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AN ULTRASTRUCTURAL STUDY ON THE HISTOLYSIS OF THE LARVAL MUSCLE IN *TENEBERIO MOLITOR* L.

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ABSTRACT

W. Y. Lee and Y. C. Lin (1970) *An Ultrastructural Study on the Histolysis of the Larval Muscle in* Teneberio molitor *L.* Bull. Inst. Zool., Academia Sinica 9(1): 27-38. The degeneration of the dorsal longitudinal muscle of the larval metathorax in *Tenebrio molitor* L. starts from the prepupal stage, but the muscles definitely show degeneration in the pupal stage, and completely degenerated at about 52 hours after pupation. The myofibrils are greatly reduced after pupation, many myofilament disappeared on the third day (48 hours after pupation) and almost disappeared completely when the pupa was 52 hours old. The sarcosomes migrate into the intermyofibrillar spaces at the level of A-bands and break down into small fragments in the prepupal stage. A big vacuole with disordered cristae is characteristics of the sarcosome after pupation. The number of sarcosomes is greatly decreased in the third day pupa, and they become the sarcosomal fragments in the muscle mass of the 52 hours old pupa. During the process of muscle degeneration, besides the glycogen granules, some lysosome-like objects and lipid inclusion can be found in the degenerated fibrils. The myoneural junction also can be found. The degeneration of the muscles which may be explained by erosion of myofibrils, the degeneration of intracellular organelles and cytolysis is discussed.

At the outset of insect metamorphosis, many special tissues and organs of the larvae will break down. The tissues or organs of larval type are transformed into new adult type, and at the same time, some new tissues are also formed.

Degeneration of muscles is by no means limited to insects, even in the insects not limited to the process of metamorphosis. Gutmann (7) observed the macroscopic changes and weight loss of muscles after denervation. He also studied the histological alteration in muscles 2-3 days after denervation (8). Pellegrine and Franzin (20) in the electron microscope study, described the process of the muscular atrophy induced by denervation in the red and white skeletal muscle fibers of rats. The degeneration of flight muscles in several adult insects was studied, such as queen ants by Janet (12), mosquitoes by Hocking (9), and aphids by Johnson (13). Stegwee *et al* (22) have described physiological and cytological phenomena of the temporary resorption of the flight muscles in diapausing potato beetles. Edwards (3) found that the histolysis of the flight muscle in the female *Dystercus* bug is related to reproduction, and is controlled by food and mating. The breakdown of the larval muscles during metamorphosis was studied in mosquitoes by Hulst (10), blowfly by

Evans (4), *Drosophila* by Robertson (21), *Thymalus* beetle by Breed (1), *Callandra* beetle by Murray and Tiegs (19) and *Rhodnius* bug by Wigglesworth (24). Finlayson (5) found that denervation induces the degeneration of the abdominal muscle in *Saturniid* moths during pupation. Lockshin and Williams (18) found that the intersegmental muscles in abdomen of the pernyi silkmoth degenerate within 28 hours after the ecdysis.

The degeneration of larval muscle of *Tenebrio* beetles was observed with light microscope in this laboratory (16). From the anatomic studies of this insect (17), one part of the dorsal longitudinal muscle of the larval metathorax is transformed into indirect flight muscle, and the other is broken down during metamorphosis. In this report, the process of degeneration in larval muscle studied with electron microscope is described.

MATERIALS AND METHODS

The larvae and pupae of *Tenebrio molitor* L., bred in the flour-yeast medium, were employed for the present investigation.

When the fully grown larvae become quiescent, a faint pigment mark is observed through the cuticule at the anterior lateral area of each abdominal notum. These quiescent larvae are considered to be in the first stage of prepupa. As these marks become prominent, the quiescent larvae are considered to be in the second stage of prepupa. In general, the duration of the first prepupal stage lasted about 48 hours. The prepupae of the second stage molt to become pupae in 24 hours.

The newly molt pupae were considered as the first day pupae in this investigation. Newly molt pupae were sacrificed at 24-hour intervals until they reached 4 days old (72 hours after the pupation). Additional fixations of the pupae were made at 2-hour intervals from 48 hours to 72 hours after the pupation.

The electron microscopic techniques modified by Kiyoshi Hama were employed. The dorsal longitudinal muscles of the metathorax, from prepupal to pupal stages were fixed and dissected in cold 6% glutaraldehyde with 0.1 M cacodylate buffer. The tissues were then washed in cold 8% sucrose solution and post-fixed in cold 2% osmium tetroxide. Dehydration was made in ethanol and propylene oxide. For embedding the Epon resin was utilized. The Epon blocks were sectioned on a Sorvall Porter-Blum MT-2 Ultra-microtome and the sections were stained with uranyl acetate and lead citrate. They were then examined with a Hitachi Electron Microscope Tpye 11-A.

