ELECTRON MICROSCOPE STUDIES OF INSECT MUSCLE. III. VARIATIONS IN ULTRA STRUCTURE.

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INTRODUCTION

Previous studies in this series (EDWARDS, SOUZA SANTOS, SOUZA SANTOS, and SAWAYA, 1953; 1954a; 1954b) have demonstrated that there exist in the insect two fundamentally different types of muscle. In the lower insects all muscle fibrils exhibit a single type of ultrastructure as well as similar general gross structure, such as coloration, tracheation and fiber size. In the higher insects the muscles divide themselves into two groups: (1) the flight muscles, characterized by reddish color and heavy tracheation of fibers, and (2) the other muscles of the body, characterized by their white color, little tracheation, and long period of striation. The myofibrils of the red fibers are uniform in diameter and show all lines and regions clearly. The myofibrils of the white muscles are non-uniform in diameter and lack some of the lines common to striated muscle. The differences appear to be related to the development of specialized wing muscles for the fast flight of the more advanced insects.

The present paper pretends to enlarge upon some of the previous findings and to present new material with a view to comparing further the muscles from various insects and to answer such questions as: (1) what happens to the myofibril during contraction, (2) what are the relations between period, width, and function of the various types of muscle fibrils, (3) what is the form, variation and significance of the various regions and cross lines, and (4) how do the insect fibrils compare with vertebrate fibrils in function and structure?

MATERIALS AND METHODS

Muscles were taken from various parts of the body of the following insects.

Aeschnidae larvae
Schistocerca infumata adults
Belostoma spp. adults
Hydrophilus ater adults
Caligo beltrao adults
Thysania agrippina larvae, pupae and adults
Periplaneta americana adults
Synthermes soldier
Lethocerus spp. adults

Dytiscus marginalis larvae and adults.
Meliponinae adults

The muscles used were: adductor of mandibles, various indirect flight muscles, elevator of coxa, femoral muscle, retractor of ovipositor, abdominal intersegmentals, and muscles from the hindgut and the rectal gill.

Various methods of preparation were tried, such as glycerol or saline maceration in the cold, and fixation in formol, Zenker's, Carnoy's, and Bouin's fluids. The most satisfactory preparations were those obtained by the following process. The insect was opened and the desired muscles cleaned of adhering foreign tissue but left attached in situ. On one side of the insect toothpicks were placed under the bellies of the muscles until they were stretched to a little beyond their original length. On the other side the muscles were cut at one end. The insect was then covered with 5% formol and left for four or more hours. In this manner fibrils were fixed in the stretched (belly of muscle), partially contracted (near the origin and insertion of the stretched muscle) and contracted states (the cut muscle usually contracting violently upon covering with fixative). After fixation the muscle was removed, washed with distilled water, cut into small sections, triturated by hand in a common mortar and then blended in distilled water at 20,000 rpm for 15 minutes in 5 minute periods in a micro-blendor. The resulting suspension was diluted with distilled water until suitable fibril distribution (as controlled with the light microscope) was obtained. The suspension was placed by means of a micro-pipette on the parlodion covered grids and the preparations dried and shadowed with chromium at an angle of 10 to 12 degress. It is of interest to note that similar results were obtained with all fixatives and macerating solutions used.

The preparations were observed in the RCA, EMU electron microscope of the Secção de Microscopia Eletrônica, and the SIEMENS UM 100d electron microscope of the Secção de Virus. Initial magnifications of 1200 to 10,000 were used, the resulting negatives being enlarged usually 5 times for the final picture. Best results were obtained were high contrast negatives (Kodak or Lumiere) and enlargement paper (Leigrano, extra hart).

RESULTS

A. *GROSS MUSCLE STRUCTURE*:

The gross structure of the muscles of the several insects investigated varied in four major characteristics: color, tracheation, number of sarcosomes (mitochondria of insect muscle), and dissectability.

In the lower insects (considered here as those below Hemiptera) all muscles of the body are of a single color, generally a translucent

white to a pale yellowish-brown. The color appears to vary with sex, e. g. muscle from the male cockroach is darker than that from the female. The color may also vary with species, e. g. *Schistocerca* muscle is darker than that of the dragonfly nymph. However, within a given species and sex there may be variations from one individual to another. This was most commonly observed in the cockroach, but occurred also in other insects. In the higher insects there is a sharp differentiation in color between flight muscle and other muscle. In the adult the flight muscle is generally pinkish to reddish brown, rarely yellowish brown and never white. All other muscles are similar to those of the lower insects, i. e, white. In the larvae and early, pupae of higher insects only white muscle is present.

In ordinary histological preparations, or even in muscle simply crushed between two slides and observed immediately in the microscope, one can observe that the red, flight muscle is filled with mitochondria surrounding the fibrils, whereas the white muscle has few cytoplasmic inclusions. By differential centrifugation it is possible to separate the mitochondria from the fibrils and observe that the color appears mainly in the mitochondrial fraction.

The tracheation of the muscles varies from one to the next but sharp difference exists between the tracheation of the red muscles and the white. Whereas the white muscles are supplied with few tracheae of few branches, the red muscles are heavily bound by trachaeae which end in tracheolar tufts around each fiber. Electron micrographs show that the flight muscle contains both annular and helical types of taenidia in the tracheoles, whereas ther other muscles of the body contain only the helical type.

During dissection the two types of muscle, red and white, show differences in ease of separation of fibers, and in breakability. The red muscle fibers are heavily invested with tracheae and tracheoles, the white fibers less so. (There appears to be no connective tissue binding the muscles of insects. This may be related to the absence of smooth muscle inasmuch as connective tissue and smooth muscle have a common origin and are very similar in appearance in their ultra structure). Despite the tracheal support the red muscle sepaartes easily into small bundles which may be dissected further by fine needles into the component, long, uniform, single fibers. The white muscle breaks into pieces and adheres more tightly to its chitinous insertions. The single white fiber is difficult to isolate, easily damaged, and tears, rather than separates, from neighboring fibers. During dissection the white fibers often show contraction waves and retraction clot formation. When blended the red fiber breaks into uniform, long, single fibrils, in contrast to which the white fiber breaks into short bundles of connected fibrils. The blended, red, single fibril tends to fracture transversely, at the Z line. The

white fibril tends to fray longitudinally so that it becomes difficult to determine when one has isolated a single white fibril.

B. *MYOFIBRIL CHARACTERISTICS*:

1. *Gross appearance*. — The red myofibril (Figs. 1-5) appears cylindrical, uniform in diameter, is long, and fractures transversely, usually at the Z line in the I region. The filaments appear to be continuous through both the A and I regions. Gross filaments are visible in both regions, thus giving the fibril the appearance of a roughly woven cloth. The white myofibril (Figs. 6-14) appears in our preparations as a flattened, non-uniform, generally frayed fibril. It is usually short, and often split longitudinally into a number of filament bundles. As in the red fibril, the longitudinal filaments are continuous. The cross filaments are more clearly visible in the white than in the red fibril. In both types of fibrils the filaments appear to occupy the entire fibril.

In isolated fibril preparations one cannot determine diameters accurately. At first glance the red fibril appears to be narrow and the white fibril to be broad and flat. However, as may be seen in the accompanying figures, the white muscle preparations usually showed small component bundles, which could be interpreted as either single fibrils or as groups of filaments. Thus one cannot determine whether several fibrils are present, connected by a continuous Z line (Fig. 6) or whether one fibril has split with concomitant division of the Z (Fig. 7). In those cases where the fibril appears to be a single unit the diameter of the white fibril appears the same as, or lesse than, that of the red fibril. Thus from isolated fibril preparations one cannot relate fibril diameter to muscle function.

2. *Length of sarcomere*. — A correlation does exist between resting sarcomere length and velocity of action of the muscle. As may be seen in Table One the red flight muscle has the shortest period of cross striation (2.5 mu) and the mandibular adductor and intestinal wall muscle the longest periods (10.0 and 9.4 mu respectively). Thus the muscle capable of rapid action, e g. red., flight muscle, is characterized by a short period, whereas the fibril of the muscle involved in more sustained action, e. g. buccal muscles, has the longer period. It is to be noted that the relation here appears to be between period and rapidity of action, not between period and locale of function. The red flight muscle of *Hydrophilus* and *Dytiscus* has a shorter period than the swimming muscle. The white, flight muscle of *Periplaneta* has the same period as the white, leg muscle. The flight muscle of *Belostoma* is similar to that of the bee flight muscle, yet the latter insect is a more powerful flier.

