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A HISTOLOGICAL STUDY OF DEVELOPMENT OF THE THORACIC MUSCULATURE FROM THE LARVAE TO ADULTS IN *TENEBRIO MOLITOR* L. (TENEBRIONIDAE, COLEOPTERA, HEXAPODA).

III. A STUDY ON THE DEVELOPMENT OF THORACIC MUSCULATURE AT THE PUPAL STAGE.[1]

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ABSTRACT

The histological changes of the thoracic musculature in *Tenebrio molitor* L. during metamorphosis has been investigated with the method of paraffin section. The prepupal and pupal stages were studied.

During the metamorphosis of the larvae of *T. molitor* into the adults, most of the larval muscles transform into the adult muscles and few degenerate, while many imaginal muscles are formed from cells of imaginal bud during the pupal stage.

INTRODUCTION

During metamorphosis many specialized tissues and organs of the larva are broken down and their constituent substances reinvested in the formation of of specialized pupal structures. Later, the process repeats itself as the pupa is transformed into the imago. Lockshin and Williams (9) have stated that metamorphosis is an orderly and predictable pattern of birth and death at the cellular level. In this paper are described observations and conclusions concerning the muscular changes found in the thoracic segments of *Tenebrio molitor* L. during metamorphosis.

The literature pertaining to insect is repleted with references to muscular changes during metamorphosis. Wigglosworth (14) has studied the formation of striated muscle fibre in Hemiptera. Korn (5) has described the musculature of the head and the thorax in Neuroptera during metamorphosis. The growth of flight muscle in Diptera has been studied by Shatoury (13) and Hinton (2). Metamorphosis of Hymenoptera has been observed by Oertel (6). The metamorphosis of one kind of Lepidoptera has been reported by Hufnagel (4). In Coleoptera, Murrey and Tiegs (10) studied the metamorphosis of *Calamdra oryzae* and Breed (1) the *Thymalus marginicollis* during metamorphosis.

MATERIALS AND METHODS

Specimens for this study were reared in the laboratory as described previously (7).

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After the larva has fully grown, it stops feeding and becomes quiescent. Meanwhile a pigment mark begins to appear gradually from beneath the cuticle in the anterior lateral area of each abdominal notum. The appearance of this mark of the resting larva is considered in the present study to designate the prepupal stage. After one day the prepupa molts to become the pupa.

Specimens from each day of seven days pupal stage were examined in this study. They were fixed in Bouin-Duboscq solution, dehydrated *via* a butanol series and embedded in tissue-mat (m. p. 60°C). The sections were cut at 6-10μ and stained with Delafield-haematoxylin and 0.5% eosin in 95% alcohol.

The preparation were viewed with 40x plan achromatic and 100x plan achromatic oil immersion lens of an Olympus research microscope. Photographs were made with a Asahi pentax SV camera.

The names of muscles for this study are those termed by Lee (6).

RESULTS

Several types of skeletal muscle fibres are found in the 3 segments of thorax of adult and the larval stages of *T. molitor* (13 and 14). The muscles of the larval type include all of the thoracic muscles of the larva; these muscles are composed of few relatively large fibres with a well marked sarcolemma and usually with nuclei at the periphery of the fibres scattered in the sarcoplasm. The muscles of the fibrillar type are only found in the direct and indirect flight muscles of the metathorax of the adult; they are composed of very large fibres with nuclei scattered throughout their substance. The muscles of the general type are presented in the most muscles of three segment thorax in the adult stage; the muscles are composed of numerous small fibres frequently arranged in a penniform or bipenniform manner and attached by a common tendon; nuclei are found at the surface of the fibres. A few muscles in the prothorax of the adult are termed by Lee and Chang (7) as the tubular general type; the nuclei arranged in a row along the axis of the fibres but the sarcostyle does not show radial structure in the cross section.

Two kinds of myoblasts, which from the imaginal muscles, can be found in the late instar of the larva: (a) Masses of isolated cells related to the imaginal buds of legs and wings. They multiply throughout the larval stage. (b) Small cells which adhere to or penetrate into certain larval muscle fibres.

I. Metamorphosis of Larval Muscles into Adult Muscles.

(A) The formation of the fibrillar muscles.