RESULTS

I. Prepupal Stage:

In the electron micrographs (Fig. 1, 2) drosal longitudinal muscles of the prepupae do not show much difference from that of the mature larva (16). The myofibrils of prepupal muscles are still closely packed. I-bands (I, Fig. 1, 2), Z-bands (Z, Fig. 1, 2) and A-bands (A, Fig. 1, 2) are well defined. The sarcoplasmic reticulum (Sr, Fig. 1, 2) is preserved at all levels, but the dyads (D, Fig. 1, 2) appear to be degenerated. There are only a few sarcosomes (M. Fig. 1, 2) located in I-bands (I, Fig. 1, 2). Some of these long rod-shaped sarcosomes (M, Fig. 1, 2) are migrated to the intermyofibrils at the level of A-band (A, Fig. 1, 2). Both in the first and the second prepupal stages, these sarcosomes are broken down into several small oval-shaped fragments. The small lysosome-like objects (L, Fig. 1, 2) and lipid inclusions (P, Fig. 1, 2) have been noted between myofibrils from the first prepupal stage. The glycogen granules (G) heavily stained by lead citric or uranyl acetate in the preparations are

irregularly distributed and appear more in the second prepupal stage (G, Fig. 2) than the first prepupal stage (G, Fig. 1).

II. Pupal Stage:

After pupation, the diameter of the fibrils is greatly reduced. The interfibrillar spaces are enlarged. From the electron micrographs of one or two days old pupa, the A-bands (A, Fig. 3, 4) the I-bands (I, Fig. 3, 4) and Z-bands (Z, Fig. 3, 4) are still recognizable. The sarcosomes (M, Fig. 3, 4) are scattered at all levels of the fibrils and are mostly oval in shape. Some of the sarcosomes are oval-shaped and mostly with vacuolated out-pouching. In others, the cristae of sarcosome are arranged in a more parallel fashion but some are in disorder. It is possible that this is an indication for progressive sarcosome degeneration. The lysosome-like objects (L, Fig. 3, 4) are also found between myofibrils. Droplets of lipid inclusion (P, Fig. 3, 4) are scattered in the region of the sarcoplasmic reticulum. The glycogen granules (G, Fig. 3, 4) are increased in number and scattered in the sarcoplasm.

Degeneration of the larval muscles in the third day pupa is well under way. The myofibrils structure is shown in Figure 5. Many myofilaments (Mf, Fig. 5) have disappeared, and been replaced by the sarcoplasmic reticulum (Sr, Fig. 5) and glycogen granules (G, Fig. 5). The interruption of Z-bands (Z, Fig. 5) can be explained in terms of histolytic changes in the fiber. The plasma membrane (Pm, Fig. 5) is infolded, so that the peripheral sarcoplasm appears to be pinched off from the fiber. The sarcosomes are of oval or much elongated oval shaped and show the changes of degeneration. In a few sarcosomes, cristae accumulated to make room for a hyalin vesicle. The lysosome-like objects (L, Fig. 5) and lipid inclusions (P, Fig. 5) are present in the degenerating region. The myoneural junction is filled with synaptic vesicles which are structures of concentric osmiophilic membrane (O, Fig. 5), in the sarcoplasm. The nuclei are elongated. The distribution of chromatin is irregular, with dense areas at the periphery.

A muscle fiber of the 52 hours (3 1/6 days) old pupa is shown in Figure 6. Almost no fibrillar structure remains, there is only a few slender filaments (Mf, Fig. 6) that trail off the isolated Z-bands (Z, Fig. 6). Sarcosomes are mostly elongated and fewer in number than in any previous period. In this stage, the lysosome-like objects (L, Fig. 6) are obviously closely associated with the degenerating sarcosomes. It seems that the sarcosomes are degraded by the lysosome-like objects. Several osmiophilic dense post-synaptic vesicles are present in the peripheral area of the degenerating fiber. The sarcoplasmic reticulum (Sr, Fig. 6) is now dispersed between the remaining Z-bands (Z, Fig. 6) and near the synaptic vesicle, but the sarcoplasmic reticulum appears similar to that before the onset of degeneration. A few number of dyads (D) can be found in this stage.

Figures 7 and 8 also show the muscle fiber of the 52 hours (3 1/6 days) pupa. Figure 7 shows that muscle fiber is completely degenerated and loses all its organization. It becomes a structureless, acidophilic mass with plasma membrane (Pm, Fig. 7), lysosome-like objects (L, Fig. 7), fragments of sarcosomes (M, Fig. 7) and glycogen granules (G, Fig. 7). In figure 8, the muscle is gradually broken down into small fragments which will fall into haemocoel.

DISCUSSION

Muscle degeneration is not uncommon in insects. Stagwee *et al* (22) reported that the degeneration of flight muscle of the colorado potato beetle, *Leptinotarsa decemlineata*, could be produced by extirpation of the postcerebral complex of endocrine glands, the corpora cardiaca and the corpora allata. In the experiments carried

out by Finlayson (5, 6) on *Saturniids*, the abdominal muscles which normally break down only in the adult insect were caused to break down in the pupa by denervation or by removing the ganglia from the segment or from the preceding segment containing the muscles. He (6) also found in *Saturniids* that the removal of the second or third abdominal ganlia from the spinning larvae causes the muscle in the third segment to degenerate. Finlayson (6) suggested that both the withdrawl of neural influence from the muscles and hormone balance probably play a part in muscle histolysis. However, Wigglesworth (24) found that the break down of the intersegmental muscles in *Rhodnius* was independent of innervation. He suggested that it is controlled by hormonal means. Johnson, (14) working on the degeneration of flight muscles in a late aphids, suggested that the hormonal environment is important in muscle breakdown.