3. *Anisotropic region*. — It has been noted by FARRANT and MERCER (1952) in the grasshopper wing muscle, and by us (EDWARDS,

SOUZA SANTOS, SOUZA SANTOS, and SAWAYA, 1954a; 1954b) that the regions and lines of the white muscle fibrils are not as clearly differentiated as those of the red type. This is particularly true of the A region, which shows considerable variation.

The white muscle in the streched state shows both A and I as clearly distinguishable regions, the A being considerably more dense. In the partially contracted fibril the density of the A region is less, and in the fully contracted fibril the A region is uniformly of little density. In the stheched fibril the A region generally is divided into three bands; two more dense bands proximal to the adjacent I regions, and a less dense band in the center (Figs. 8, 9, 10, 11). It is believed that the central band represents the disc of HENSEN (H. band). In the partially contracted fibril the H band becomes narrower (Fig. 7), and the two proximal portions of the A region less dense. In the contracted fibril the H band is lacking (Fig. 12), or may be represented by a very narrow trough (Fig. 13). The length of the A region does not appear to change during contraction. In extreme contraction the A region appears as a thin, concave region of little density with only the longitudinal filaments visible (Fig. 14).

In the white fibril the M line never was seen as a distinct line, but only as a variety of modifications of the central portions of the A region (Figs. 9, 13). It is difficult to decide whether this actually state of contraction of the fibril, but rather with the type of muscle and species of insect. The modifications ran the gamut from complete absence of a line (Fig. 12) up to a series of heavy, dense, irregular, raised spots aligned unevenly across the H band (Figs. 7, 8, 11). Most commonly observed was a single, narrow depression across the center of the A region (Figs. 9, 13). It ss difficult to decide whether this actually represents a reduced H band, is indeed the M line, or an entirely new structure characteristic of this type of fibril. Variations of the furrow type of line were found as: (a) a faint, partial furrow, (b) deep, triangular furrow, (c) several furrows parallel and irregular in outline, and (d) irregularly perforated furrow. In all cases the myofilaments were continuous throughout the A region. Breaks did not seem to occur in the furrow. In some white muscle preparations the furrow appeared to continue from one fibril to the next, but such continuity could well be an artifact due to the clumping of the fibril luring blending and drying. The second most common appearance of the M line region in the white fibrils was that of a transverse series of irregular, raised spots. The spots were sometimes small in diameter and regularly aligned, giving the appearance of one or more discontinuous lines through the H band (Figs. 6, 7, 8, 10, 11). Sometimes they appeared to be aligned beside a raised, continuous region in the H band, thus giving the appearance of an M line with ragged edges. In the extreme the spots were large, extremely irregular in outline, and scattered throughout the less

dense band of the A region. The interpretation of these spots is rather difficult in the light of present knowledge. One possibility is that the white fibril lacks a true M line, but that the reticular system (cf. EDWARDS and RUSKA, 1954) surrounds the fibril in the A region in a regular manner, and that when the fibril is isolated, as in blending, some of the reticular system dries down in the H band giving the appearance of the irregular spots. Some credence to this possibility is given by the fact that in certain photos (Figs. 8, 9, 10) one can see connecting filaments between the spots on the fibril, and that similar irregular patches of non-muscular material may be found on the grid beyond the periphery of the fibril, either as isolated or connected spots. A second possibility is that the insect white fibril has an M line and H band but that it differs from the insect red muscle and vertebrate muscle ,in that the M line is discontinuous. One cannot consider these structures as artifacts due to preparation inasmuch as they are characteristic of all white muscle independent of the method of maceration or fixation used.

In partial contraction of the white fibril the M line material generally is absent and the H band appears as a narrow, shallow, incomplete cross trough (Figs. 7, 13). Thus the center of the A region is characterized by a less dense depression in contradistinction to the dense, raised Z line of the I region, giving the fibril the appearance of alternating concavities and convexities. In *Hydrophilus* white muscle an M line was visible at times and in certain *Periplaneta* preparations the M sometimes appeared as an incomplete row of small spots in partial contraction.

In contraction the M and H bands are completely lacking (Fig. 12) and the A region of little density, i. e. the A and I regions appear to have interchanged density, giving the fibril an accordion appearance (Fig. 14).

By way of contrast to the white fibril, the A region of the red fibril was quite constant in appearance. In the stretched, or relaxed, fibril the A appeared as two distinct, dense regions separated by a uniformly less dense H band containing a dense M line in the center (Figs. 1, 3, 4). Thus the A, H, and M of the red fibril appeared as classically described for the vertebrates. In partial contraction the A region lost some of its density, the H band became less clear, the M line remaining as a distinct line (Fig. 5). In certain preparations sub M lines were visible, appearing as 1 to 3 smaller lines on either side of the main M line (Fig. 4). In complete contraction of the red fibril the H band and M line disappeared, the A region appearing as a concave region of little density, thus resembling the white fibril in this stage.

4. *Isotropic region*: — The isotropic region (I) of the insect muscle fibril is more constant in form than is the A region. It does show modification, however, from one type of fibril to another (red to white) and changes in size during contraction.

In the stretched, red fibril the I region appears to be of little density, the Z line is prominent, and the filaments are quite visible. At this stage the I region is almost equal to the A in length. No N lines are visible. During partial contraction the I region becomes shorter, more dense and more convex; the Z line appearing to be greater in height. In full contraction the I region practically disappears; appearing as an extremely dense, heightened, short region of width practically not much greater than the Z line. In certain preparations of *Hydrophilus* muscle it appeared that N lines were formed during contraction, but this was not repeated in other preparations. It is to be noted that in the red fibril the Z line always appeared as a dense, solid ring of substance encircling the fibril. It did not, however, extend beyond the periphery of the fibril.

Similar changes occurred in the white fibril I region, i. e. the shortening during contraction and the increase in density and elevation. The white fibril differed from the red, however, in the form of the Z line. In its simplest form the Z appeared as a thin, dense line across the fibril. In one preparation the line showed a rubbly structure rather than being a continuous, solid line. In some fibrils the Z appeared as a distinct ring, usually seen in elliptical form due to the flattening of the fibril during drying. In certain fibrils the ring appeared to be embedded within the I substance, in others it appeared to be peripheral to the filaments of the I region. More generally the latter was true. In the isolated, white fibrils the Z line often appeared to extend beyond the periphery of the fibril, or in those preparations in which more than one fibril was present in a group the Z appeared as a continuous line across the several fibrils (Figs. 6, 7). This could be considered as an artifact caused by the union of the fibrils during the drying process, or it could be considered as characteristic of the white fibrils. (Since this work was completed ultrathin sections have been made (cf. EDWARDS and RUSKA, 1954) of several insect white muscles. It was found that the Z line in general was restricted to the fibril, but in *Hydrophilus* coxal levator muscle the Z appeared as a continuous line. This contrasts sharply with other fibers in which the endoplasmic reticulum connects adjacent Z lines, giving, at low magnifications, the impression of a continuous line). In some preparations the Z line appeared as a hollow ring, i. e. double line (Fig. 10), and in some it was seen definitely to split along its long axis (Fig. 9). In all preparations the Z line appeared to be raised considerably above the surounding I region. In *Hydrophilus* coxal levatorfibrils in the contracted state, in several cases, the Z line was seen to throw a long shadow (Fig. 13) filled with a very finely veined network.

These variations in form of the Z line did not appear to be related specifically to the function of the given muscle or to its stage of contraction.

DISCUSSION AND SUMMARY

The process of contraction seems to be the same in both red and white type insect fibrils. In the extended, or relaxed, fibril the I region and the A region approximate each other in length, the A being slightly longer than the I. In this stage all lines and bands that the two types contain are visible. In the partially contracted fibril the A region becomes less dense and the H band begins to disappear. Meanwhile the I region shortens. In the fully contracted fibril the A region, still of initial length, is of little density and appears to be more concave, i. e. of little substance. The I region appears the opposite, i. e. quite dense and convex. Thus, during contraction the two regions appear superficially to have exchanged substance. The exchange appears to be actually a migration of the two proximal halves of the A region into the adjacent I regions. We are thus more inclined to the classical view of the changes during contraction, rather than to the view of BENNETT and PORTER (1953) of a sarcoplasmic and reticular migration in and out of the fibril.

The relation of the various forms of the myofibrils to the specific function of the muscle involved is more difficult to interpret. It is quite evident that the width of the fibril, in isolated fibril preparations, cannot be related to function. The major difficulty lies in the fact that the white fibrils do not easily separate but occur in chunks of several fibrils' width. For this reason it is also impossible to follow any changes in diameter that might occur during contraction. The relation between period and function is more easily visualized As pointed out by SZEKESSY (cited in SZENT-GYORGYI, 1947) there is a relation between length of sarcomere and rate of muscular motion. In corroboration of his findings we have found that the muscles capable of slow, sustained activity have the longer period, whereas those muscles involved in rapid motion, e. g. wing muscles, have the shorter period. It must be noted in relation to periodicity and function that we are speaking here of primary function. For example, a swimming muscle of a secondarily aquatic insect has a long period equivalent to that of the leg muscle of a non-swimming insect.

