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COMPARATIVE STUDIES ON ULTRASTRUCTURES OF THE THORACIC MUSCLES OF THE LARVAL AND ADULT STAGES IN *TENEBRIO MOLITOR* L.

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ABSTRACT

W. Y. Lee, Y. C. Lin and F. Y. Shen (1970) *Comparative Studies on Ultrastructures of the Thoracic Muscles of the Larval and Adult Stages in Tenebrio molitor L.* Bull. Inst. Zool. Academia Sinica 9(1): 15-21. The dorsal longitudinal muscles of the metathorax of larval muscles in *Tenebrio molitor* L. named as larval muscles here and the indirect flight muscles of adult muscles named as adult muscles, were examined by electron microscope. In larval muscles, the Z-bands show as wavy and sinuous in appearance, I-bands are of two-fifths in length of A-bands and the H-band is absent. In adult muscles, Z-bands are of linear in shape, I-bands are much shorter, and H-bands are present. The ratio of thin to thick myofilaments in larval muscles is of 6:1, while in adult muscles is of 3:1. Abundant sarcoplasmic reticulum envelopes each myofibril; and T-systems are at two sides of A-bands. However, the sarcoplasmic reticulum of adult muscles is much reduced, and T-systems are located at the level of H-bands. Sarcosomes of larval muscles are distributed in I-bands at either side of Z-bands. The sarcosomal sizes of larval muscles are much smaller in comparison with those of adult muscles. The sarcosomes of larval muscles contain a few cristae in parallel arrangements. The sarcosomes of adult muscles are arranged in row and inserted into spaces between intermyofibrils, and they contain a large number of cristae with compact arrangements. The sarcosomal sizes of adult muscles are much larger in general. Different shapes of sarcosomes can be observed in both larval and adult muscles.

In the histological comparisons of thoracic muscles of adults and larvae of *Tenebrio molitor* L., there are three types of muscles shown in the adults: general muscles, tubular general muscles and fibrillar muscler(10); while in the larvae, only one type has been described, which is morphologically similar to the general muscle of the adults(11).

In the present report, the fine structure of thoracic muscle of adults and larvae, that is the direct flight muscles of the adult, and the dorsal longitudinal muscles of metathorax of the larvae, will be described. In histological aspect, the direct flight muscles belong to fibrillar muscles, while the dorsal longitudinal muscles of the larval metathorax is closed to the general muscle. In the postembryonic development, one of the indirect flight muscles are transformed from the dorsal longitudinal muscles of the larval metathorax during metamorphosis(12). The organization of myofibrils, the membrane system of sarcoplasmic reticulum and sarcosomes of these two kinds of muscle have been compared and described in the present report.

MATERIALS AND METHODS

Ten day old adults and mature larvae of *Tenebrio molitor* L. were used in this investigation. The electron microscopic techniques modified by Kiyoshi Hama (1968, personal communication) were employed. The insects were injected ca 0.2-0.5ml of the cold 6% glutaraldehyde with 0.1M cacodylate buffer, and then split mid-sagitally. The dorsal longitudinal muscles of the larvae and the indirect flight muscles of the adults were cut and fixed in the above fixative for 3 hours. The tissues were teased into small pieces, then washed with cold 8% sucrose for 30 minutes, and post-fixation were in cold 2% osmium tetroxide for 2 hours. The fixed tissues were dehydrated in 50% and 70% ethanol in cold, and 90% and 100% ethanol at room temperature, and lastly propyleneoxide each for 15 min. The tissues were embedded in Epon 812 resin(13), and polymerized at 60°C. The tissues were stained with uranyl acetate either prior to dehydration, or these sections were stained with uranyl acetate and lead citrate after the sections were mounted. God and silver sections (ca 600-1,500Å) were cut by Sorvall Porter-Blum MT-2 Ultra-microtome with glass knife and the sections were collected on uncoated copper grids. All the electron micrographs were taken under Hitachi Electron Microscope Type 11-A with 50KV and 75KV.

RESULTS

The larval muscles and the adult muscles described hereinafter are representing dorsal longitudinal muscles of the larval metathorax and the indirect flight muscles of adult respectively.