The dorsal longitudinal muscle of larval metathorax in *Tenebrio molitro* L. consists of 2 layers of fibers, the outer fibers which lie near the body wall, are tranfromed into the adult muscles during pupal stage, while the inner fibers undergo complete histolysis in prepupa and the first three days pupa (15). If inner muscle histolysis is played by hormone balance, why outer muscle fibers do not degenerate? The breakdown of the muscles in this study agreed with the suggestion proposed by Finlayson (5, 6). However, it may also be accounted by the erosion of myofibrils, the degeneration of the intracellular orgenelles and cytolysis.

In the prepupal stage the degenerating fibrils still maintain a fairly good structure, some sarcosomes migrate into the intramyofibrillar spaces at the level of A-bands. There are numerous membrane limited osmiophilic bodies and the small lipid inclusion in the muscles.

Degeneration of myofibrils occurs during the first two day after pupation. The myofibrils decrease in their diameter and are replaced by glycogen granules and sarcoplasmic reticulum. In the third day pupa characterized by the disappearance of myofilaments, it seems some destructive proteins have spread in the muscle fibrils, but Z-bands apparently resist the attack, and in the periphery of the plasma membrane. Fifty-two hours after pupation, the plasma membrane infolds markedly and the muscle fibril appears to be separated into a large pieces of fibril fragments which will be discarded into the haemocoel. There is no evidence for the involving of phagocytosis in the muscle degeneration.

As regards to sarcosomes, they migrated from I-bands into the intermyofibrils at the level of A-bands, then disintegrate into small sphere-shaped fragments and then disappeared gradually. After pupation sarcosomes are swollen to form big vacuoles. Cristae decreased in number or formed an accumulated mass in the vacuolized sarcosomes. Later, sarcosomes decrease in number roughly in proportion to the reduction of the contractile material in the fibers. De Robertis *et al* (2) indicated that there are three types of degeneration in mitochondria: (i) fragmentation into granules followed by lysis and dispersion; (ii) intense swelling with transformation into large vacuoles; (iii) large accumulation of materials with transformation of mitochondria into hyalin granules. Trandler *el al* (23) reported that the degenerative changes of sarcosomes include swelling, disruption and complete loss of the cristae, and rarely fraction of the matrix. The process of sarcosome degeneration during the prepupal stage in the present studies is similar to that of the first type as described by De Robertis *et al*. The degenerative processes of the first two days of the pupae are similar to that of the second

and the third types of De Robertis. Some of the sarcosomes, after pupation, are associated with the formation of the lysosome-like objects. Stegwee *et al* (22), Lockshin and Williams (18) observed the formation of a starshaped deposit of lipoprotein, in degenerating muscles and interpreted that these objects are degenerated mitochondria. Our evidence suggests that these lysosomes-like objects probably are similar to the star-shaped bodies derived from the mitochondria.

The appearance of the lysosome-like objects started at the prepupal stage and increased in size and number after pupation. These organelles resembled structures observed in the denervated rat muscle (20), and in the degenerated intersegmental muscle of the pernyi silkmoth (18). The lysosome has ever been detected by electron microscope. Jackson (11) suggested that lysosome might involve in resorption and degradative processes in connective tissue. Therefore, a clear correlation can be established between the reduction and degeneration of the muscle components and the appearence of lysosome-like objects.

The nuclei show no changes although changes are going on within the cytoplasm. When the muscle is in process of degeneration, only some nuclei become pycnotic and some appear to be unaffected even in the 52 hours pupa. In *Rhodnius* (24) and *Leptinotarsa* (22), certain muscles are resorbed, and later, regenerated, but the nuclei are undisturbed by the marked cytoplasmic changes. The muscle in this present study is unable to regenerate.

In conclusion, the degeneration of the dorsal longitudinal muscles of larval metathorax in *Tenebrio molitor* L. similar to the intersegmental muscles of pernyi silkmoth, is resulted by the intervention of the destruction of sarcosomes.

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REFERENCES

1. Breed, R. S.: (1903) The changes which occur in the muscles of a beetle, *Thymalus marginicollis* Chev. during metamorphosis. *Bull. of Mus. of Comparative Zool. at Harvard College Vol. XI No. 7.*
2. De Robertis, E. D. P., W. W. Nowinski and F. A. Saez: (1960) "General Cytology, Ch. 7." *W. B. Saunders Co. Philadephia Pa.* pp. 169-197.
3. Edwards, F. J.: (1969) Environmental control of flight muscle histolysis in the bug, *Dysdercus intermedius. J. Insect Physiol.* 15: 2013-2020.
4. Evans, A.: (1936) Histolysis of muscle in the pupa of the blowfly *Lucilia sercata* Meig. *Proc. Roy. Ento. Soc.* (*A*) 11: 52-59.
5. Finlayson, L. H.: (1956) Normal and induced degeneration of abdominal muscles during metamorphosis in the Lepidoptera *Quart. J. Mscro. Sci.* 97: 215-234.
6. Finlayson, L. H.: (1960) A comparative study of the effects of denervation on the abdominal muscles of *Saturniid* moths during pupation. *J. Ins. Physiol.* 5: 108-119.
7. Gutmann, E.: (1945) The reinnervation of muscle by sensory nerve fibers. *J. Anat.* 79: 1-28.
8. Gutmann, E.: (1948) Effect of delay of innervation on recovery of muscle after nerve lesion. *J. Neurophysiolgy* 11: 279-290.
9. Hocking, B.: (1954) Flight muscle outoysis in *Aedes communis. Mosquito News* 14: 121-123.
10. Hulst, F. A.: (1906) The histolysis of the musculature of *Culex pungens* during metamorphosis. *Biol. Bull. Woods Hole* 11: 277-304.
11. Jackson, S.F.: (1964) "Connective tissue cell." in "The Cell, Vol. VI" ed. by Bracket, Jean and A. E. Mirsky P. 387-590.
12. Janet, C.: (1907) Anatomie du Corselet et Histolyse des Muscles Vibrateurs apres. *Le Vol. Nuptial Chez la Reinede la Fourini* (*Lasius niger*) *150 pp du Courtieax et Gout Limoges.*
13. Johnson, B.: (1957) Studies on the degenera-

