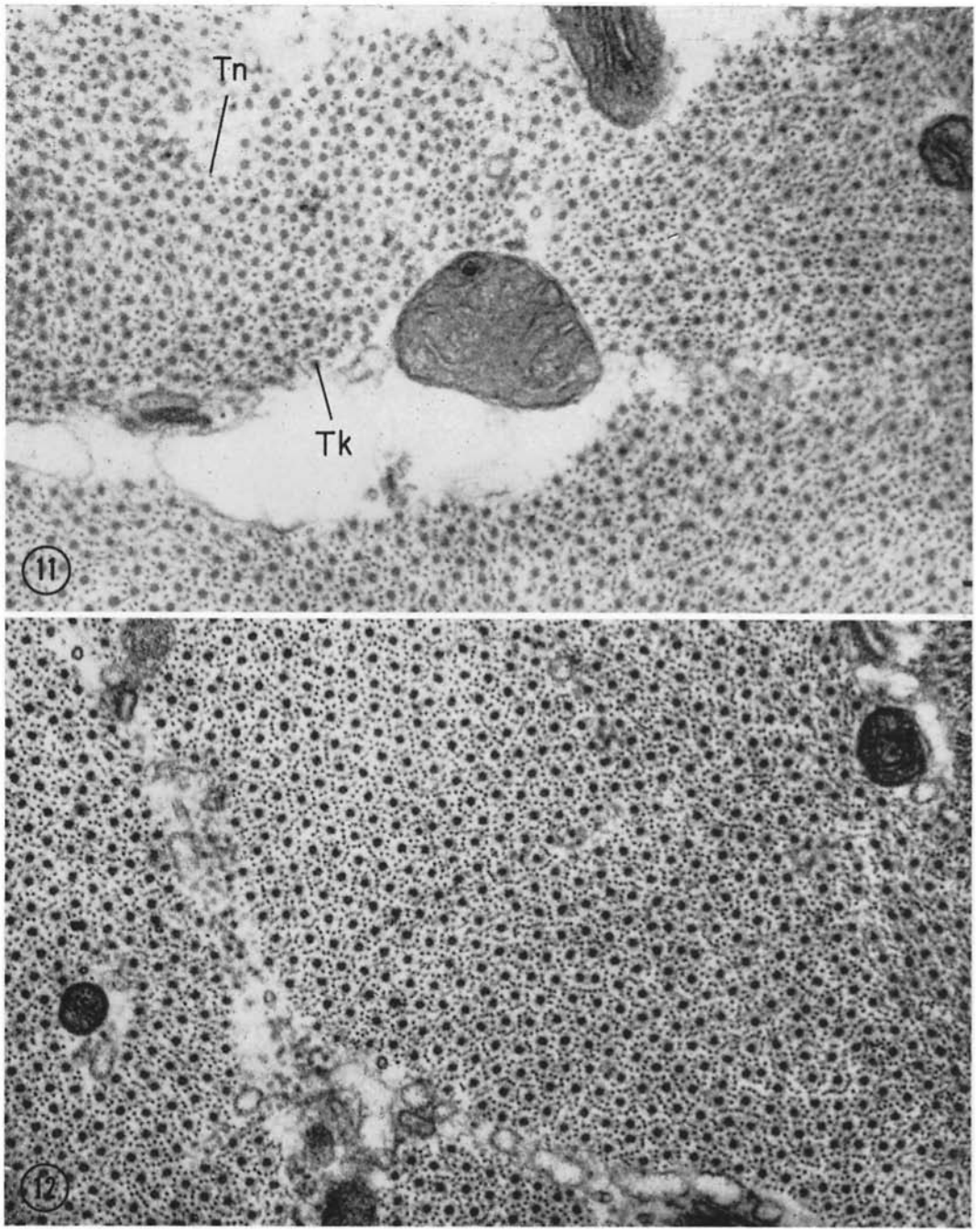

FIGURE 11 Transverse section through a fully developed ventral intersegmental abdominal muscle through the center of the A band. The number of thin filaments (*Tn*) surrounding each thick filament (*Tk*) decreases from right to left. Stained with PTA and UrAc. × 72,500.

FIGURE 12 Transverse section through the lateral region of the A band of a fully developed ventral intersegmental abdominal muscle. Thick filaments are in a hexagonal array. In the best aligned areas, 12 thin filaments can be seen surrounding each thick filament. Stained with UrAc and lead citrate. × 72,500.

