ULTRASTRUCTURE OF THE SKELETAL MUSCLES OF THE ADULT AND HYPOPUS OF THE MITE *CALOGLYPHUS MYCOPHAGUS*

BY J. S. KUO *

ANATOMY
MUSCLES
ADULT
HYPOPUS

ABSTRACT : The muscles of both adult and hypopial stages of *Caloglyphus mycophagus* are striated, with rather long sarcomeres (ca. 5 μm) and the fibres have a poorly developed sarcotubular system and are not divided into distinguishable myofibrils. Thick filaments of the hypopial muscles are smaller than those of the adult, and the ratio of thin to thick filaments is 6 : 1 and 8.5 : 1 in the hypopus and adult respectively.

ANATOMIE
MUSCLES
ADULTE
HYPOPE

RÉSUMÉ : À la stase adulte et à la stase hypopiale de *Caloglyphus mycophagus* les muscles sont striés, avec des sarcomères assez longs (ca. 5 μm), les fibres possèdent un système sarcotubulaire pauvrement développé et ne se divisent pas en myofibrilles discernables. Les filaments des muscles de l'hypope sont plus petits que ceux de l'adulte, le rapport entre filaments fins et épais chez l'hypope et l'adulte étant respectivement de 6 : 1 et 8,5 : 1.

INTRODUCTION

There have been a considerable number of studies of the fine structure of the muscles of arthropods including a recent review on the subject by PRINGLE (1972). Most of these studies have been on insect and crustacean muscles. With the exception of a very brief mention of the muscle structure of a mite, *Acarus siro* (Hughes, 1969), there have been no fine structural studies of the muscles of species of the group Acarina. Though there was an excellent light microscopy study on the developing skeletal muscles of a tarsonemid mite by ARNOSON (1961).

This paper presents a comparative study of the fine structure of the dorso-ventral body skeletal muscles of two quite different morphological stages, the adult and the hypopus of the mite *Caloglyphus mycophagus*. The position of these muscles are observable with a ligth microscope, and the fine structure of their attachment to the cuticle has been described earlier (KUO and NESBITT, 1970 ; 1971 ; KUO, MCCULLY and HAGGIS, 1971).

MATERIALS AND METHODS

Adult and hypopial stages of *Caloglyphus mycophagus* (Megnin) (Acaridae) were obtained from cultures maintained at 20°C (for details of cultural method see KUO and NESBITT, 1970).

* Department of Biology, Carleton University, Ottawa, Canada.
Present Address : Electron Microscopy Centre, University of Western Australia, Nedlands, Western Australia, 6009. Australia.

The animals were fixed in 6 % glutaraldehyde in 0.025 M phosphate buffer pH 6.9, for 48 hours at 0°C, and then were postfixed in 2 % osmium tetroxide in phosphate buffer for 24 hours at room temperature. After dehydration in an ethanol series, the animals were embedded in Araldite through propylene oxide.

Sections were cut with glass or diamond knives and put on grids, 75 × 300 mesh uncoated or 200 mesh with supporting films. Grids were stained for 15 minutes with freshly prepared 5 % uranyl acetate in 50 % ethanol, followed by lead citrate for 10 minutes. Specimens were examined in a Siemens Elmiskop 1A at 80 Kv.

OBSERVATIONS

ADULT

General organization of the muscle fibres.

Muscle fibres are oval in transverse section, ca. 1 μm and 4 μm in diameter. They are very compact when viewed in transverse section and are not obviously divided into myofibrils. The muscle fibres are penetrated by invaginations of the plasma membrane, the T system, which links to the sarcoplasmic reticulum to form the sarcotubular system. This system, however, is relatively poorly developed in these muscles.

The Contractile System.

The most conspicuous striations seen in longitudinal sections of the myofribrils are very electron dense Z lines. These lines tend to be distorted into wavy transverse lines which reflect the variable length of the sarcomeres with a range of 4.0-5.5 μm. Transverse striations corresponding to the A and I bands of vertebrate skeletal and insect flight and visceral muscles can also be observed, but the boundaries of these two bands are indistinct. The H and M lines, usually present in the striated muscles of other arthropods and of vertebrates are not identifiable. Thus it is not possible to determine the length of the thin filaments. The length of the A band is about 4.0 μm, with a range of 3.5-4.8 μm. The I band of the thin filaments is about 0.37 μm long with a range of 0.35-0.38 μm. The Z line usually has irregular margins and is ca. 0.25 μm long with a range of 0.19-0.33 μm.