tion of flight muscle in alate aphids. I. A comparative study of occurrence of muscle breakdown in relation to reproduction in several speices. *J. Insect Physiol.* 1: 248-256.
14. Johnson, B.: (1959) Studies on the degeneration of flight muscle in alate aphids. II. Histology and control of muscle breakdown. *J. Insect Physiol.* 3: 367-377.
15. Lee, Wen-Yung: (1964) A study of development of the thoracic musculature from larvae to adults in *Tenebrio molitor* L. *Ph. D. Thesis in the University of Minnesota, U.S.A.*
16. Lee, W. Y. and K. K. Chang: (1968) A histological study of development of the thoracic musculature from the larvae to adults in *Tenebrio molitor* L. (*Tenebrionitae, Coleoptera, Hexapoda*). III. A study on the developement of thoracic musculature at the pupal stage. *Bull. Inst. Zool. Academia Sinica.* 7: 71-81.
17. Lee, W. Y., 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.
18. Lockshin, R. A. and Carroll M. Williams: (1965) Programmed cell death. I. cytology of degeneration in the intersegmental muscles of the *Pernyi* silkmoth. *J. Ins. Physiol.* 11: 123-133.
19. Murray, R. V. and O. W. Tiegs: (1935) The metamorphosis of *Calendra oryzae. Quart. J. Micro. Sci.* 77: 405-495.
20. Pellegrino, C. and C. Franzini: (1963) An electron microscope study of denervation atrophy in red and white skeletal muscle Fibers *J. Cell Biol.* 17: 327-350.
21. Robertson, C. W.: (1936) The metamorphosis of *Drosophila melanogaster,* including an accurately timed account of the principal mrophological changes. *J. Morph.* 59: 351-3?0.
22. Stegwee, D., D. C. Kimmel, J. A. Boer and S. Henstra: (1963) Hormonal control of reversible degeneration of flight Muscle in the colorado potato beetle *Leptinotarsa decemlineata* Say (Coleoptra). *J. Cell Biol.* 19: 513-527.
23. Tandler, B., R. A. Erlandson, A. L. Smith and E. L. Wynder: (1969) Riboflavin and mouse hepatic cell structure and function. II. Division of mitochondria during recovey from sample deficiency. *Anat Rec.* 151: 427-440.
24. Wigglesworth, V. B.: (1956) Formation and involution of striated Muscle fibers during the growth and moulting Cycles of *Rhodnius prolixus* (Hemiptera) *Qnart. J. Micro. Sci.* 97: 465-480.

Fig. 1. An electron micrograph of muscle fiber from the first prepupal stage. The structure of muscle fiber is almost the same as normal larval muscle. A-bands (A), I-bands (I) and Z-bands (Z) are distinct. Some sarcosomes (M) are migrated to the intermyofibrillar space at the level of the A-band and break into small fragments, others are remained in I-bands. The lipid inclusion (P) and the lysosome-like objects (L) are present. D, Dyads. Sr, Sarcoplasmic reticulum. G, Glycogen granules. 15,000. x

Fig. 2. An electron micrograph of muscle fiber from the second prepupal stage It appears similar to Fig. 1. Ds, Degenerating sarcosome. 15,000. x

Fig. 3. A section of muscle fiber of the first day pupa. The myofibrils decrease their diameter. However, I-bands (I), Z-bands (Z) and A-bands (A) can be identified. Degenerating sarcosomes (a,b,c,—g,) are not uncommon. Numbers of the lysosome-like objects (L) and the glycogen granule (G) increase. P, Lipid inclusion. Sr, Sarcoplasmic reticulum. D, Dyads. 15,000. x