All flight muscles except the dorsal longitudinal medium muscle undergo rapid and complete change when the larva ceases feeding and the wing bud everts. In the prepupal stage the larval muscle bundle divide lengthwise into from four to thirty strands, the division being complete at this stage. Figures 1 and 2 show the cross and the longitudinal sections of the larval muscle; Figures 3 and 4 show, respectively, cross and longitudinal sections in which division has been completely accomplished. Figure 4 shows the cross section of different size of strands which will form the adult muscle fibres. In Figures 3 and 4, there are many nuclei and spindle shaped cells of myoblasts and the cells of the trachea lying at the periphery of the strands; the sarcolemma of the larval muscle and the cross striation have disappeared entirely. At this time, muscle fibres stain blue.

Up to the second day of pupal stage the muscle nuclei undergo rapid mitotic division. Figures 5 and 6 show that many elongated nuclei and numerous chain of nuclei are present not only at the periphery of the fibre but are deeply embedded in the muscle substance. The larval nuclei scatter at the periphery of the fibres and are going to die. The sarcoplasm of the muscle is found to increase gradually.

Figure 7 shows that new fibrillae appear in the cross section. The nuclei deeply embed in the fibril and lie at the periphery of the muscle surface. During the later half of the pupal life, a number of important changes take place, the most noteworthy being growth in size, the increase of volume of muscles day by day, and the migration and scattering of nuclei throughout the muscle substance (Fig. 8.). The striation of the muscle begin to show on the sixth day of the pupal stage (Fig. 9).

The metamorphosis of the larval muscle into the adult fibrillae muscle of the dorsal median longitudinal muscle are similar to those mentioned above, except that only the destructive period of the larval muscle occurs at the later stage of the pupa. It begins to change while the larva ceases feeding. At the outer row of the larval muscle bundles (Fig. 10), metamorphosis takes place from one muscle bundle to another, and then, the entire rows of muscle are destroyed on the third day of the pupal stage and the adult fibrillar muscles are reconstructed on the sixth day. The inner rows of the larval muscles gradually degenerate and completely disappear on the third day of the pupal life.

(B) The formation of the general muscles:

These muscles may be divided into three groups according to the period in which they begin their metamorphosis. Those of Group I begin their metamorphosis into numerous muscle strands when the larva ceases feeding. The cross striations appear faintly, and the nuclei and the myoblasts lie at the surface of the muscle strand. The structure and the change of the longitudinal section of this muscle are shown in Figures 11 and 12; the muscle strands become divergent by the change of the integument when the insect molts as the pupa. When the muscles grow, they become fan-shaped.

On the third day of the pupa (Fig. 13), nuclei divide rapidly by mitosis and form many chains. Meanwhile, many degenerating larval nuclei lie at the periphery of the muscle fibres. At the same time, in the muscle fibres at the portion near the base, the sarcoplasm much decreases in volume, becomes fibrous and decreases in the number of nuclei. This portion forms the tendon on the sixth day of pupa (Fig. 14) and chitinolize before the emergence of adult. Figures 15 and 16 show the longitudinal section and the cross section of the leg muscle of the adult before emergence respectively.

Group II includes the lateral dorsal oblique muscle of the prothorax and the mesothorax. The muscle begins its metamorphosis as the muscles of Group I when the larva ceases feeding. However, the color of the stain of this muscle is different. In it, nuclei are stained blue and sarcoplasm red even the muscle has already divided into several strands. Up to the pupal stage the color of sarcoplasm changes as blue. The formation of the adult muscle and of the tendon are similar to those of the muscles of Group I.

Group III includes the dorsal longitudinal muscles of the prothorax and

the mesothorax, the ventral longitudinal muscles of three segments, the intersegmental dorsoventral muscle and the intersegmental lateral muscles of the larva. Some bundles of these muscles degenerate, while some transform as the adult muscle. The transformative muscles remain as the larval muscles in the prepupal stage and begin to metamorphosis after larval-pupal ecdysis. They gradually change until the third day of pupa. The reconstructive period of the muscles starts from the second day old pupa, as the nuclei and the myoblasts divide. The elongated nuclei and the numerous chains lying on the muscle fibres are present on the third day of pupa (Fig. 18); the muscle fibres grow afterward. Up to the sixth day of pupa these muscles have already formed developed into the adult muscle. In most of these muscles, nuclei lie at the periphery of the muscle fibre and a few nuclei embed in the sarcoplasm (Fig. 15 and 16).