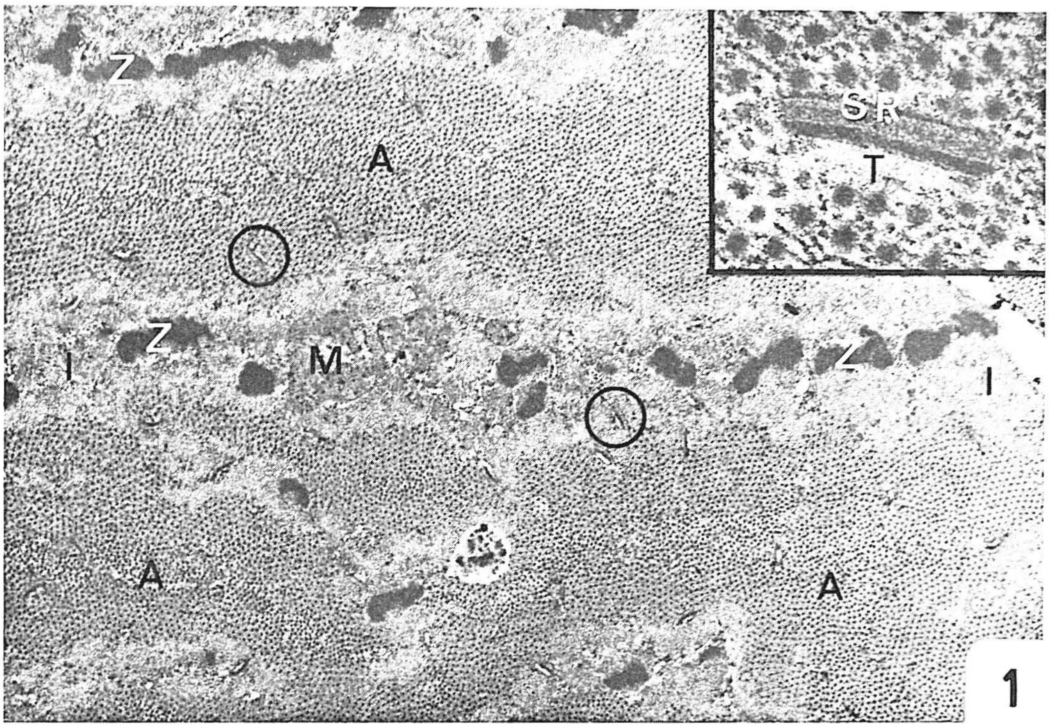
Clear orbits containing twelve thin filaments surrounding each thick filament are usual in the A band region (Fig. 3). Occasionally there are thirteen thin filaments associated with each thick filament (Fig. 5). A given thin filament often appears associated with more than one thick filament, but the count of thin and thick filaments in wide areas of favourably oriented transverse sections indicates approximately a 8.5 : 1 ratio of thin to thick filaments.

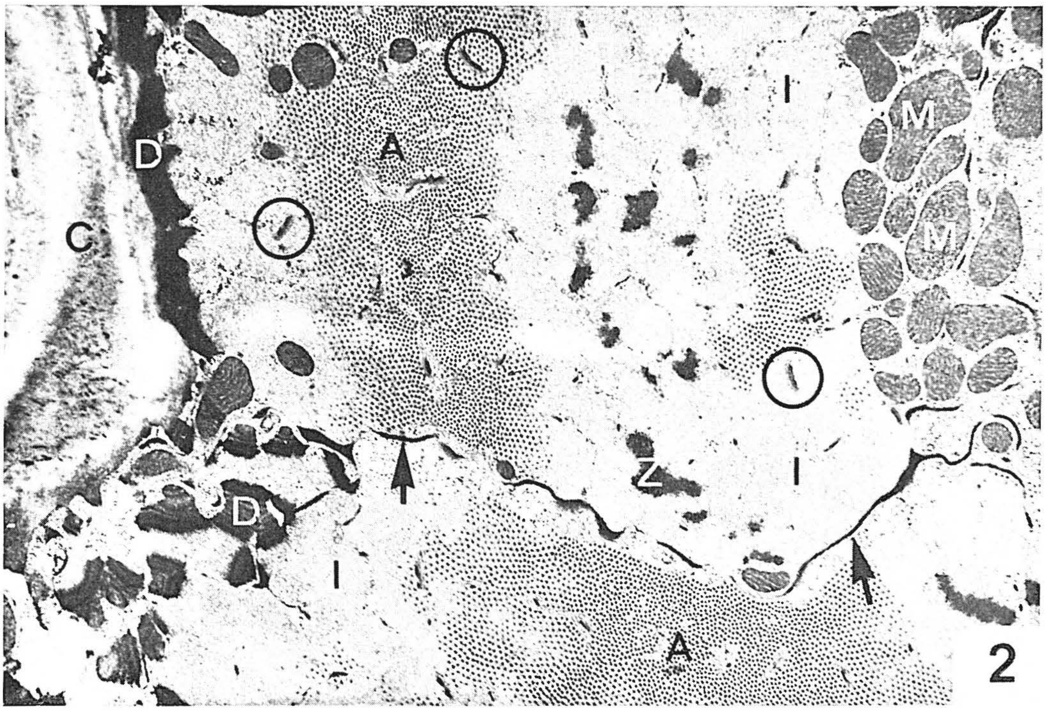
The thick filaments are about 23 nm in diameter, and appear to be made up of electron dense sub units (Fig. 5). The thin filaments measure ca. 6.5 nm in diameter. The thick filaments have a centre to centre spacing of 40-45 nm. The spaces between thin and thick filaments are usually of low electron density, but at a very high resolution, cross bridges can sometimes be seen linking the two types of filaments (Fig. 5). In the I band region, the thin filament density per unit area of cross section is approximately 3 times that in the A band. These thin filaments have a centre to centre spacing of ca. 10-12 nm and do not seem to be arranged in groups (Fig. 3).