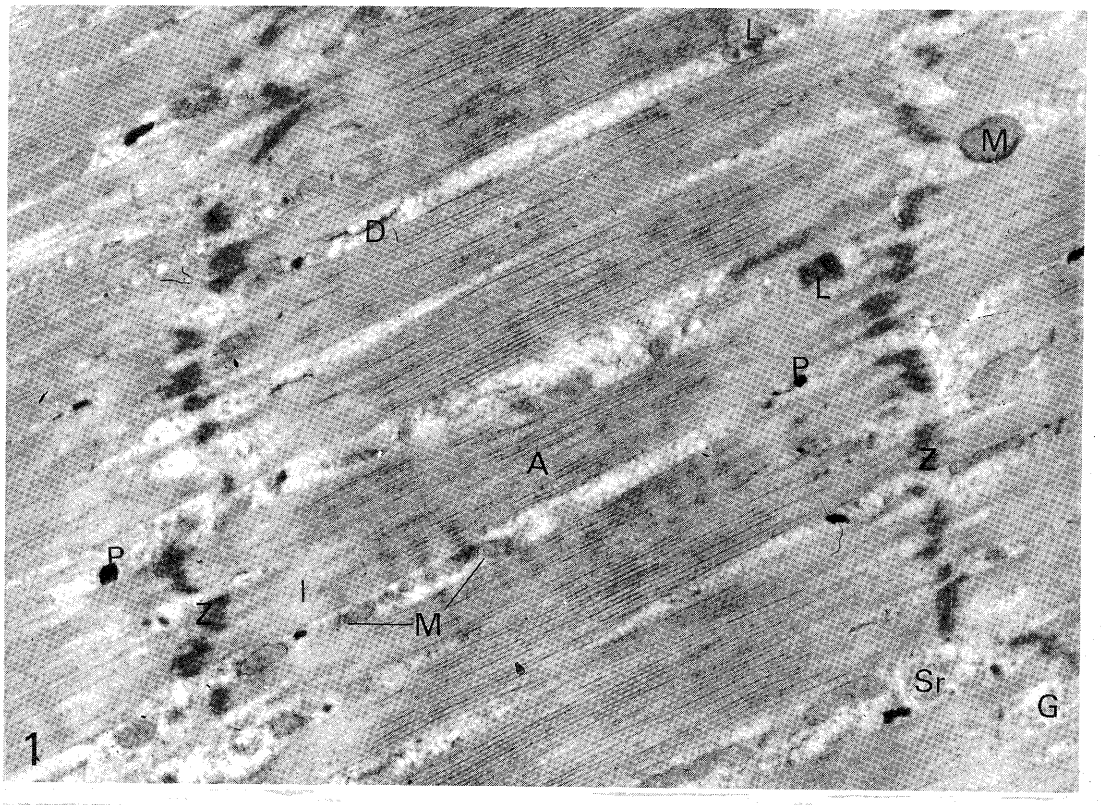
Fig. 4. A section of muscle fiber of the second day pupa. The myofilaments greatly reduced in number. The degenerating sarcosomes (a,b,c,—) swell to form a big vacuole. A, A-bands. I, I-bands. Z, Z-bands. L, Lysosome-like objects. P, Lipid inclusions. 15,000. x

Fig. 5. An electron micrograph of the muscle fiber of the third day pupa. Degeneration is well under way. Many myofilaments (Mf) have disappeared, number of sarcosome is much reduced. The sarcosomes (a, e) are still in good shape, but the cristae begin to degenerate, sarcosome (e) becomes a hyalin vacuole. The plasma membrane (Pm) is infolded. Z, Z-bands. Sr, Sarcoplasmic reticulum. P, Lipid inclusion. O, Myelin figures. L, Lysosome-like objects. G, Glycogen granules. D, Dyads. 15,000. x

Fig. 6. An electron micrograph of the muscle fiber of 52 hours old pupa. The orientation of the myofibrils can be determined from the remnants of Z-bands and A-bands and the myofilaments (Mf.) The lysosome-like objects (L) are closely associated with the degenerating sarcosome (M), some materials are jettisoned from the sarcosome to lysosome-like object. The plasma membrane (Pm) is deeply infolded. O, Myalin figures of the myoneural junction. D, Dyads. 15,000. x

Fig. 7. An electron micrograph of the muscle fiber of 52 hours pupa. The muscle fiber is completely destroyed to become a disorganized cytoplasmic mass. There are some degenerating sarcosomal fragments, the lysosome-like objects and the plasma membrane (Pm) remained. The muscle mass is in histolysis. 30,000. x

Fig. 8. An electron micrograph of the muscle fiber of 52 hours old pupa. The muscle mass has been broken down into small pieces. Pm, Plasma membrane. L, Lysosome-like object remnants. 20,000. x



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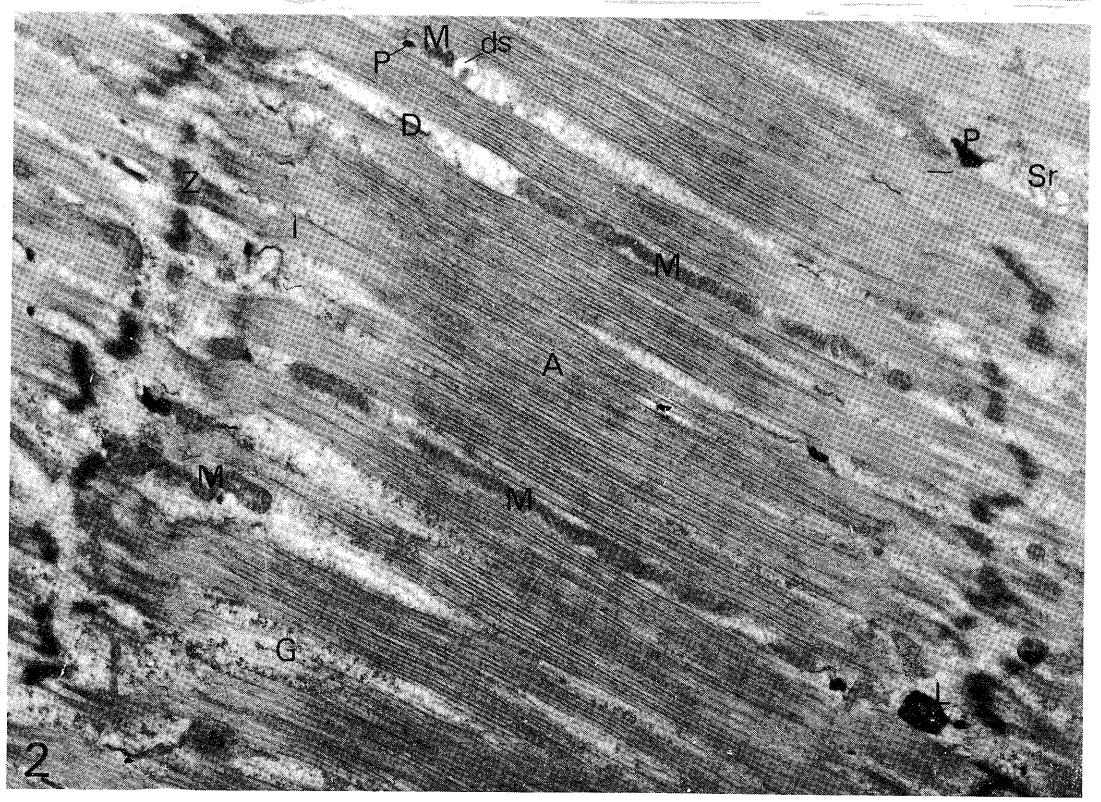