The outstanding result of the present study is the observation that variations can occur within a given muscle fibril in regard to the lines and regions. Of most interest is the variation in the M line. We observed, as had DRAPER and HODGE (1949) and others in vertebrate muscle, sub M lines in the red fibrils and variation of the H band with various stages of contraction. More interesting, however, was the variation that occurred in the white fibrils. The M line was absent in some preparations but in the majority apeeared as a series of raised, irregular spots giving the impression of a discontinuous, almost diffuse line. It could be (as suggested by ROZSA, WYCKOFF and SZENT-GYORGYI, 1950,

and FARRANT and MERCER, 1952) that the line becomes lost during preparation of the fibrils. However, the same phenomenon was observed in fibrils following a variety of treatments in preparation, hence we believe it is a characteristic of the white fibril. We must thus believe that (1) the M line of the white fibril is in the form of a series of raised, irregular spots, or (2) the M line is missing and that the spots represent remnants of the endoplasmic reticulum (cf. EDWARDS and RUSKA, 1954) which have stuck to the fibril during preparation. In all events it is not possible at the moment to determine the significance of this line in the insect fibril. The variations observed in the Z line of the insect fibril are those previously reported for vertebrate muscle, i. e. cylindrical form separate from the contractile substance, splitting of the line, double line, etc. We concur with the prevailing opinion that the Z line is not part of the contractile substance. In the red fibrils it is definitely restricted to the confines of the fibril. In certain white muscle fibrils (e. g. *Hydrophilus* coxal levator) the Z line appears to be continuous from one fibril to the next and gives the impression of a series of linked circles. It is interesting to note that in only one preparation were apparent N lines visible. This is not easily explained inasmuch as vertebrate muscle prepared in exactly the same manner shows definite N and sub N lines.

It is believed that one of the most important results of the present work is the demonstration of variations that may occur within the various specialized muscles of a given animal. This opens the door for a more thorough investigation of the specialized muscles of various animals throughout the animal kingdom. It appears that there is no such thing as "the striated muscle", but that the structure of the muscle varies with function and phylogenetic position of the animal.

SUMÁRIO

Com a finalidade de aumentar resultados anteriores e de apresentar novo material para comparar os diversos músculos de vários insetos foi feito um estudo por meio do microscópio eletrônico. Usamos 8 músculos diferentes de cada um de 14 insetos, representativos de 8 ordens.

As fibrilas dos músculos foram preparadas por meio de maceração em glicerina ou fixação em formol a 5%, seguida por trituração num microblendor, secagem na telinha e assombreadas com cromo a um ângulo de 12 graus.

Em estrutura grossa os músculos dos diversos insetos variam em 4 caracteres, i. e. côr, traqueação, quantidade de mitocôndrias e facilidade de separação de fibras. Nos insetos inferiores todos os músculos são do tipo "branco", com pequenas variações em côr devido ao sexo ou idade do indivíduo. Nos insetos superiores os músculos dividem-se em tipo "vermelho" do vôo, e tipo "branco" dos demais músculos do corpo. O tipo branco tem sarcolema espesso, pouca traquealização, poucas mitocôndrias, e as fibrilas separam-se com dificuldade devido ao sistema reticular e arranjo sinfibrilar. O tipo vermelho tem sarcolema fino, muita traquealização, sistema traqueolar-mitocondrial em redor das fibrilas espaçadas, assim as fibrilas separam-se fàcilmente.

Em aparência grossa a fibrila vermelha e cilíndrica, uniforme em diâmetro, comprida, e fratura-se tranversalmente na região da linha Z. A fibrila branca parece chata, não uniforme, desfia-se fàcilmente e fratura-se longitudinalmente. Em ambos os tipos os miofilamentos são contínuos através das regiões A e I. O diâmetro da fibrila branca é igual ou menor que o da fibrila vermelha.

Uma relação existe entre comprimento do sarcômero e velocidade de ação da fibrila, p. e. uma fibrila do músculo de vôo de *Hydrophilus* tem um período de 2,5 mu, enquanto um fibrila branca da perna de *Caligo* tem período de 5,9 mu e a do músculo adultor mandibularis de *Synthermes* tem período de 10,0 mu.

Os processos de contração nos dois tipos de fibrilas parecem ser semelhantes. Na fibrila relaxada as regiões A e I aproximam-se em comprimento, a região A sendo ligeiramente mais comprida do que a I. Neste estágio tôdas as regiões e linhas que pertencem aos dois tipos

de músculos são visíveis. Na fibrila parcialmente contraída a região A torna-se menos densa e a faixa H começa desaparecer. No entretanto, a região I encurta-se. Na fibrila completamente contraída a região A, mantendo-se no comprimento original, torna-se de menor densidade e mais côncava, i. é. parece conter pouca substância. Ao contrário, a região I aparece densa e convexa. Assim sendo, acreditamos que durante contração as duas regiões trocam de substância. A troca provàvelmente é uma migração da substância das duas metades próximas da região A na direção das regiões I adjacentes. Assim, tendemos mais a admitir a descrição clássica das mudanças durante a contração em veí de adotar a idéia de BENNETT e PORTER (1953) duma migração de sarcoplasma e retículo dentro e fora da fibrila durante a contração.

O resultado principal do estudo aqui apresentado é a observação de que podem ocorrer variações nas linhas e regiões numa dada fibrila dum músculo. O maior interêsse encontra-se nas variações na linha M. Observamos, como já fizeram DRAPER e HODGE (1949) e outros nos estudos de músculos de vertebrados, linhas acessórias M nas fibrilas vermelhas e uma variação na faixa H ligada aos vários estágios de contração. Variação maior ocorre nas fibrilas brancas. A linha M se ausenta em algumas preparações, mas na maioria aparece sob a forma duma série de manchas irregulares levantadas, dando assim a impressão duma linha descontínua, quase difusa. Pode ser que a linha M verdadeira se perde durante a preparação das fibrilas, mas devemos lembrar que o mesmo fenômeno ocorreu em tôdas as fibrilas brancas preparadas de várias maneiras, e assim acreditamos ser característica da fibrila branca. Devemos crer que (1) a linha M da fibrila branca do inseto existe na forma descrita, ou (2) a linha M não se encontra nessas fibrilas e que as manchas representam o resto do sistema reticular grudado à fibrila durante a secagem.

As variações observadas da forma da linha Z são aquelas já vistas no músculo dos vertebrados, i. e. forma cilíndrica da linha, divisão longitudinal da linha, linha dupla etc. Concordamos com a opinião prevalecente que a linha Z não forma parte da substância contractil da fibrila. Na fibrila vermelha a linha Z restringe-se definitivamente à fibrila. Em certas fibrilas brancas, no outro lado, (p. e. músculo elevador da coxa de *Hydrophilus*) a linha Z parece ser contínua de fibrila a fibrila, assim dando a impressão duma série de círculos ligados. As linhas N eram visíveis em uma só preparação. Não podemos explicar êste fato fàcilmente uma vez que nas fibrilas dos vertebrados preparadas na mesma maneira as linhas N e linhas acessórias N sempre se mostram.

Acreditamos que um dos resultados mais importantes neste trabalho é a demonstração das variações que podem ocorrer nos músculos especializados dum dado animal. Assim, a porta acha-se aberta para outras pesquisas sôbre os vários músculos especializados de vários ani-

mais no reino animal. Parece que não existe "o músculo estriado", mas que a estrutura do músculo varia com a sua função e com a posição filogenética do animal.

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TABLE ONE

Equilibrium sarcomere lengths of insect fibrils (in microns).

Insect	Muscle	Length	Insect	Muscle	Length
Aeschnid larva	abdom. intersegmental intestinal	6.6 9.4	*Periplaneta americana*	flight femoral	4.1 4.3
Schistocerca infumata	dorso-long. flight ovipositor retractor tergo-sternal	3.5 8.6 4.1	*Gryllotalpa*	femoral	6.2
Synthermes	adductor mandibularis	10.0	*Belostoma*	flight coxal	2.8 4.1
Hydrophilus ater	flight muscle coxal levator	2.5 4.2	*Dytiscus*	larval interseg. coxal flight	5.0 5.2 3.4
Caligo beltrao	dorso-longitudinal furco-pleural femoral	3.4 3.7 5.9	Meliponid	coxal	7.5

LEGENDS FOR FIGURES

All fibrils were shadowed with chromium at an angle of 12°. Other preparation described in the text. The magniifcation is indicated by the line denoting the length of 1 mu. Symbols as fallows: A — anisotropic region; H — Hensen's disc; I — isotropic region; M — mesophragma; Sar — sarcosome (mitochondria); Tr — tracheole; Z — telophragma.