In longitudinal sections the thin filaments can be seen to extend from both the A and I regions into the electron dense material of the Z line (Fig. 4). However, because of its great electron density, it is not possible to resolve much structure in the Z line region. Occasionally a few structures similar in diameter to thin filaments can be seen in transverse sections of this region (Fig. 3).

The Sarcotubular System.

The sarcotubular system of *Caloglyphus* muscle is much reduced (Fig. 1), compared to that of the muscles of other arthropods. Nevertheless, as in other striated muscles, this system in the mite muscle is composed of a transverse component, the transverse T tubular system of invagination of

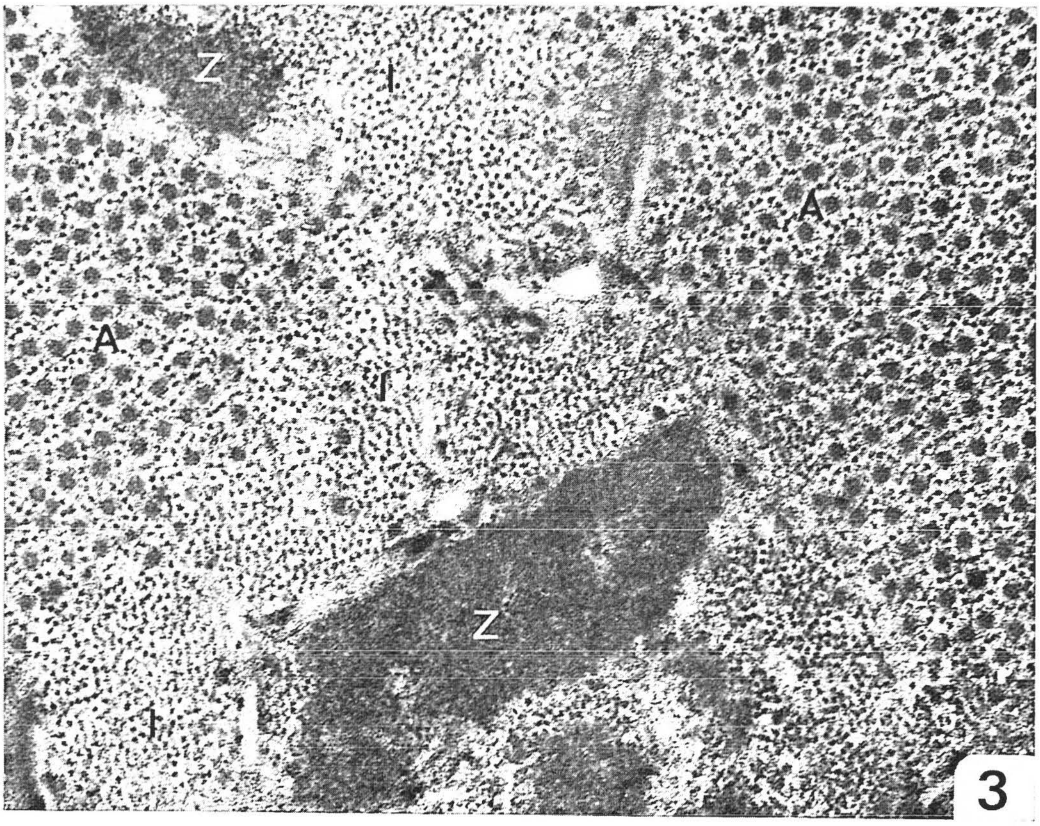


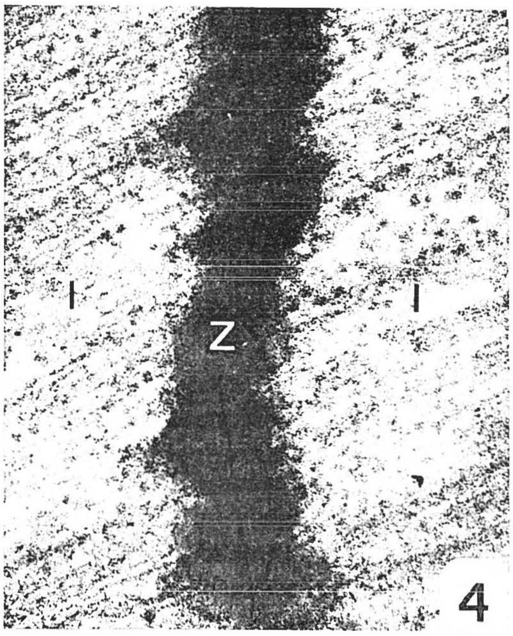


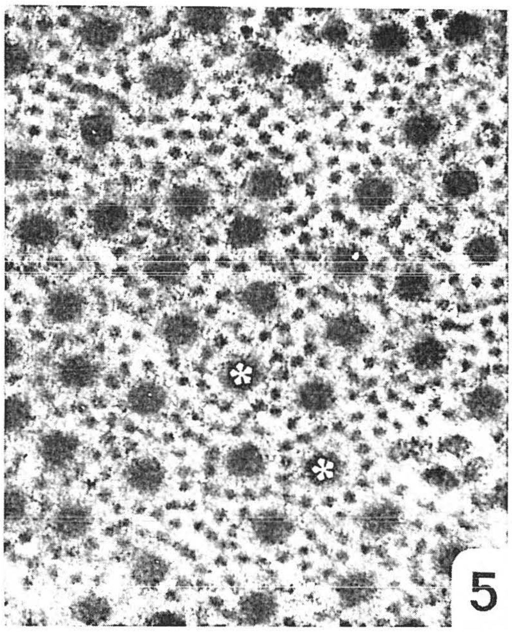
FIGS 1-2 : 1) A transverse section of a skeletal muscle fibre of an adult mite. Portions of Z lines (Z), I bands (I) and A bands (A) can be seen. Dyadic structures (circles) are widely distributed. Mitochondria (M) are occasionally found deep in the muscle. × 15,500.

Inset : Hight magnification view of a dyadic structure. In such a region the sarcoplasmic reticulum (SR) and a T system tubule (T) come into close apposition. The region between a sarcoplasmic reticulum membrane and a tubule membrane is very electron dense. × 130,000.

2) A transverse section of portions of a skeletal muscle fibre of a hypopus. Note that the peripheries (arrows) of the muscle cells have a discontinuous electron dense appearance. Numerous mitochondria (M) are located in the periphery of the fibres. Various bands of the sarcomere can be seen, A (A), I (I), Z (Z) bands.Various dyadic structures (circles) also scattered throughout the sarcomere. × 18,350.