The sarcomeres of larval muscles in the relaxed fiber (Fig. 1) are 3.0-3.6μ in length. Their myofibrils show irregular shape in the transverse sections (Fig. 5). The sarcomere lengths of adult muscles (Fig. 3) are much shorter than those of larval muscles, only 1.2-1.5μ. The myofibrils are mostly oval in shape in the transverse muscles sections (Fig. 7).

I-bands (I, Fig. 1) are ca two-fifths length of A-bands (A, Fig. 1) in larval muscles. Z-bands (Z, Fig. 1) go across the myofibrils, and they are very clear; the sinuous appearance of Z-bands is the characteristic of larval muscles. H-bands can not be defined in these muscles. In adult muscles, I-bands (I, Fig. 3) are much shorter than A-bands (A, Fig. 3). The former (I, Fig. 1) is 0.12-0.15μ and the latter (I, Fig. 3) is 1.08-1.35μ. H-bands (H, Fig. 3) of myofibrils are obscure in appearance. Z-bands (Z, Fig. 3) are definitely shown as a stright line.

Abundant sarcoplasmic reticulum (Sr, Fig. 1, 5) envelopes each surrounding myofibril in larval muscles. T-systems and dyads (D, Fig. 1, 5, 6) are in the inter-myofibrillar spaces at the lateral margins of A-bands. The sarcoplasmic reticulum of the adult muscles (Sr, Fig. 3, 7) lightly surrounds myofibrils. T-systems and dyads (D, Fig. 3, 7) are mostly distributed in the areas between myofibrils near H-bands and sarcosomes. In comparing the larval with the adult muscle, no great difference in the number per sarcomere of diadic contacts is evident.

In cross-sections, the arrangements of thick and thin filaments differ in larval and adult muscles. Each thick filament (Tk, Fig. 6) of larval muscles is surrounded by an orbit of 10-13 (usually 12) thin filaments (Th, Fig. 6), the overall ratio of thin to thick filaments is 6 : 1. In the myofilaments of adult muscles (Fig. 8), each thick filament is surrounded by 6 thin filaments with overall ratio of thin to thick filaments is 3 : 1. It shows a regular hexagonal arrangement.

In larval muscles, the sarcosomes which contain a few number of cristae (M, Fig. 2, 6), are scattered in I-bands only at each side of Z-bands (M, Fig. 1, 5). The sizes of sarcosomes of larval muscles are smaller in dimension than that of adult muscles ($0.2\text{-}1.15 \times 0.2\text{-}0.6\mu^2$). They are varied in shapes, such as elongate and irregular. On the contrary, the sarcosomes in the adult muscles contain a large number of cristae with compact parallel structure (M, Fig. 4, 8) and they are located in row of the full myofibrillar length of muscle fibers (M, Fig. 3). The sizes of sarcosomes of adult muscles are mostly much larger than those of larval muscles, the largest is of the length of ca 4μ in the longitudinal section. There are also many different shapes.

DISCUSSION

In anatomic aspect, the dorsal longitudinal muscles of the larval metathorax of *Tenebrio molitor* L. differ from the indirect flight muscle of the adult. The fibers of the former are flattened in shape and the latter forms circular or elongate shape in transverse section(9). In histological aspect, larval muscles that morphologically closed the general type of muscle(11), and indirect flight muscles of the adult belong to the fibrillar muscles of which fibers are invaded by a rich tracheolar system(10). In electron microscopic studies, the fine structure of myofibrils of larval muscles shows differences in the foregoing results. All of these results may aid in explaning functinal difference between two muscles in the discussion.

The significance of density of tracheolar penetration of different muscles is related to their metabolic activity. Much evidence already exists relating the amount of tracheation directly to insect muscles moving speeds(14).

The patterns of muscle striations of larval muscles and adult muscles appeared different. Larval muscles have longer length of the sarcomere while adult muscles appeared to be shorter. Some evidence have shown that I-band in resting muscle occupies ca 50% of the total length of the sarcomere in the muscles of insect leg whereas it is reduced to 20-30% in synchrouous flight muscles of *Libellala* and *Schistocerca* and very small figure in asynchronous fibrillar muscles of *Calliphora*(14). The patterns of muscle striation in larval muscles mostly resemble those of the muscles of insect leg. The adult muscles belong to asynchronous fibrillar muscles.