The dorsal longitudinal muscles and the intersegmental lateral muscle of the prothorax are shown in Figure 19. Cross striations of the muscle are present and the nuclei arrange on a row in the center of the muscle fibres. From these are formed the tubular general muscles.

II. The Histogenesis of the New Adult Muscles.

In the late instar of the larva, the future thoracic region contains two main groups of the imaginal buds. On each side there is a dorso-lateral set of a dorsal mesothoracic elytral and a dorsal metathoracic wing bud. The ventro-lateral group contains the leg buds of each thoracic segment. A few myoblasts associate with these imaginal buds. They divide and appear as spindle shaped cells which develop into fine fibrils and come into association with adjacent epidermal cells (Fig. 20). This region with the epidermal cells marks the place as the muscle origin or insertion. The myoblasts continue to divide and form the fibrils. The epimero subalar muscle of the third day pupa are shown in Figure 21; in these new muscle, fibres are already formed as other muscles. From this time the muscle fibres increase their volume and the nuclei continue to divide. On the sixth day of pupa, the cross striations are formed and the new adult muscles are already developed. Figure 22 shows the longitudinal section of the new adult muscle, the epimero-subalar muscle of the metathorax.

There are nine new muscles formed in the mesothorax and eight new muscles in the metathorax. No muscle is newly formed in the prothorax during metamorphosis.

III. The Histolysis of the larval muscles.

The degeneration of the larval muscles takes place at the prepupal stage and ends before the half life of the pupal stage. The degeneration of the larval muscle, according to the time of histolysis, may be distinguished into two groups. Group I are found in the leg muscle of the larva. In this group, striations have disappeared, the volume of the fibres has decreased and the substances of this degenerating muscle stain blue with haematoxylin when the larva cease feeding; the nuclei undergo chromatolysis, as shown in Figure 23. The degeneration of these muscle fibres is accomplished very repidly. All of these degenerating muscles disappear entirely on the second day of the pupa.

Group II includes the dorsal and the ventral longitudinal muscles and the intersegmental dorsoventral muscles. These muscles appear as a strong larval muscle in the prepupal stage. After the larval-pupal ecdysis, the cross striation

and fibrillation appear faintly. On the second day of the pupa, these muscles are flaccid and poorly contractile. The nuclei also appear undergoing chromatolysis (Fig. 24). On the third day of the pupal stage, they completely disappear.

DISCUSSION

Pringle (12) has listed the different ways in which the indirect flight muscles of insects develop. He includes all types of development under the following six categories: (1) fibre proliferation; (2) from nymphal muscles; (3) fibre cleavage; (4) fibre cleavage and myoblast incorporation; (5) myoblast incorporation on rudiment; and (6) myoblast organization.

Hinton (3) has given five different processes in which the indirect flight muscles of insects grow: (1) Nuclei of rudimentary fibre divide and there is no incorporation of free myoblasts. In many exopterygotan insects, the fibril formation takes place precociously and develops from the fibre rudiment. However, in the higher insects, the fibril formation begins at about the time when nuclear division ceases. The fibre continues to grow in volume until some time after the pupal-adult molt. (2) Free myoblasts are incorporated into the muscle rudiment and the muscle nuclei do not divide, this occurs in some insects of Homoptera. (3) Free myoblasts are incorporated into the muscle rudiment and the muscle nuclei also divide. (4) All myoblasts of the indirect flight muscles are contained within the larval muscle. The flight muscles, even the most general muscles which transform from the larval muscle, develop in this manner. Nuclei of larval muscles degenerate and the myoblasts divide to give rise to the imaginal nuclei. (5) Free myoblasts adhere to or penetrate into degenerating larval muscle. This occurs in some Hymenoptera and Diptera-Cyclorrhaphan.

Breed (1) divides the metamorphosis of larval muscles into adult muscle in a beetle *Thymalus marginicollis* into three periods. The period of destructive changes occurs in the resting larva when the larva ceases feeding. The reconstructive period takes place at the pupal stage and the imaginal period. In this period, the adult muscles are already formed and cross striation is readily distinguishable. In the present study, the metamorphosis of larval muscles into adult muscles can also be divided into the period of destructive changes, the period of reconstructive period and the imaginal period. In the direct flight muscle and the leg muscle, the period of destructive changes takes place in the resting larva, and in the dorsal and the ventral longitudinal muscles and the intersegmental muscles, the period is present in the first three days of the pupal stage. The days before the sixth day of pupa are the reconstructive period, and the sixth and seventh days before emergence are the imaginal period.