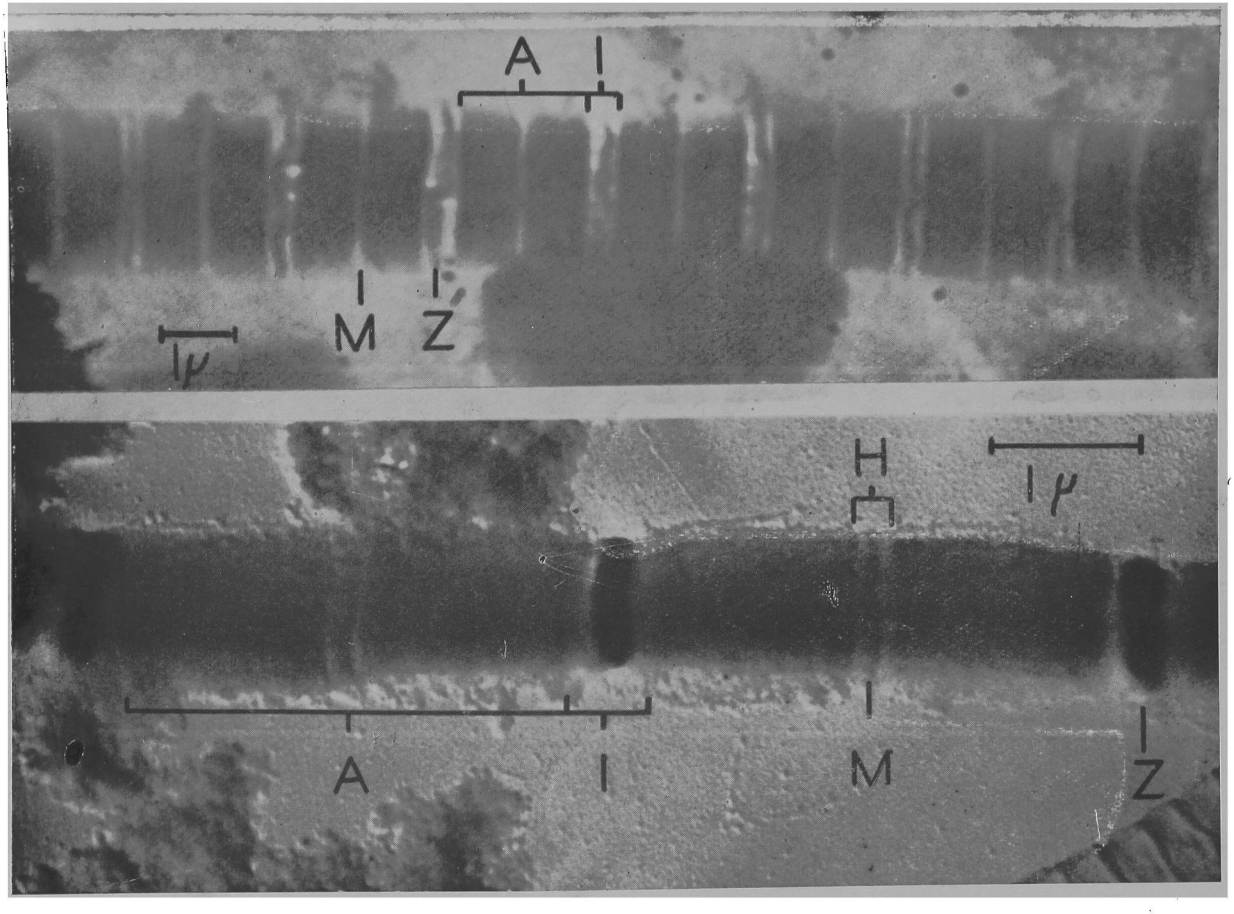

Fig. 1. — A. Red, flight muscle fibril from *Hydrophilus*. Note breaking of fibril in I region into distinct sarcomeres. B. Red, flight muscle fibril from *Belostoma*.

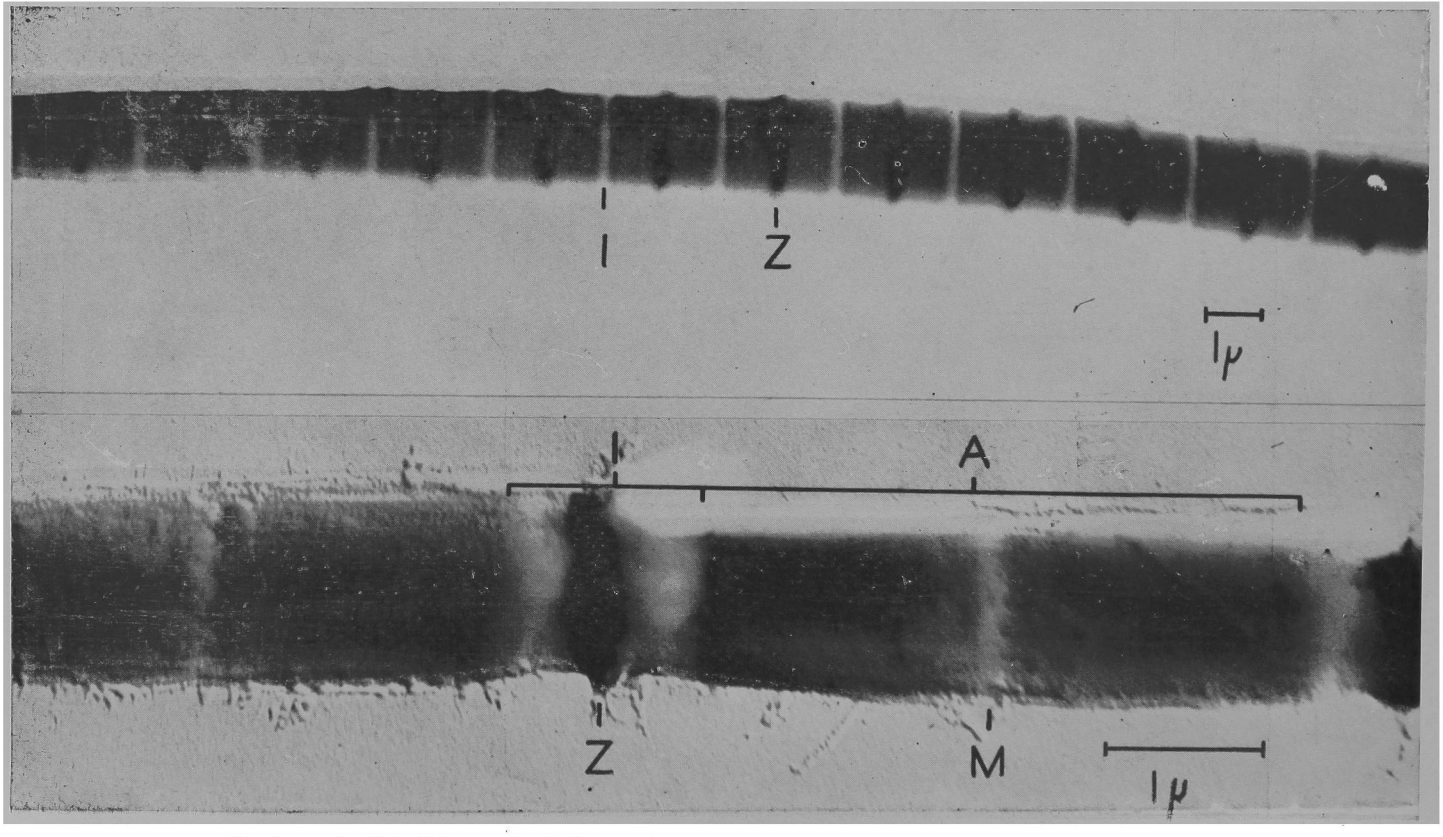

Fig. 2. — A. Red, flight muscle fibril from Meliponidae. B. Red, flight muscle fibril from *Caligo*.