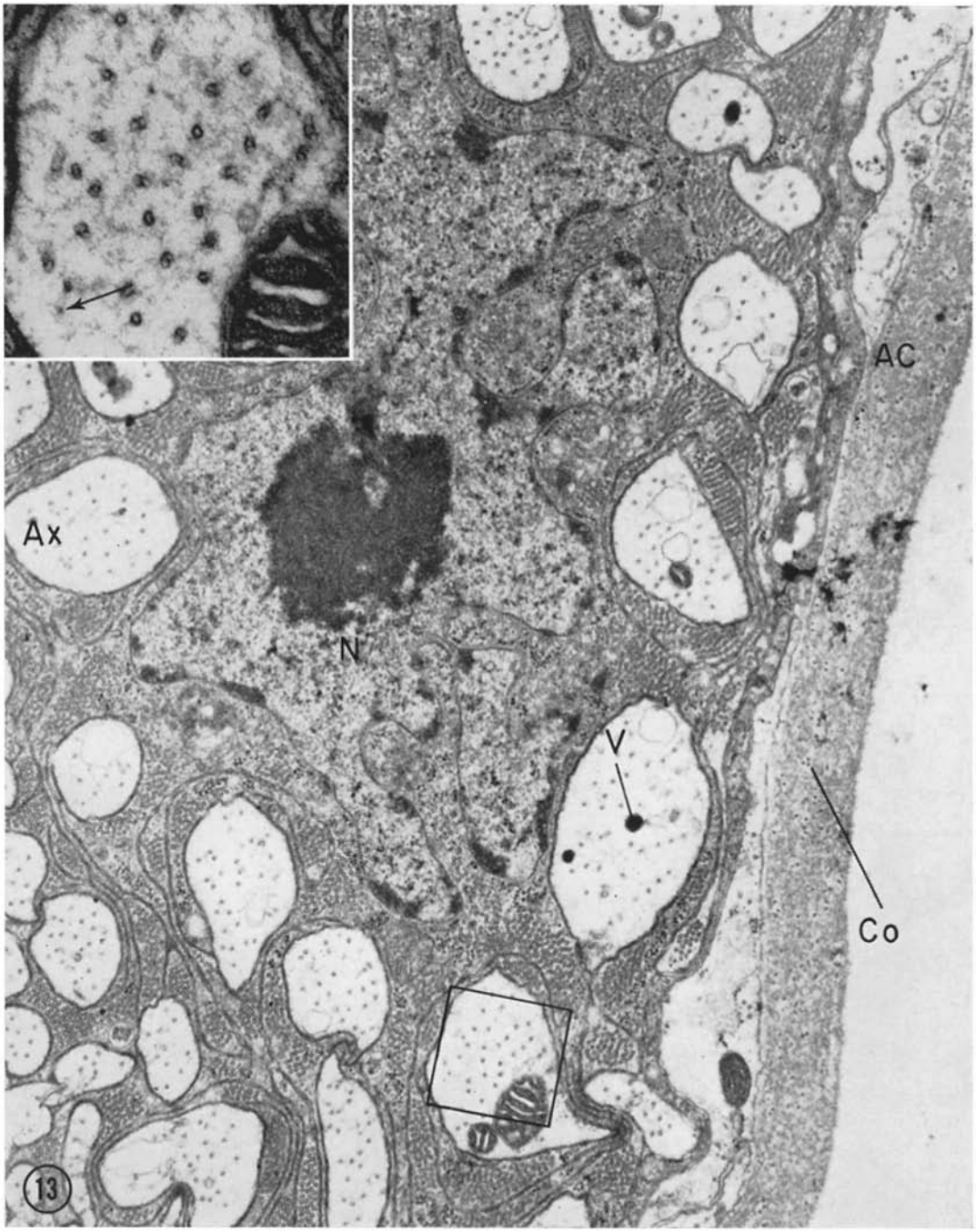

FIGURE 13 Transverse section through the ventral nerve cord at molting. Note the nucleus (*N*) of the Schwann cell in the center of the micrograph. The cytoplasm of the Schwann cell is packed with longitudinally aligned microtubules. Axon (*Ax*); vesicle containing dense material (*V*); collagen (*Co*); extracellular amorphous coat (*AC*). Insert: higher magnificantion of outlined axon showing axofilaments (arrow) 100 A in diameter lying parallel to axotubules. Stained with PTA, UrAc, and lead citrate. × 24,500; insert, × 77,500.