Shatoury (13) has studied the development of the imaginal muscles of *Drosophila* (Diptera) and found that muscle of trochanteral depressors are formed from the mesoderm cells of the leg imaginal buds. In the histogenesis of the new adult muscle of *Tenebrio molitor*, more than ten muscles in the mesothorax and the metathorax are formed from the wing buds or the leg buds during the early half of the pupal life.

Breed (1) found that the process of the histolysis of the larval muscle is similar to that of *T. molitor*, however, it takes place at the pupal stage. The muscles are gradually atrophied from the young pupa until the old pupa, and

the fibres become slender strands. The nuclei undergo a typical chromatolysis. There is no evidence of the phagocytosis about the muscle degeneration. Lockshin and Williams (9) studied the degeneration in the intersegmental muscle of the pernyi silkmoth where the breakdown of the muscle can be accounted for by lytosis brought about the rupture of lysosomes in the apparently viable tissue. This event is followed by the erosion and eventual loss of the myofibrils and by the degeneration of the intracellular organelles.

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EXPLANATION OF ILLUSTRATION

Fig. 1. Cross section of the Tergo Remotor in the larval stage —800x

Fig. 2. Longitudinal section of the Tergo Remotor in the larval stage —800x

Fig. 3. Longitudinal section of the Anterior Lateral muscle of the adult, taken from the prepupal stage and showing the larval muscle divide lengthwise into several muscle strands. —800x

Fig. 4. Cross section of the Anterior Lateral muscle (Stated in Fig. 3) —800x

Fig. 5. Longitudinal section of the Anterior Lateral muscle. Taken from the second day of the pupa and showing the nuclei lie the muscle fibres as numerous chains. —800x

Fig. 6. Cross section of the Anterior Lateral muscle. (Stated in Fig. 5) —800x

Fig. 7. Cross section of the Anterior Lateral muscle. Taken from the third day of the pupa and showing the muscle fibrils formed —800x

Fig. 8. Cross section of the Anterior Lateral muscle. Taken from the sixth day of the pupa and showing this muscle already formed as adult muscle—Fibrillae muscle. —800x

Fig. 9. Longitudinal section of the Anterior Lateral muscle as Fig. 8. —800x

Fig. 10. Longitudinal section of the Tergo Remotor of the prothorax in the prepupal stage. Showing the muscle begin the metamorphosis. —800x

Fig. 11. Longitudinal section of the Tergo Remotor of the prothorax on the first day of the pupa, showing the muscle becomes divergent. —400x

Fig. 12. Longitudinal section of the Tergo Remotor of the prothorax on the third day of the pupa, showing the nuclei divide and lie at the muscle fibres as the chains, the end of the muscle become fibrous. —800x

Fig. 13. Longitudinal section of the Tergo Remotor of the prothorax on the sixth day of the pupa, Showing the mucle fibres attach to the tendon. —800x

Fig. 14. Longitudinal section of the Tergo Remotor of the prothorax on the sixth day of the pupa, Showing the muscle already formed as the adult muscle (the general muscle). 800x

Fig. 15. Cross section of the Tergo Remotor of the prothorax on the sixth day of the pupa stated in the Fig. 15. —800x

Fig. 16. Longitudinal section of the Dorsal Longitudinal muscle on the first day of the pupa. Showing the muscle fibre is going to divide. —800x

Fig. 17. Longitudinal section of the Dorsal Longitudinal muscles on the second day of the pupa. Showing the muscle is in the period of destructive changes. —800x.