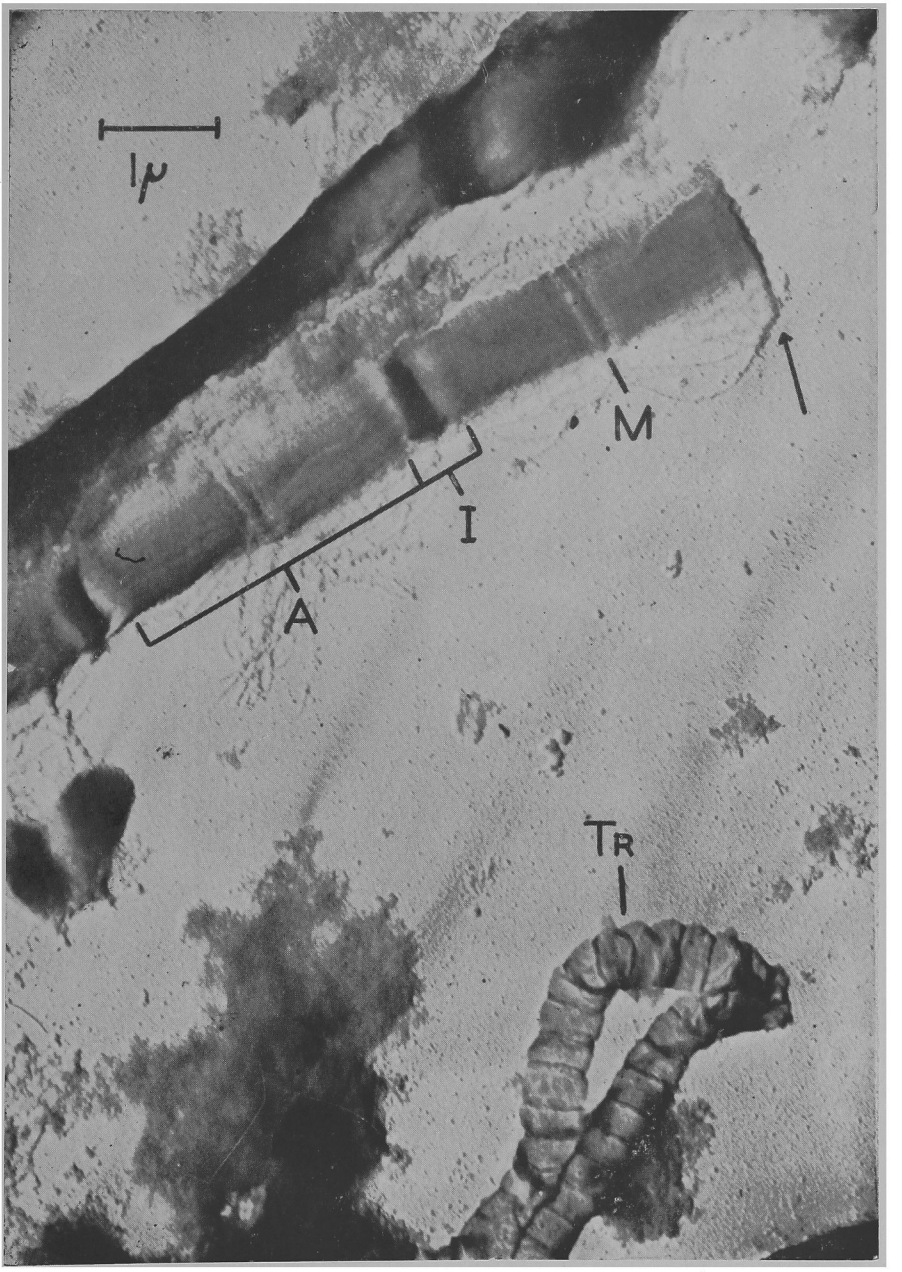

Fig. 3. — Red, flight muscle fibril from *Belostoma* showing characteristic break at **Z** line. Annular type tracheole shown is characteristic of flight muscle.

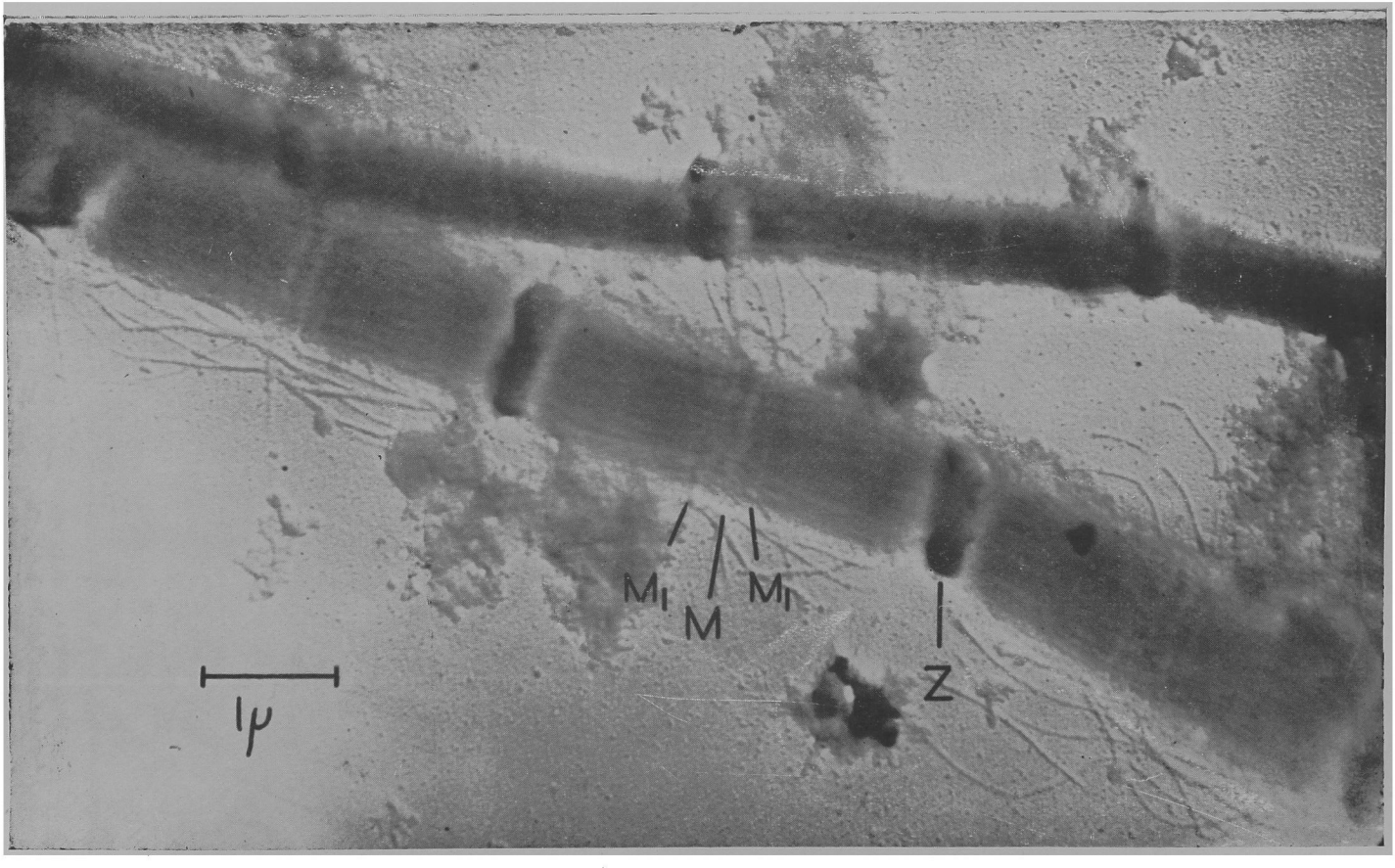

Fig. 4. — Red, flight muscle fibril from *Belostoma* showing sub-M-lines.