FIGS. 3-5 : A skeletal muscle fibre of an adult mite. — 3) A transverse section showing one arrangement of myofilaments in the different regions of the sarcomere. In the A band (A), the arrangement of thick filaments does not have a particular geometrical pattern. Each thick filament is surrounded by eleven to twelve thin filaments. The electron dense Z line material (Z) contains numerous indistinct filament-structures which seem to have similar diameters as thin filaments in I band (I). × 96,000.

4) A longitudinal section including a portion of a Z line (Z). Note the very electron dense Z line with irregular margins and the adjoining I bands (I) containing only thin filaments, some of these filaments apparently extend into the Z line. × 100,000.

5) High magnification of a transverse section showing the thick-thin filament relationship in the A band. The centre of the thick filament is solid but does not have a completely homogenous electron dense appearance which may be caused by the presence of subunits of these filaments. Sometimes thirteen thin filaments orbit one thick filament (asterisks). Numerous cross bridges are visible between thick and thin filaments. × 220,000.

the plasma membrane, and an intracelluar, more or less longitudinal component, the sarcoplasmic reticulum.

At certain points, the T system tubules and the sarcoplasmic reticulum come into close apposition forming a dyad junction. These dyads appear to be evenly distributed among the muscle fibres in the A and I bands but not in the Z line (Fig. 1). In the region of the dyads (Fig. 1 Insert), the T system has no electron dense contents and its limited membranes can just be distinguished. Here the membranes of the sarcoplasmic reticulum and the T system are only about 14 nm apart and the area between them is very electron dense. The sarcoplasmic reticulum in such a jonction is approximately 25 nm wide and filled with moderately electron dense material. The membranes of the sarcoplasmic reticulum in this region are more electron dense than elsewhere.