Fig. 18. Longitudinal section of the Dorsal Longitudinal muscle on the third day of the pupa. Showing the nuclei divide and lie the muscle fibres as chains. —800x

Fig. 19. Longitudinal section of the Dorsal Longitudinal muscle of the prothorax on the sixth day of the pupa. Showing the adult muscle already formed and the nuclei arranges as a row. —800x

Fig. 20. Longitudinal section of the myoblasts which form the Epimero Subalar muscle. —800x

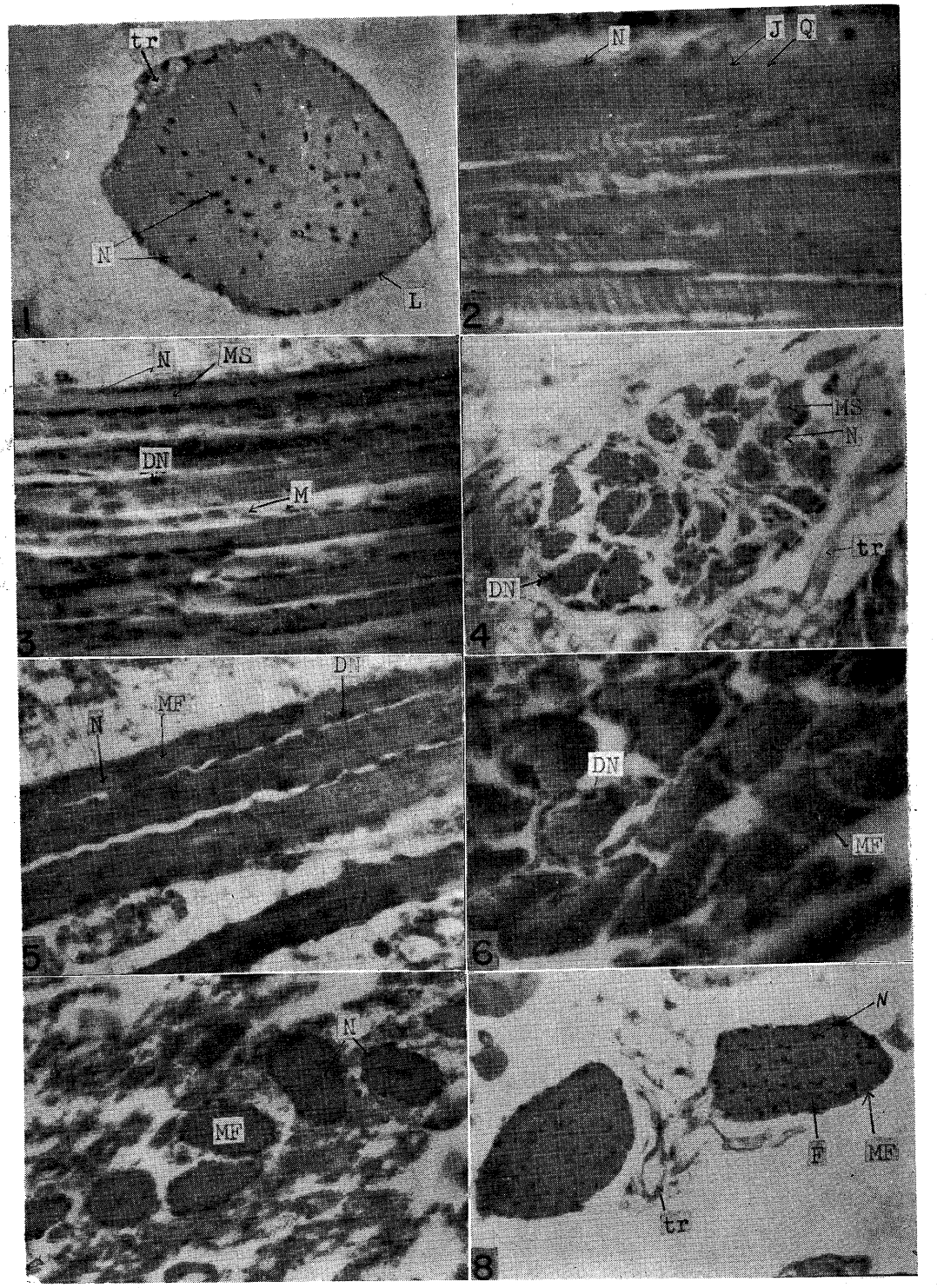
Fig. 21. Longitudinal section of the same muscle. Showing the muscle is in the period of contraction. —800x

Fig. 22. Longitudinal section of the same muscle. Showing this muscle has already formed as the adult muscle. (the general muscle) —800x

Fig. 23 and 24. Showing the degenerating muscle, the nuclei are chromatolysis. —2000x