Organization of myofilaments in insect and crustacean muscles with different functional characteristic is becoming clear (7, 8 16). Thin and thick filament ratio in larval muscles is similar to those reported in the femoral muscle of cockroach, *Leucophaea maderae* F.(3), the tibial extensor muscle of mesothoracic leg of the american cookroach, *Periplaneta americana* L.(7), the intersegmental muscle of the same insect(23), the tibial extensor muscle of the leg of the Lepidoptera, *Achalarus lyciadas*(16), and other invertebrate muscles: such as tonic muscle fibers of the crayfish(6), abductor claw muscles of the crab, *Pinnixia* sp.(15) the muscle known to be slow acting, and the superficial extensor muscle in abdomen of the lobster, *Homarus americanus*(8). They are all 6 : 1 in the ratio of thin to thick filaments. The filament ratios in adult muscles are similar to those reported for the synchronous filght muscle in the dragon-fly, *Aeshna* sp.(22), basalar muscle of the wing of the Lepidoptera, *Achalarus lyciades*(27), the asynchronous flight muscles in the giant water-bugs(1), Dipterans: *Drosophila melanogaster*(19), *Calliphora erythrocephala*(20), in aphis, *Megoura viciae*(21), the coxal muscle of mesothoracic leg of the american cockroach(7), and other invertebrate muscles known to be fast acting such as deep extensor muscle of abdomen in the lobster, *Homarus americanus*(8), the phasic muscle fibers of crayfish(6), and type A muscle of first antenna of *Pinnixia* sp(15). The evidence may be used to be explained the reason why thin to thick filament ratios are related to the action of insect muscles. Auber(2) reported that the flight muscles of some moths, *Phytometa* and *Agroti* are like those of Diptera which have faster work rhythm, and each thick filament is surrounded by 6 thin filaments. However, in the low contraction frequency muscles, as in *Pieris* bufferflies wing-beat is not more than 10/sec., each thick filament was found to be surrounded by 7-9 thin filaments.

Significant differences in the organization of sarcoplasmic reticulum and associated T-system between the larval and the adult muscles are apparent. The organization of sarcoplasmic reticulum and T-system in larval muscles is similar to the leg-muscles of cockroach, *Leucophaea maderae*(3) and the moth, *Achalarus lyciades*(16) and the intersegmental muscles of the american cockroach, *Periplaneta americana*(23). Sarcoplasmic reticulum is abundant and consists of fenestrated envelope surrounding all levels of the sarcomere. Diadic contacts are similar to abductor claw muscle of *Pinnixia* sp.(15) at the area near A/I junctions. The organization and disposition of sarcoplasmic reticulum and diadic contacts are similar to other insects with asynchronous flight muscle fibers, such as the flight muscle of *Calliphora*(20). They contain a highly modified sarcoplasmic reticulum. The cisternae of sarcoplasmic reticulum is extremely reduced and it appears to be represented by a few small isolated vesicles. However, in synchronous insect flight muscle, sarcoplasmic reticulum is abundant and is associated with a well developed cisternae(7,22). Dyads in adult muscles extend in among the myofibrils at the level of M-bands, which is similar to those of fight muscles of giant water-bugs(1). The function of sarcoplasmic reticulum may serve in calcium exchange, as the calcium pumping system of taking up the calcium that enters from outside as well as the calcium that released by the sarcoplasmic reticulum from inside(18). These membrane systems are responsible for the conduction of the excitation contraction of the muscle fibers. The great reduction in the amount sarcoplasmic reticulum in *Tenebrio* adult muscles may be correlated with the limited part it plays in those muscles. Pringle(14) suggested that while the onset of contraction may occur normally via T-system excitation, and subsequent myogenic length changes may not be coordinated with synchronized cyclic supply of calcium ions to the myofibrils. Ashhurst(1) suggested that certain insects have adapted the method for the production of a fast rate of contraction and relaxation, the mechanismis found in the asynchronous flight muscle that the active state is maintained by the arrival of nerve impulses.