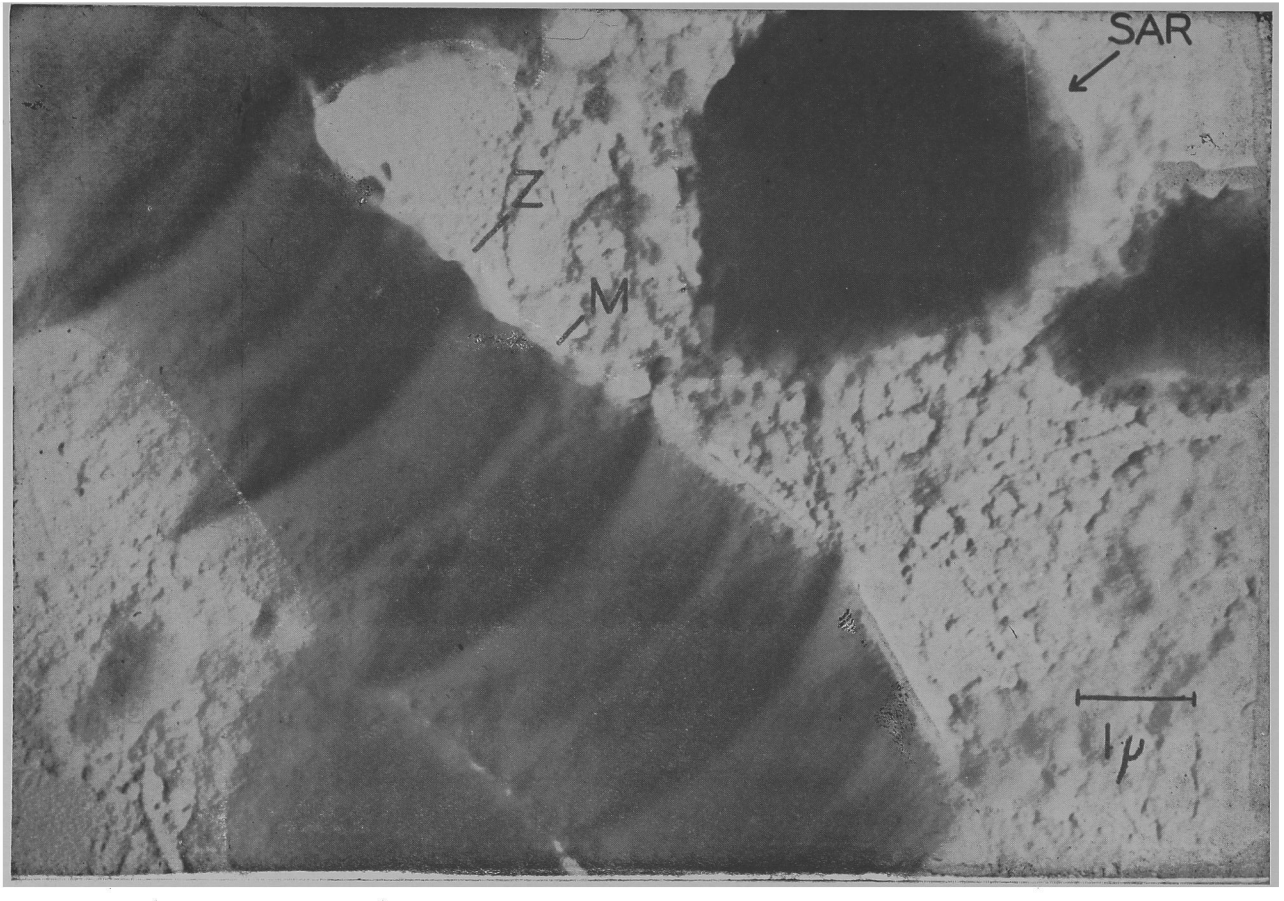

Fig. 5. — Red, flight muscle fibril from *Hydrophilus* in partial co ntraction. Note accompanying sarcosomes.

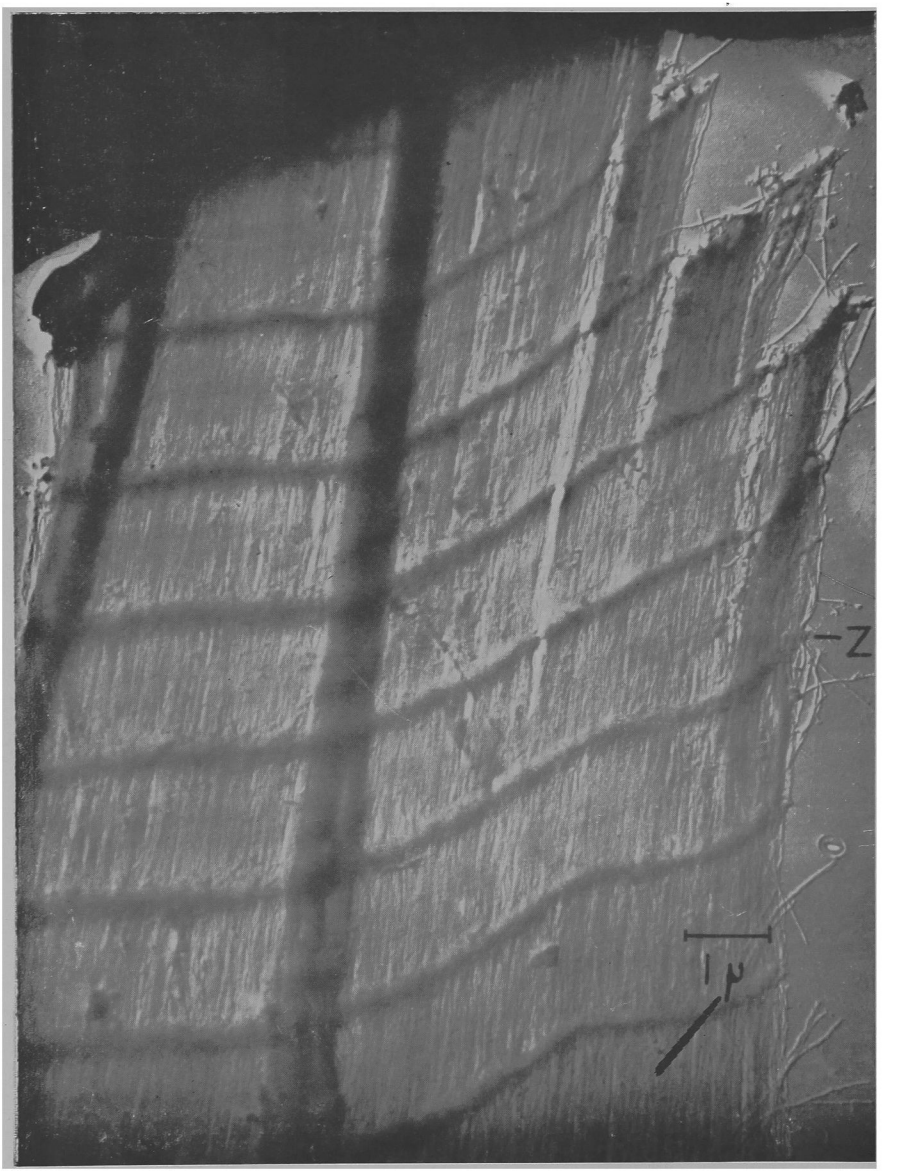

Fig. 6. — Contracted, white, coxal levator myofibrils from *Dytiscus*. Observe continuous Z line and longitudinal fracture of fibrils.