ABBREVIATIONS

C—Cuticle
E—Epidermis
DN—Dead nucleus
F—Fibril
J—Light Zone
Lc_3—Levator muscle of the coxa
M—Myoblast cell
MS—Muscle strand
MF—Muscle fibre
N—Nucleus
NC—Nuclear chain
Q—Dark Zone
Tr—Trachea
T—Tendon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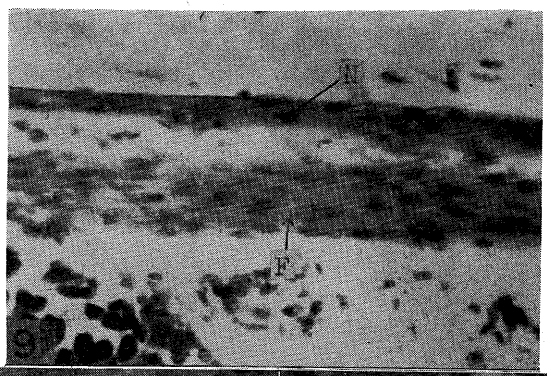
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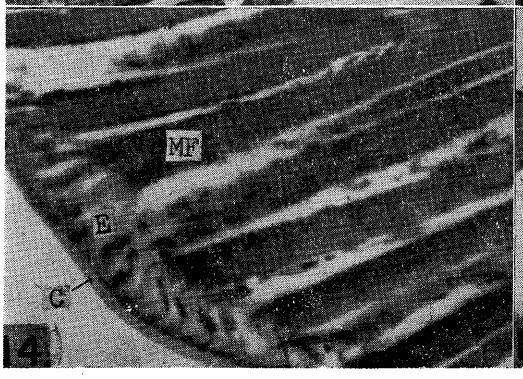