The distribution and the architecture of sarcosomes, described in the present study, have great differences between adult muscles and larval muscles. The sarcosome content of adult muscles is ca 40 per cent of the fiber volume like in flight muscles of the dragon-fly, *Aeshna* sp.(22), of *Calliphora erythyrocephala*(20), of aphids, *Megours viciae*(21) and of *Drosophila melanogaster*(19). Sarcosomes of flight muscles of *Aeshna* contain large number of whorled and irregularly arranged cristae. Those of *Calliphora* are parallel plates containing circular fenestrated cylindrical channels within the matrix. Sarcosomes of *Tenebrio* indirect flight muscles, *Drosophila* indriect flight muscles and *Megoura* flight muscles have great number of cristae uniformly parallel, though frequently the cristae orientation changes abruptly by the shape of the sarcosome. The sarcosome content of larval muscles is ca 5 per cent of the fiber volume. This is similar to leg muscles of the cockroach, *Leucophaea maderae*(4) and the moth, *Achalanus lyciades*(17). The cristae of sarcosomes in these muscles contain few numbers mostly with parallel regular arrangement.

It is well known that insect sarcosomes, in common with other mitochondria, play a role of the function of glucose metabolism and energy conservation. They contain the enzyme for the citric acid (Krebs cycle) by means of which glucose break down to produce energy. The Krebs cycle enzymes are mostly situated in the mitochondrial matrix. Sarcosomes with large number of cristae structurelly indicate that these sarcosomes have high metabolic activity.

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Fig. 1. Longitudinal section of the larval muscle. Z-bands (Z) are sinuous appearance, H-band is absent. Sarcoplasmic reticulums (Sr) occur abundantly surounding myofibrils. Dyads (D) occur at the edge of A-bands (A). Sarcosomes locate in I-bands (I). x 25,000.

Fig. 2. Enlargement of the sarcosome (M) of the larval muscle. Few numbers of cristae parallel each other. x 50,000.

Fig. 3. Longitudinal section of the adult muscle. Sarcomeres are shorter than those of the larval muscle (Fig. 1). Z-bands (Z) are liner shapes. I-bands (I) are short, H-bands (H) are present. Sarcosomes (M) are in rows at the space of intermyofibrils. Sarcoplasmic retuculum (Sr.) is reduced, few vesicle surround myofibrils and sarcosomes. Dyads (D) occur at the level of H-bands (H). x 25,000.

Fig. 4. Sarcosome of the adult muscle. This electron micrograph shows the sarcosome in longitudinal section which is particularly well illustrated the arrangment of cristae with parallel compacting structure. x 50,000.

Fig. 5. Transverse section of the larval muscle. This micrograph shows Z-bands (Z), I-bands (I) and A-bands (A) of the myofibril. Sarcosomes (M) locate in I-bands and the area of I-bands and A-bands overlapping. Sarcoplasmic reticulum (Sr) surrounds myofibrils. D, Dyads, Pm, Plasmic Membrane, Tr, Tracheloes. x 35,000.

Fig. 6. Transverse section of larval muscle, 10-13 (mostly 12) thin myofilaments (Th) surround each thick myofilament (Tk). Sarcosomal (M) cristae are also parallely arranged in the transverse section. A, A-bands. I, I-band. Z, Z-band. x 70,000.

Fig. 7. Transverse section of the adult muscle. A, A-band. I, I-band. Z, Z-band. D, Dyads. Sr, Sarcoplasmic reticulum. M, Sarcosomes. N, Nucleus. Tr, Tracheoles x 17,500.

Fig. 8. Transverse section of the adult muscle. Each thick myofilament (Tk) is surrounded by 6 thin myofilaments (Th). M, Sarcosomes. x 80,000.