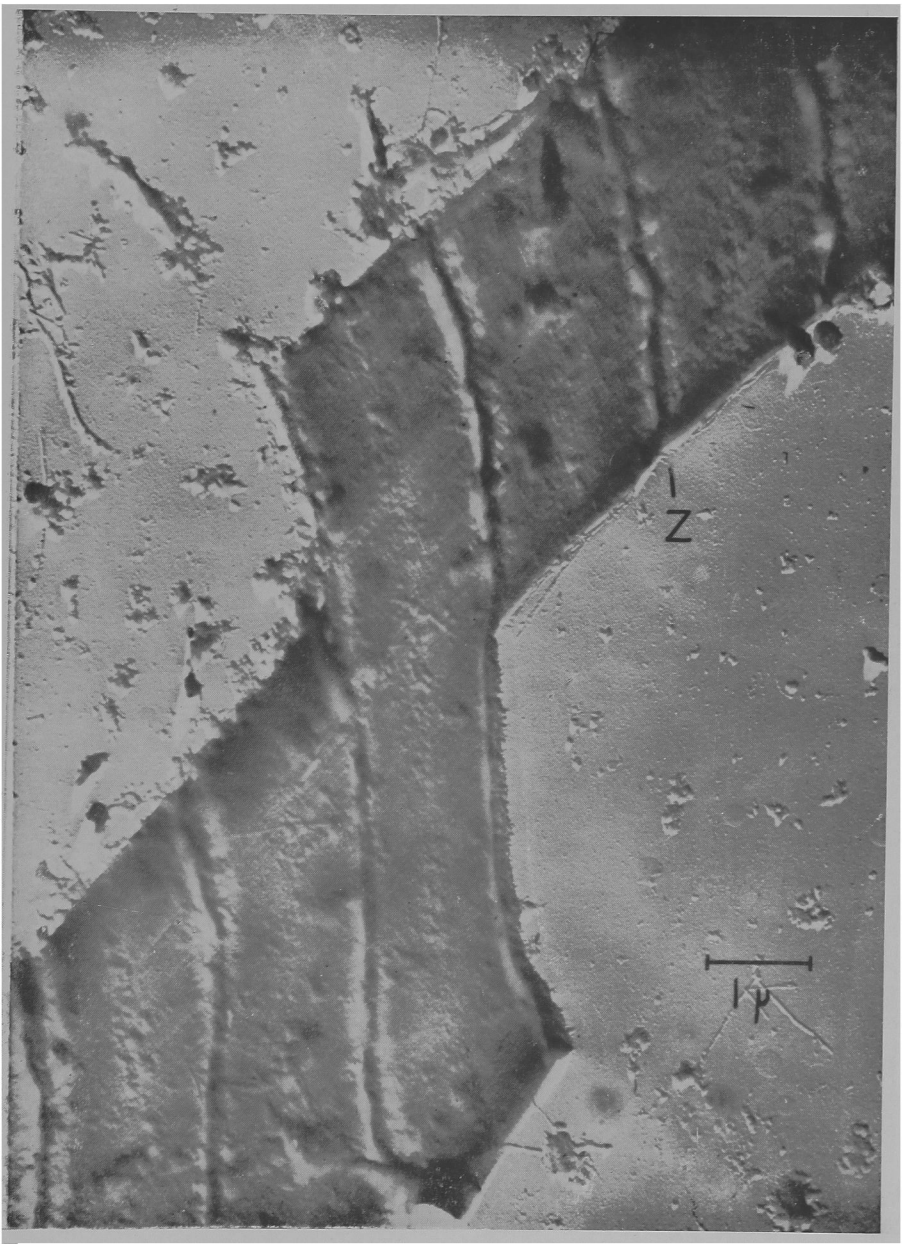

Fig. 7. — Partially contracted, white, coxal levator myofibrils from *Dytiscus* showing dotted M line and break at *Z*.

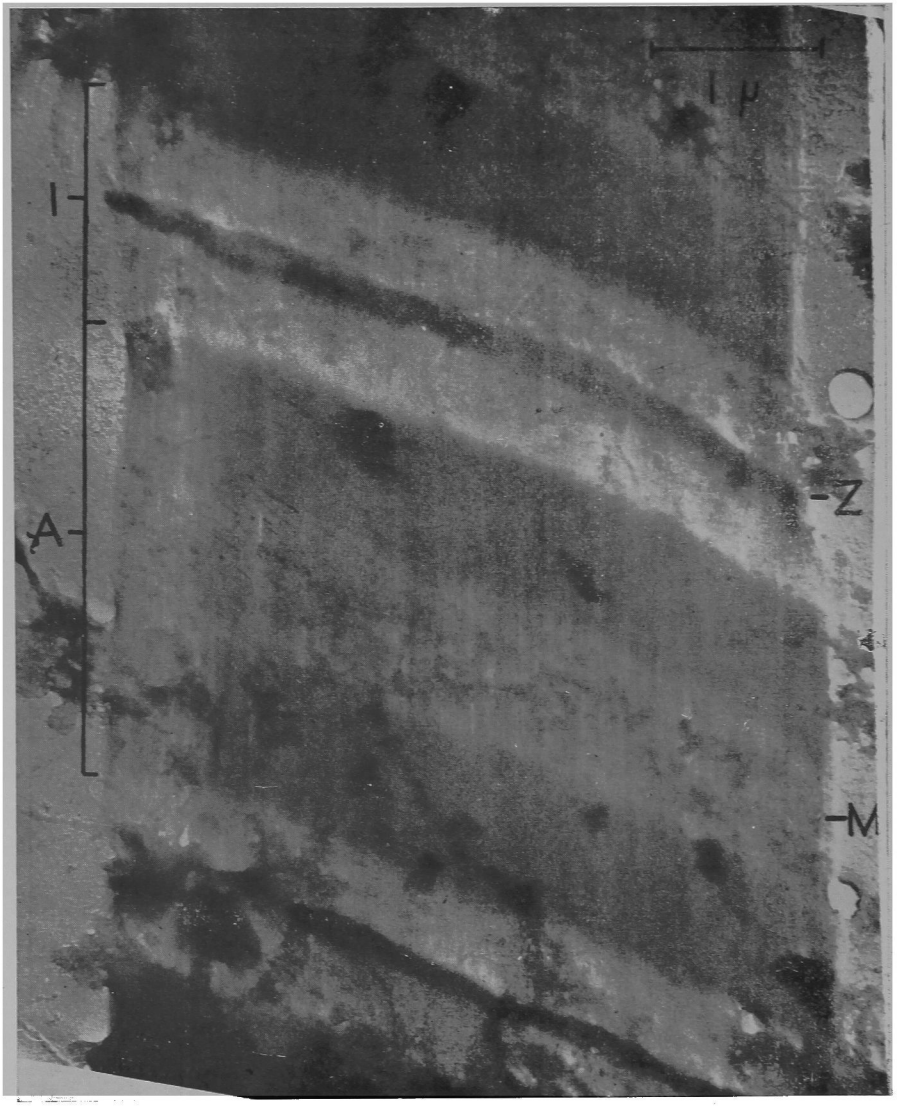

Fig. 8. — Extended myofibrils from *Dytiscus* coxal levator showing dotted M line and probable remnants of endoplasmic reticulum on surface of fibril.

Fig. 9. — Extended myofibrils from *Dytiscus* coxal levator with divided **Z** line and **M** line consisting of single row of fine dots.

Fig. 10. — Extended myofibrils from *Dytiscus* coxal levator showing continuous myofilaments and double Z line.

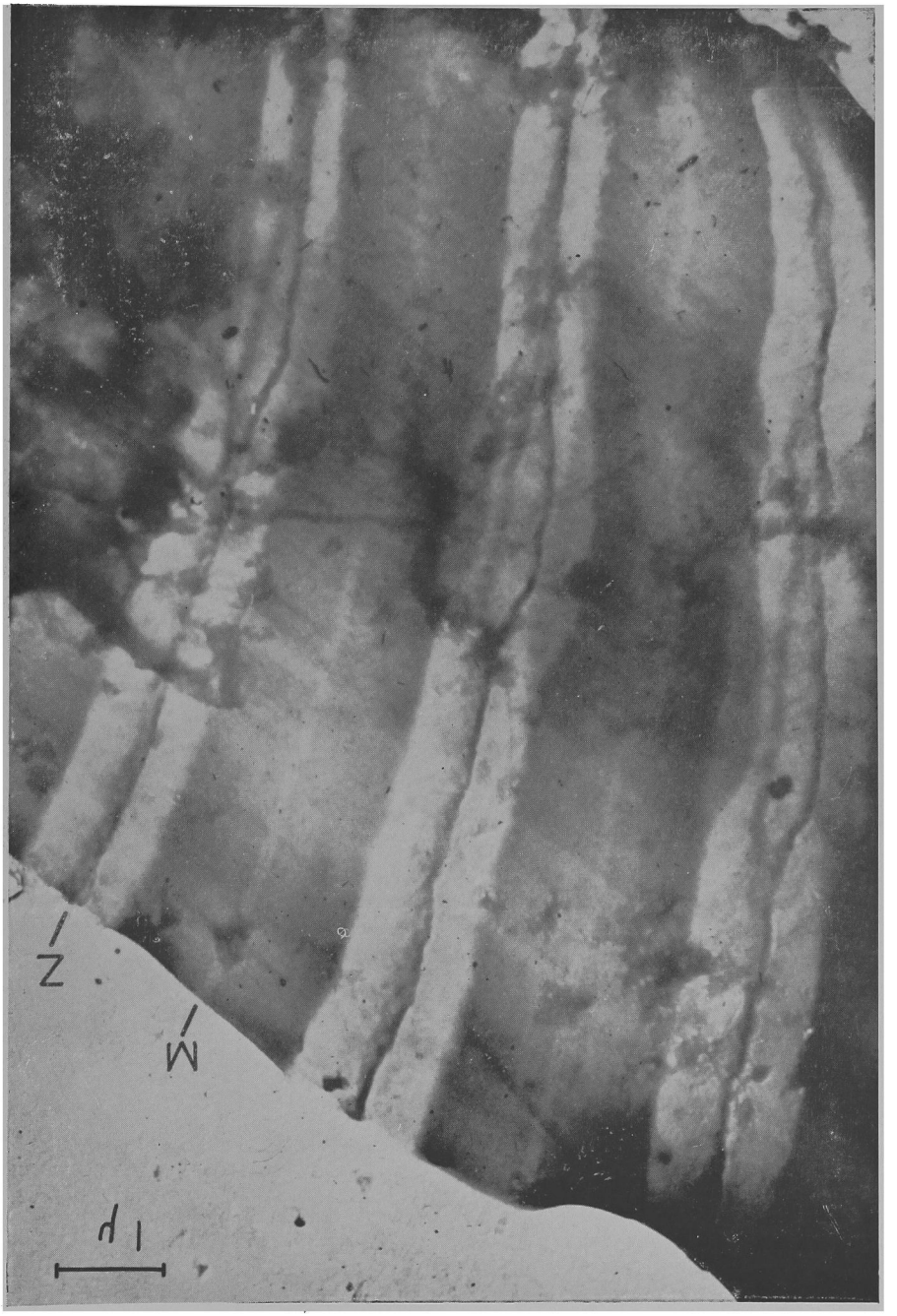

Fig. 11. — Equilibrium length myofibrils from *Dytiscus* coxal levator. M line consists of multiple rows of fine dots.