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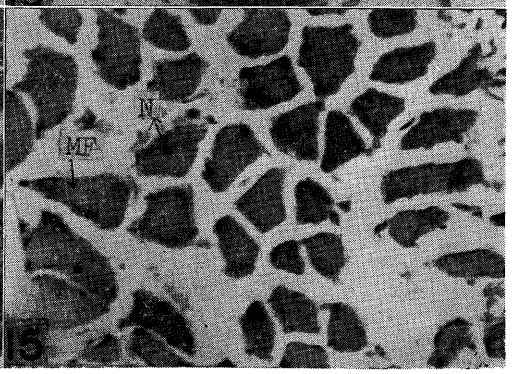
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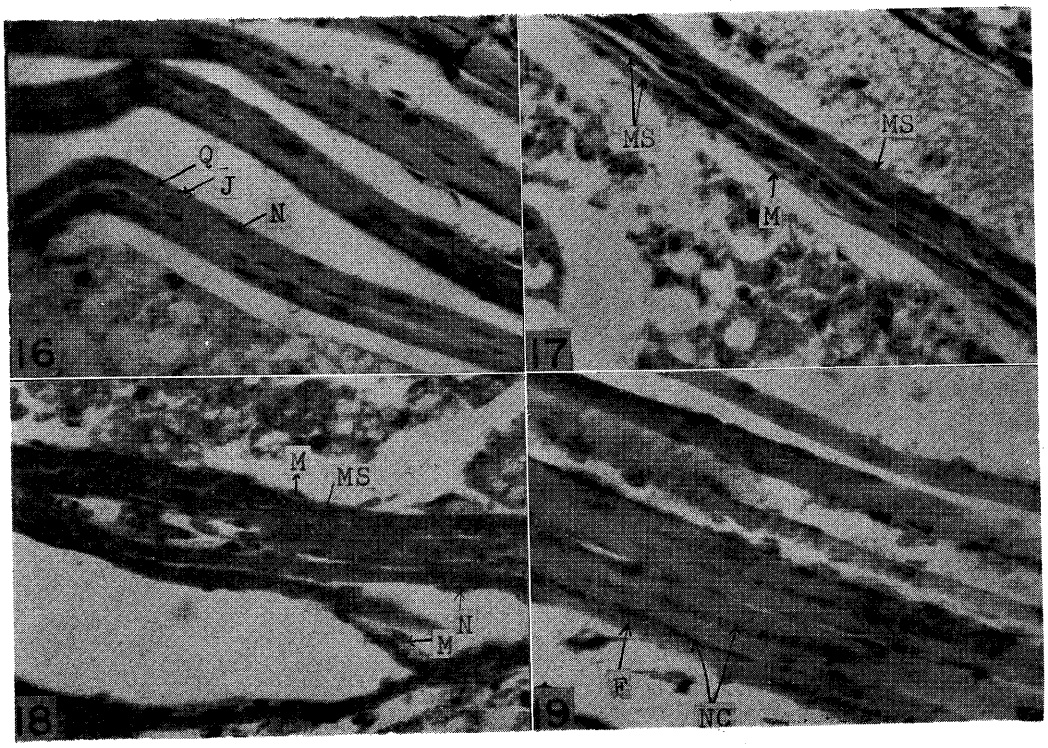
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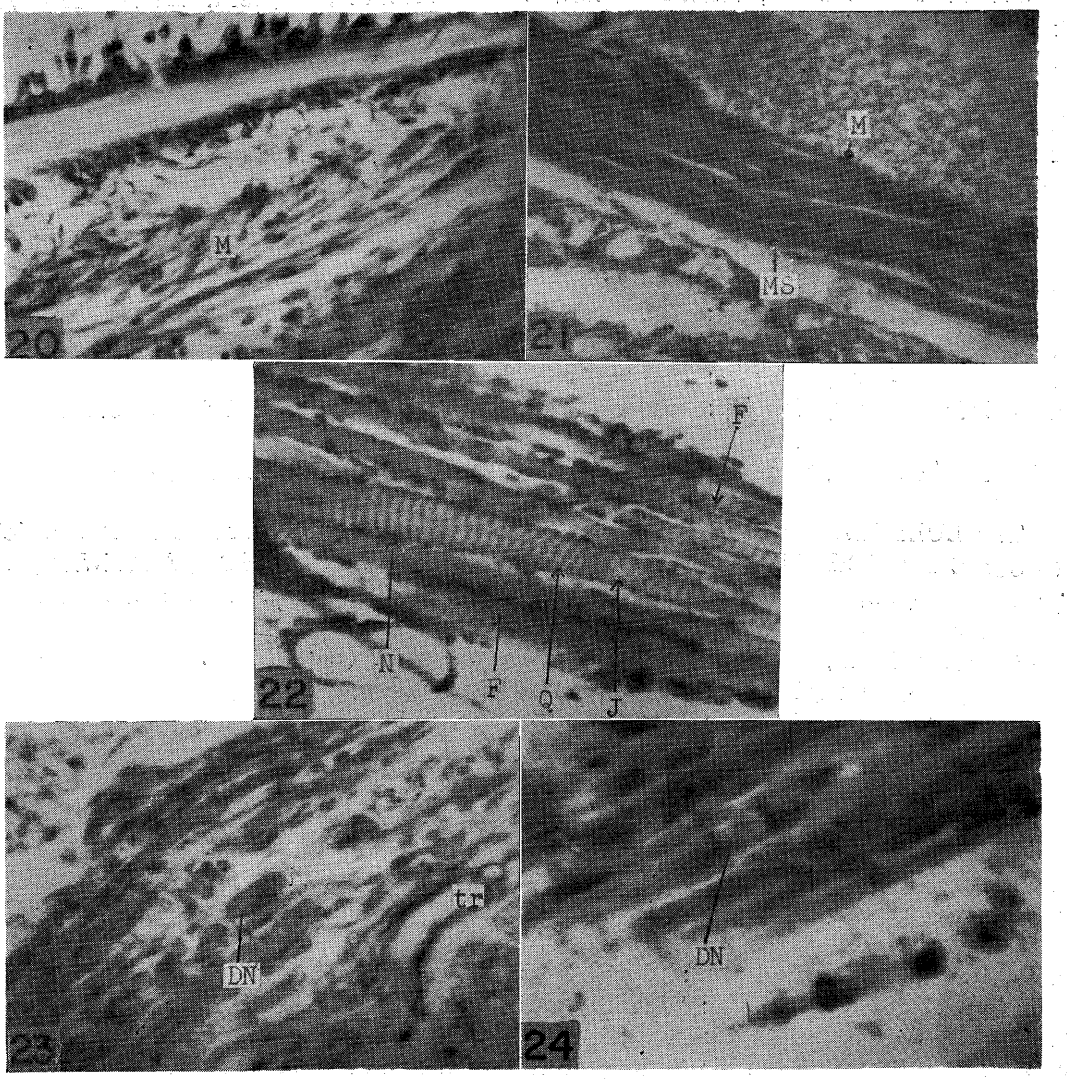
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