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D
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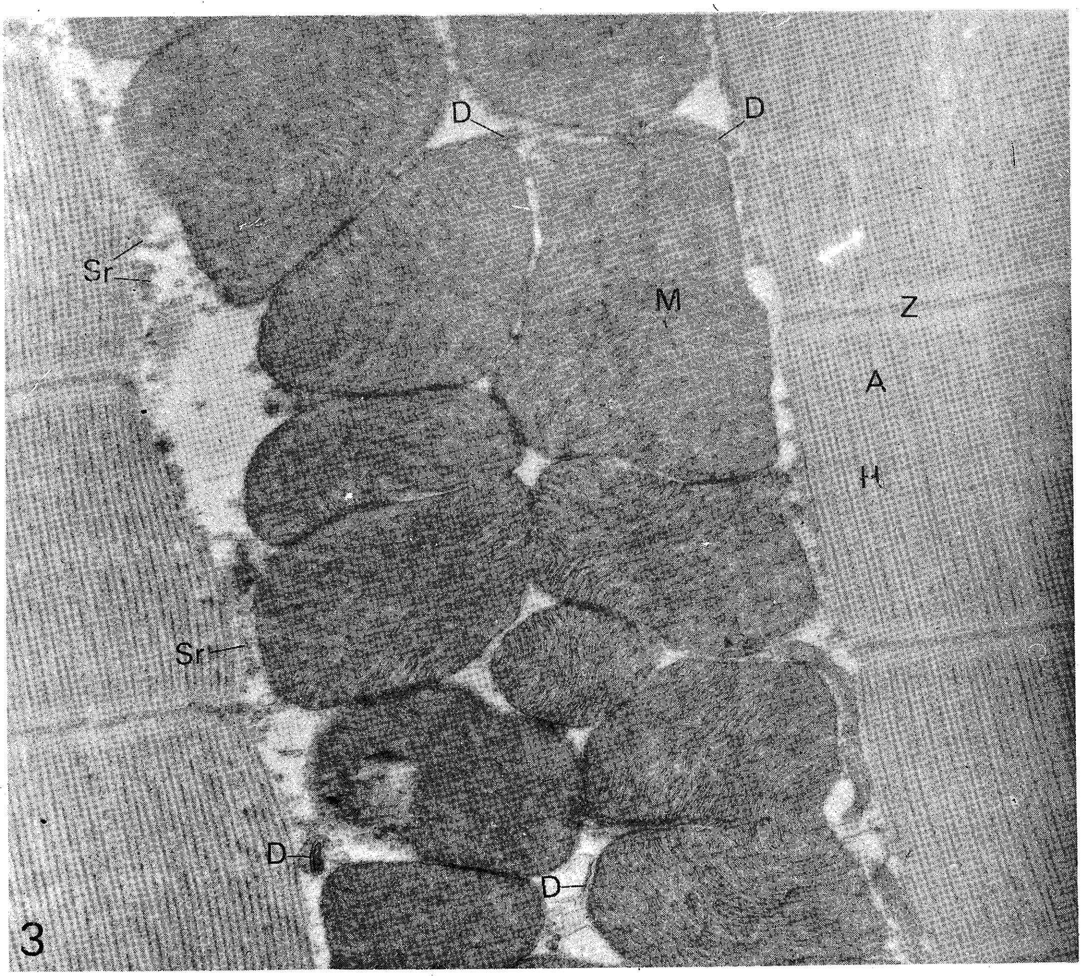