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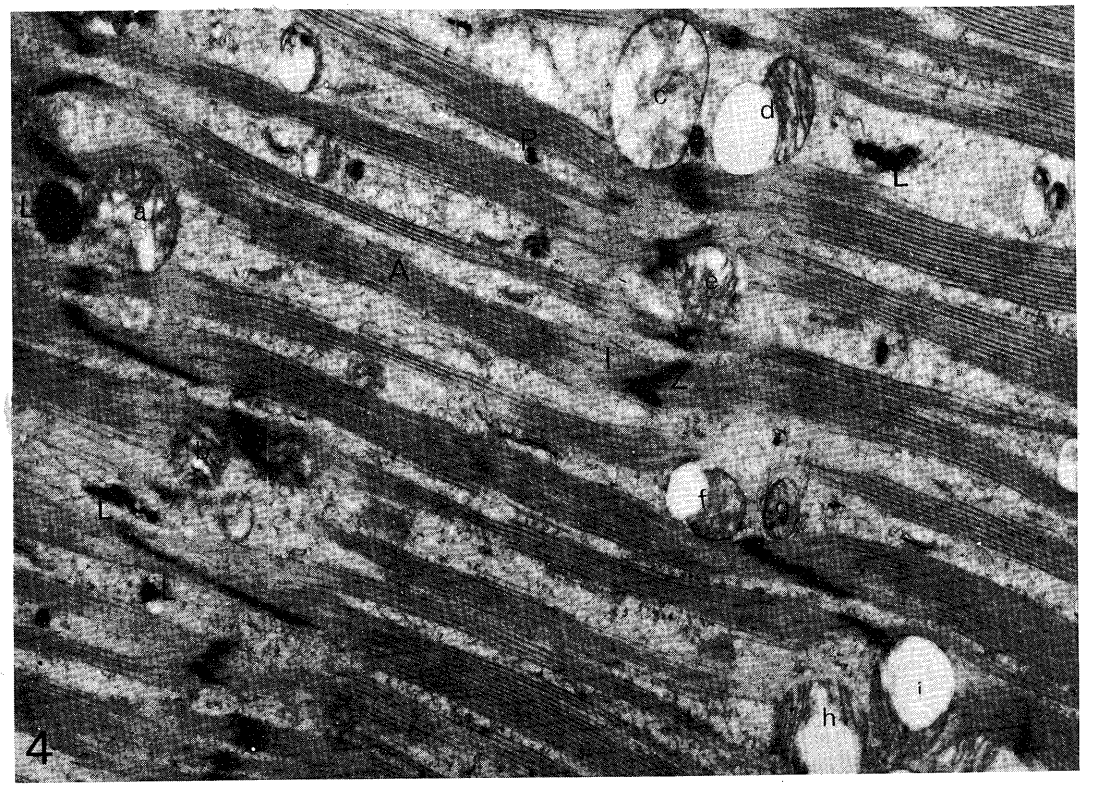
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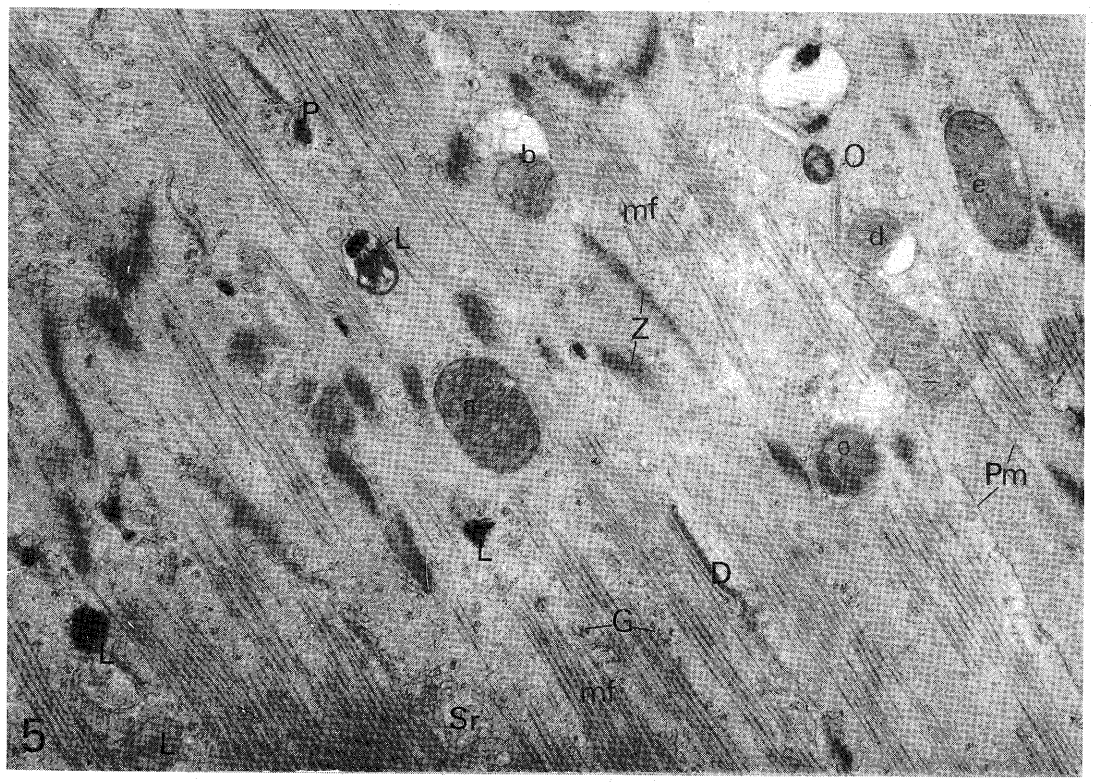