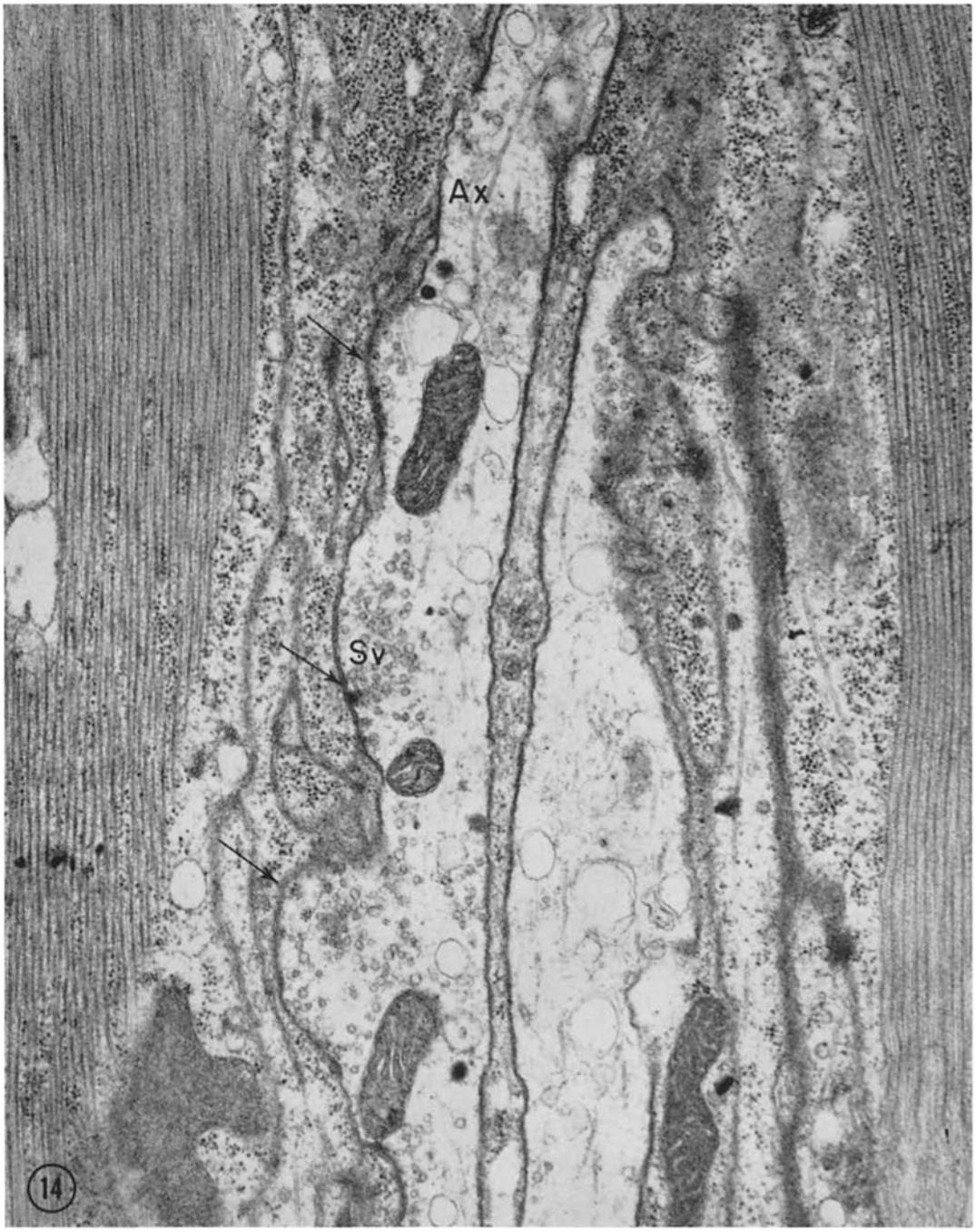

FIGURE 14 Longitudinal section through the ventral intersegmental abdominal muscle at molting showing several neuromuscular junctions. Septate junctions (arrows) and synaptic vesicles (*Sv*) occur at the synaptic terminal. Axon (*Ax*). Stained with UrAc and lead citrate. × 35,000.