2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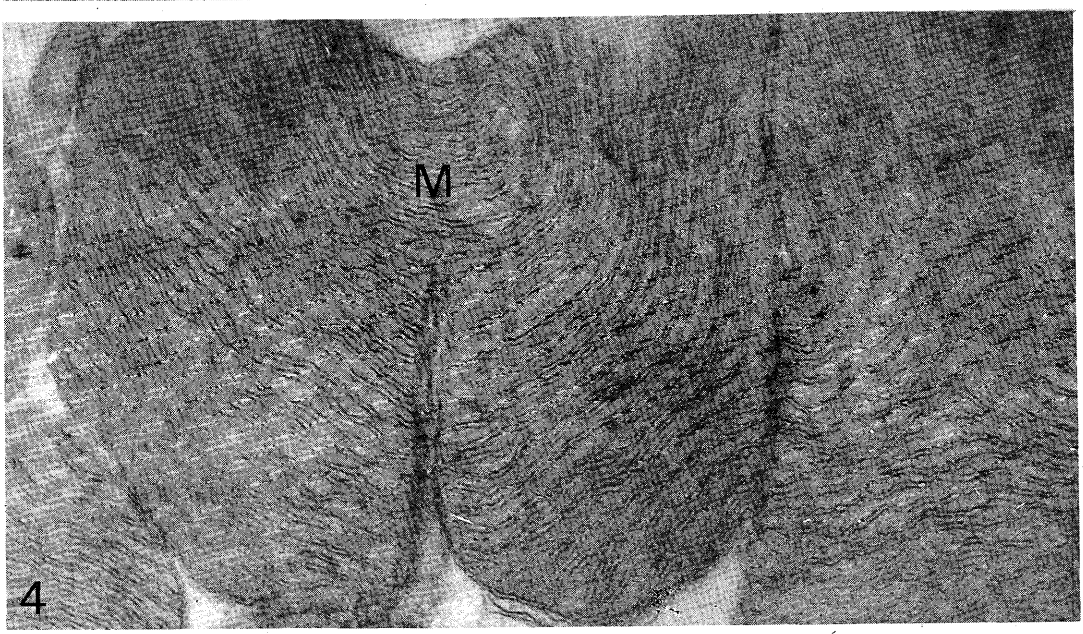
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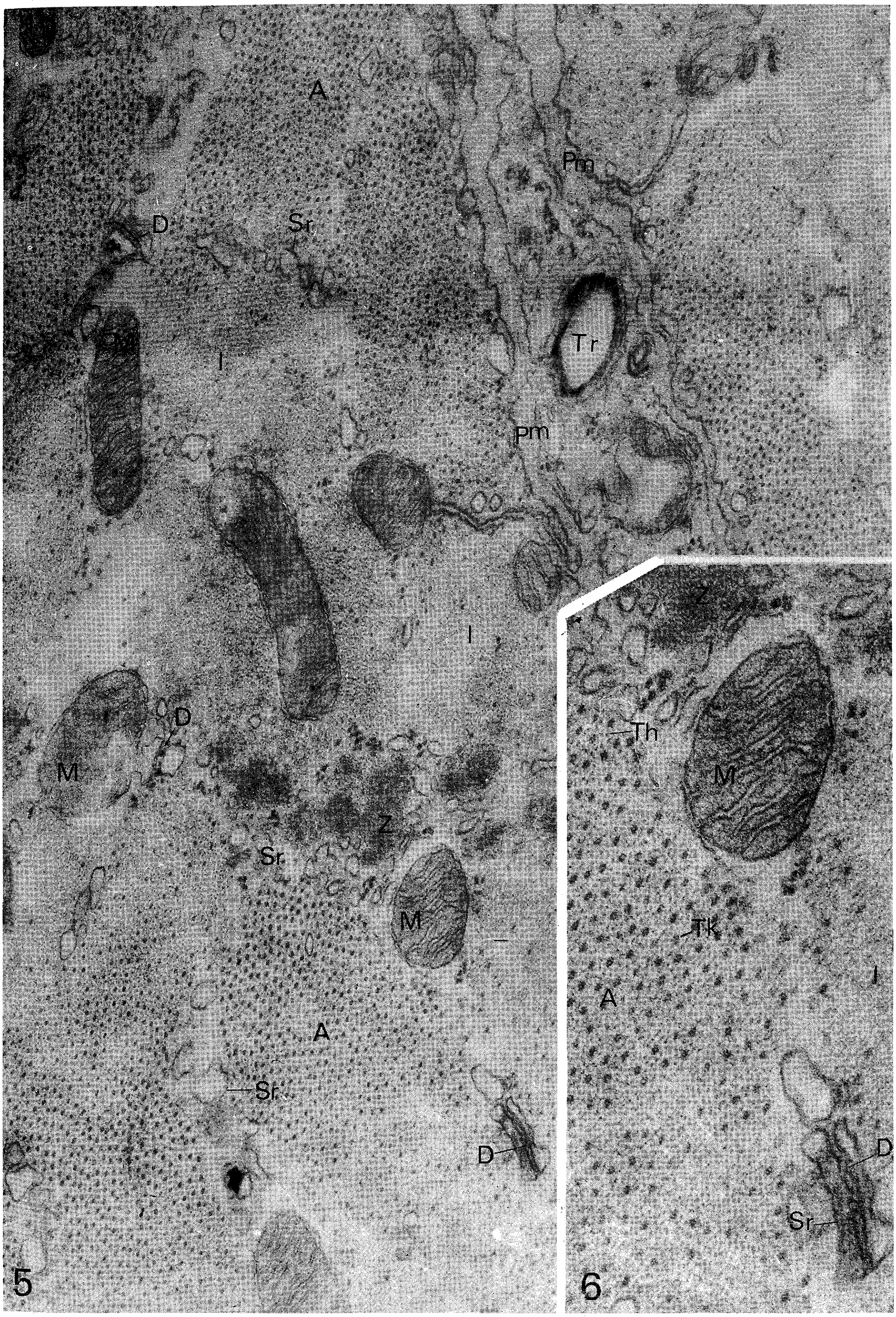