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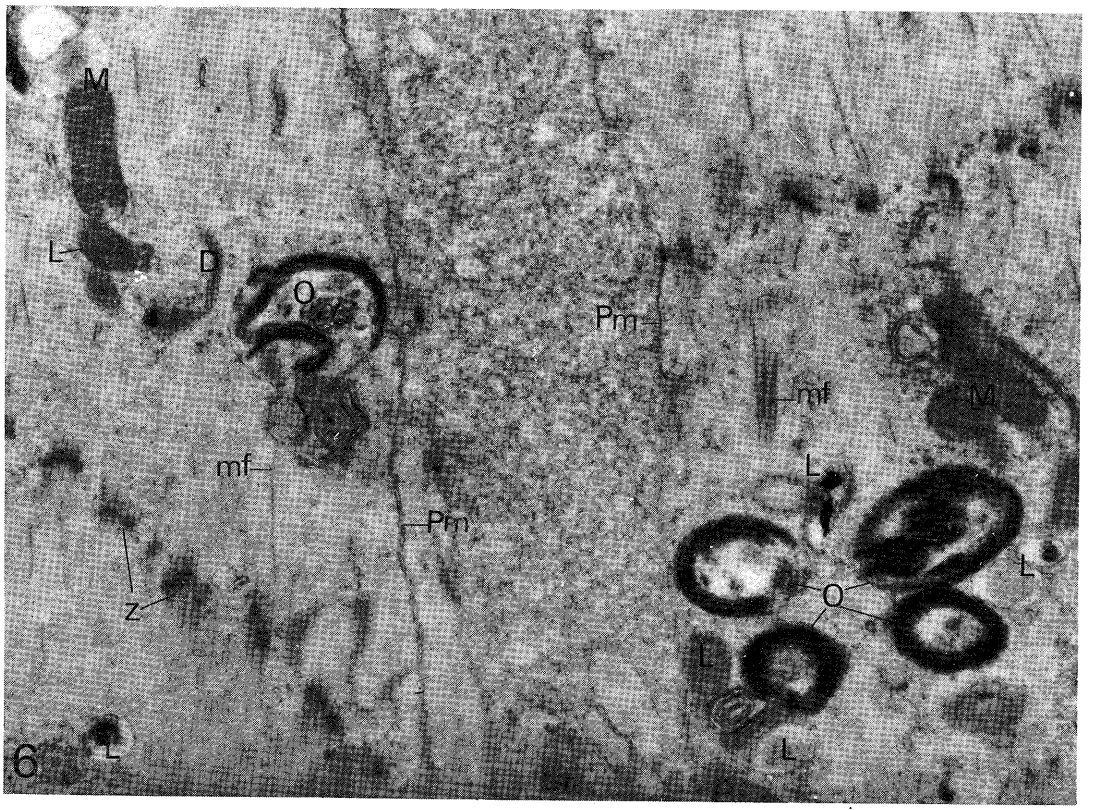