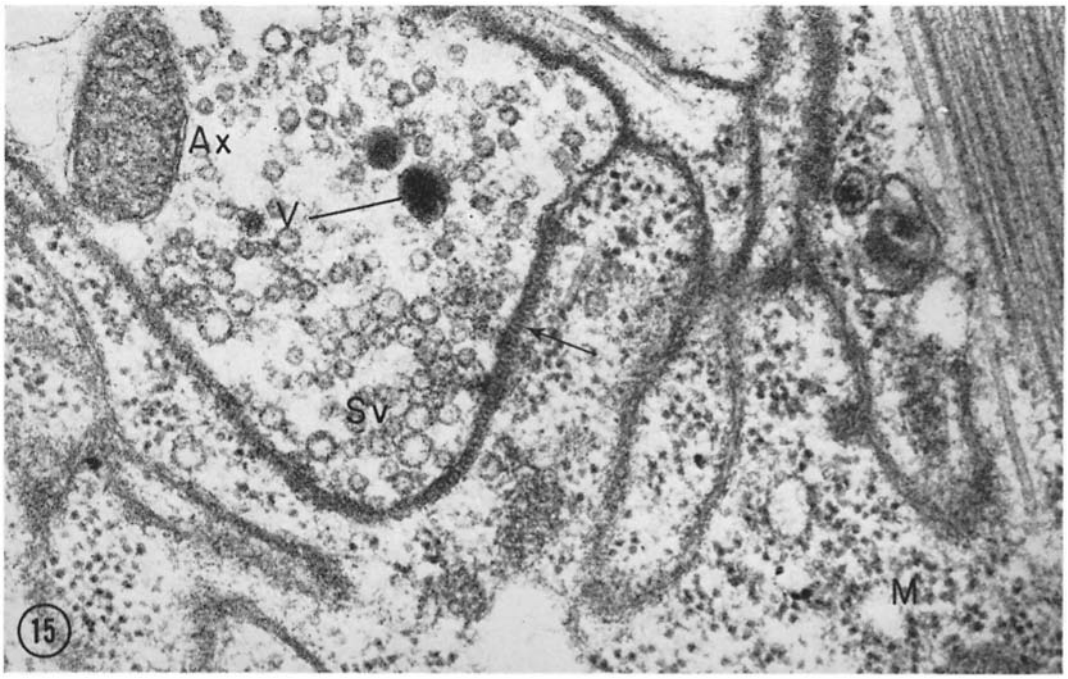

FIGURE 15 Longitudinal section through the ventral intersegmental abdominal muscle at molting showing a higher magnification of the septate junction (arrow) at the synaptic terminal. Axon (*Ax*); synaptic vesicle (*Sv*); vesicle containing dense material (*V*); muscle (*M*). Stained with UrAc and lead citrate. × 65,000.

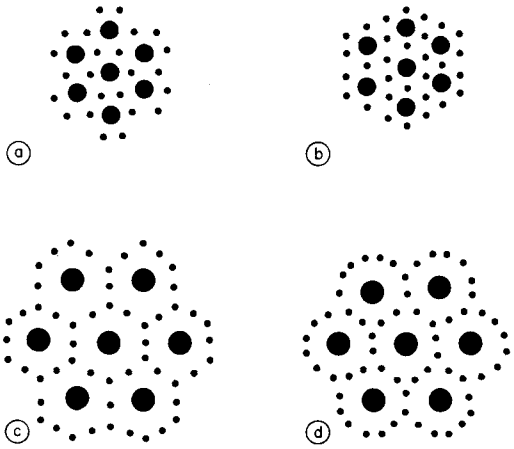

FIGURE 16 Diagrammatic representation of the arrangement of thick and thin myofilaments in the lateral A-band region. *a*, Vertebrate skeletal muscle. 2 thin: 1 thick. *b*, Insect flight muscle. 3 thin: 1 thick. *c*, 5 thin: 1 thick. *d*, 6 thin: 1 thick.

if the muscle is not maintained under tension. For a stable lattice of thick and thin filaments to be obtained, adjacent thick filaments must be connected through cross-bridges to neighboring thin filaments. Smith et al. (30) also suggested the arrangement shown in Fig. 16, diagram *d*, for various invertebrate muscles. Their results were based on direct observations without filament counts or consideration of the sharing. Morita (20) counted up to 12 thin filaments surrounding each thick filament in the Planarian head muscle and suggested a sharing of each thin filament by two thick filaments.

INNERVATION OF THE VENTRAL ABDOMINAL MUSCLE

Ventral Nerve Cord

The most unusual feature of the ventral nerve cord is the large number of microtubules present in the Schwann cells. Similar Schwann cells have recently been described in the ventral nerve cord of the shrimp, *Penaeus japonicus*, by Hama (9). He proposed that the function of the Schwann cell's

microtubules is to provide structural strength for protection from mechanical injury. This may also be the case for *Rhodnius*.

Neuromuscular Junction

In observing various muscles of the cat, rat, and monkey, Hess (11) noted two types of nerve endings which can be histochemically differentiated by cholinesterase staining. These are the *en plaque* and *en grappe* endings. The *en plaque* endings strain intensely and show the same configurations as the motor end-plates of vertebrate skeletal muscle. The *en grappe* endings are smaller, less intensely stained, and the terminal axons make several contacts along the same muscle fiber. *En plaque* endings occur only on *Fibrillenstruktur* fibers, and *en grappe* endings occur only on *Felderstrucktur* fibers. These results were confirmed by Cheng and Goodwin (3), using the electron microscope. In addition, Cheng and Goodwin saw vesicles containing dense material in the nerve endings of *Felderstruktur* fibers. This work is consistent with the results presented here for the ventral intersegmental abdominal muscles of *Rhodnius* which are of the *Felderstruktur* type. The terminal axons make several contacts along the same muscle fiber, and large vesicles containing dense material can be seen in the axons. The septate junctions seen in *Rhodnius prolixus* at the neuromuscular junction are similar to those described by Graziadei (7) in the muscles of Cephalopods.

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