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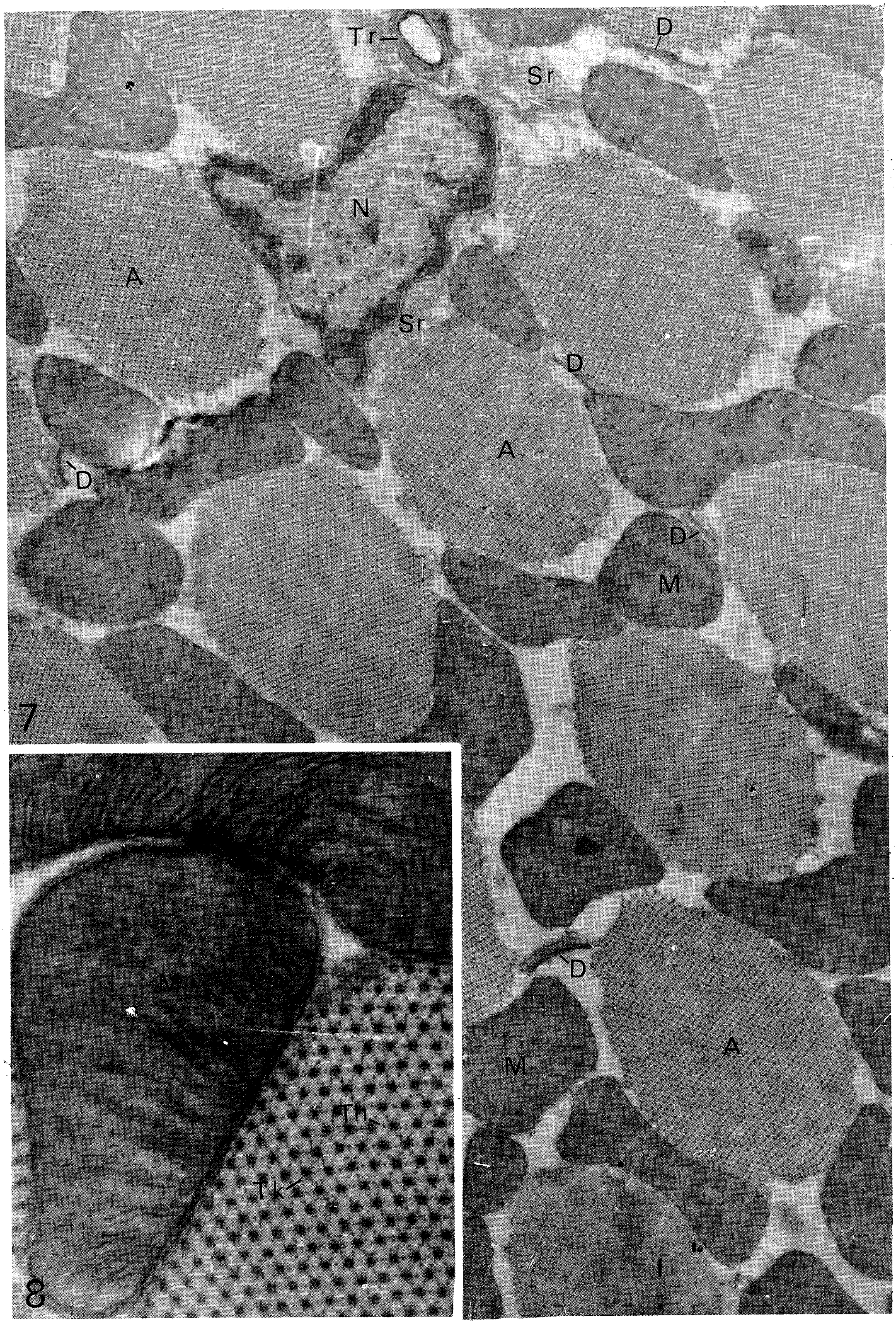

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