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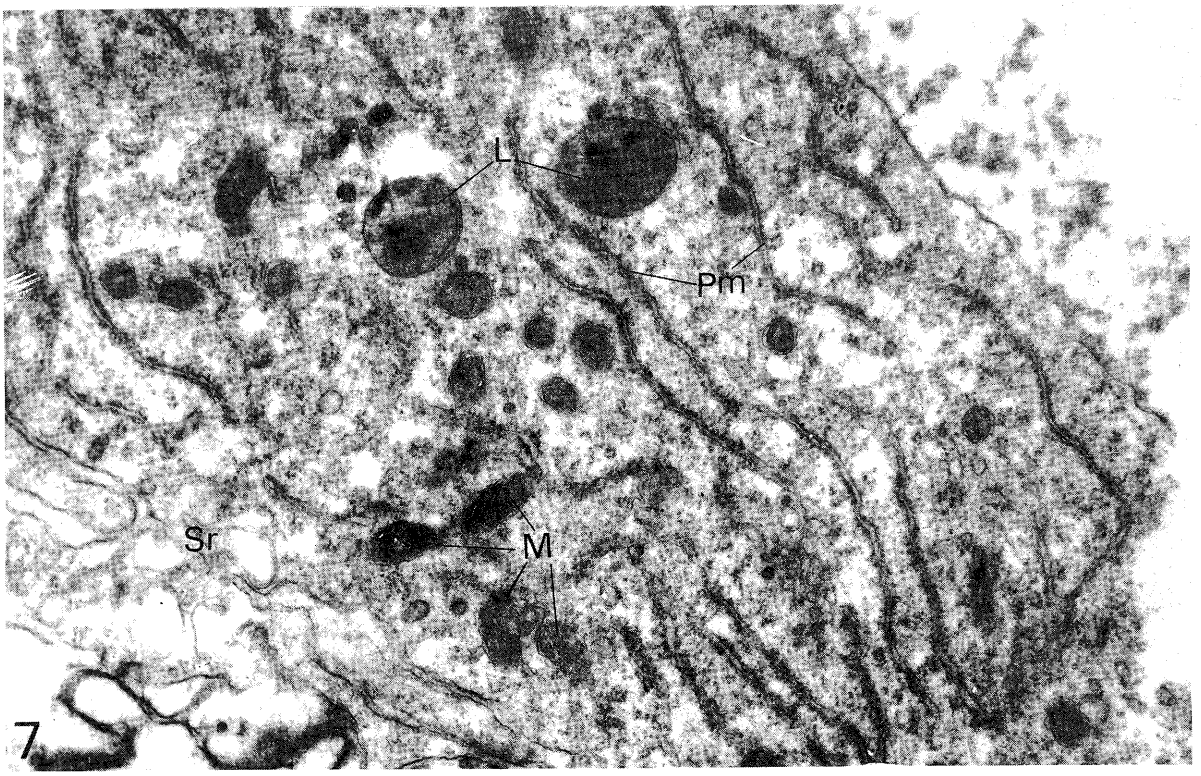

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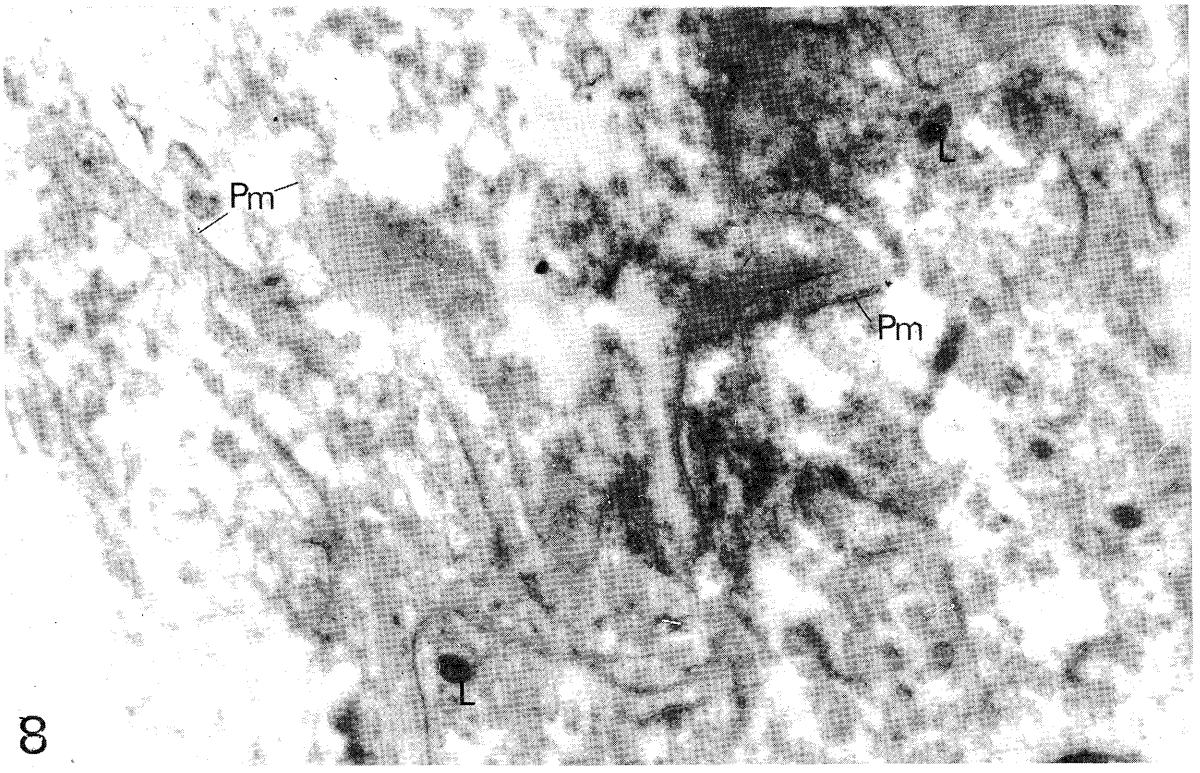

8