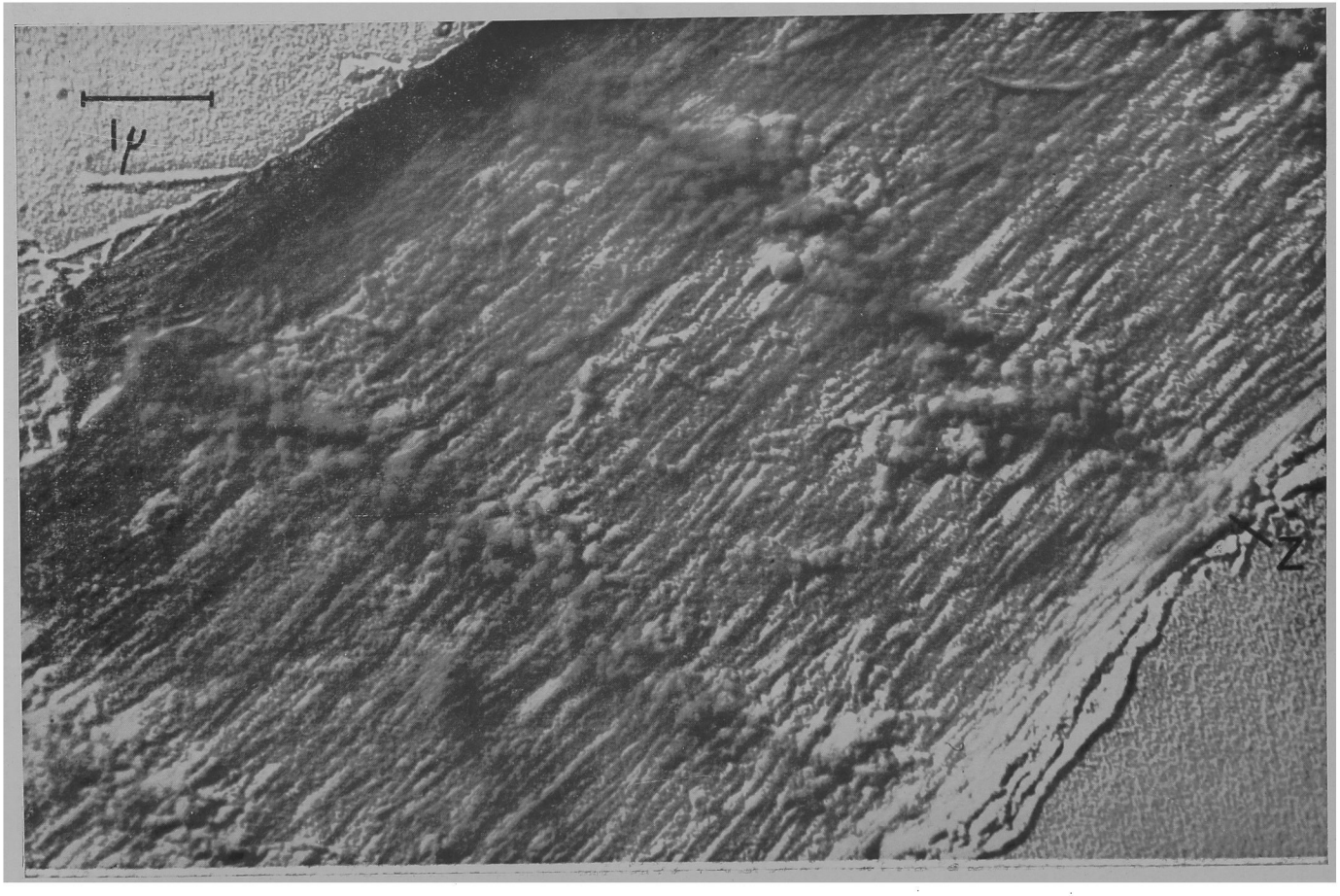

Fig. 12. — Contracted myofibrils from *Dytiscus* coxal levator. M line ab sent. Z line appears as row of small lumps, probably including remnants of reticular system.

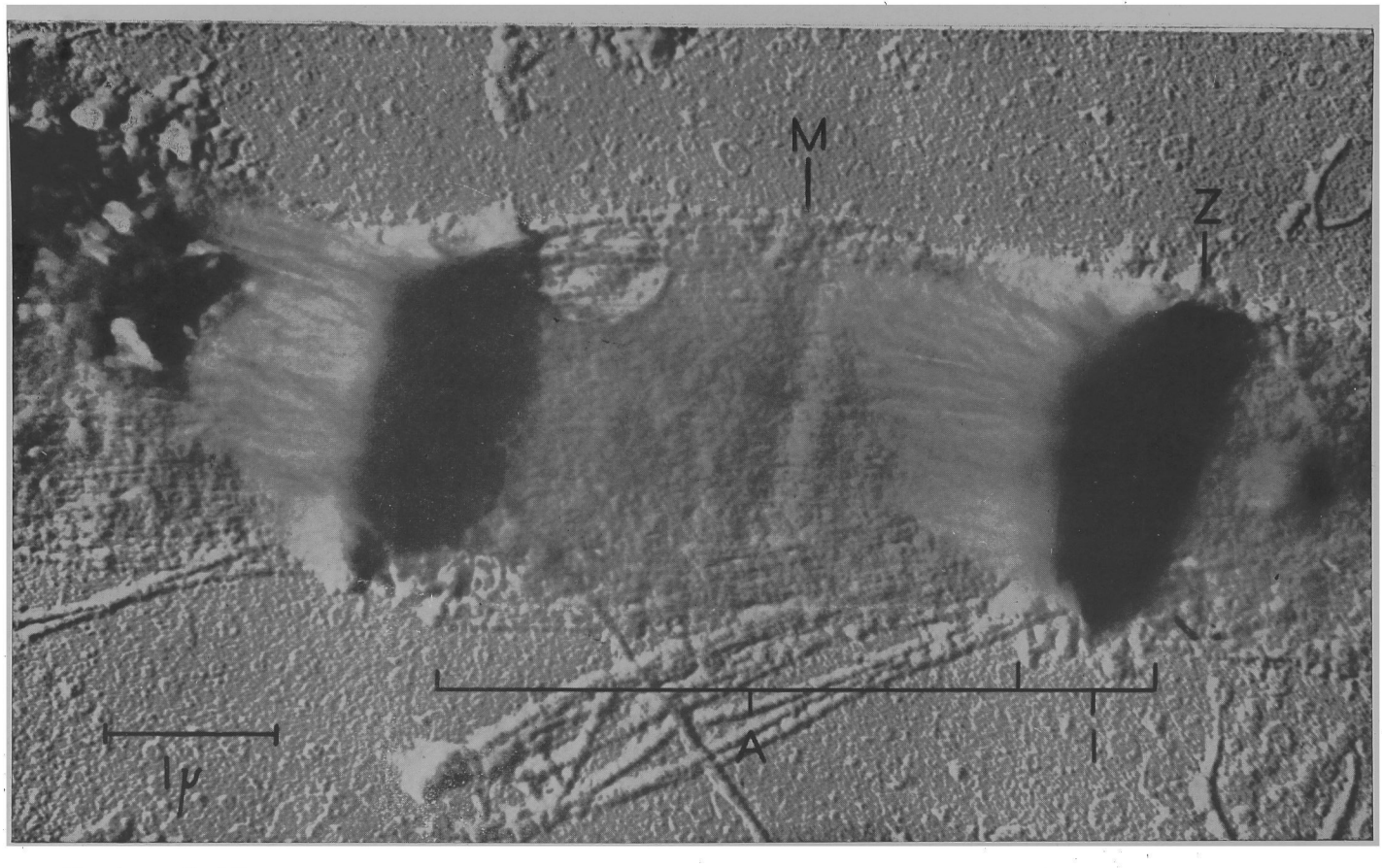

Fig. 13. — Partially contracted myofibril from *Hydrophilus* coxal levator. M appears as narrow trough; Z line throws finely veined shadow.

Fig. 14. — Fully contracted myofibril from *Dytiscus* coxal levator. Accordion appearance. Density of regions apparently reversed.