HYPOPUS

■ *General organization of the muscle fibres.*

The muscle fibres in a transverse section are ca. 4 and 8 μm in width. As in the adult muscles, the fibres are not clearly broken up into separate myofibrils by development of the membrane systems. There are many more mitochondria associated with the hypopial muscles than with the adult muscles. These mitochondria are larger and their ground substance more electron dense than those of the adult muscles (Fig. 2).

■ *The Contractile System.*

The A band is composed of two regions. The first region is one in which both thick and thin filaments are found. The thick filaments are ca. 20.5 nm in diameter, run the length of the A band, and in a cross section they frequently appear to have a central core of lower electron density. These thick filaments are generally arranged in a fairly well ordered hexagonal array and each filament of the array is surrounded by six others at equal spacing. The centre-to-centre spacing of these thick filaments is ca. 55-60 nm (Figs. 6 and 7). Thus the thick filaments of the hypopus differ in diameter, structure and arrangement from those of the adult. Surrounding each filament is an orbit of 11-12 thin filaments, each 6 nm in diameter (Figs. 6 and 7). Cross sections cut through the A band show clearly lateral projections (i. e. cross bridges) extending between thick and thin filaments (Fig. 6). The count of thin and thick filaments in a wide area of favourably oriented transverse sections gives approximately a 6 : 1 ratio of thin to thick filaments, compared to a 8.5 : 1 ratio in the adult. The second region of the A band is a true H zone, formed in the central region of the A band, in which only thick filaments are noted. These thick filaments are also arranged in fairly ordered hexagonal arrays with a centre-to-centre spacing of ca. 45 nm (Fig. 9).

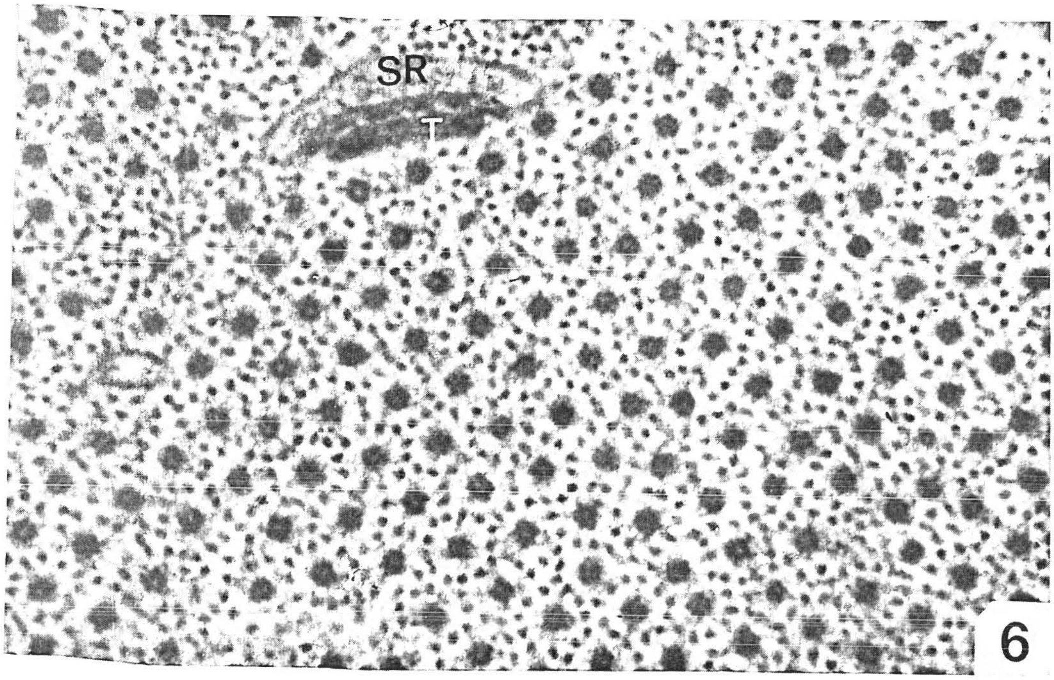
In some cases a third type of filaments is found in the positon of thick filaments. Unlike the ordinary thick filaments, these are only ca. 10-12 nm in diameter. Like the regular thick filaments, these thinner filaments appear to have a cenral core of lower density, ca. 5 nm in diameter. These thinner thick filaments are also surrounded by 11-12 normal thin filaments within regular orbits (Fig. 7).

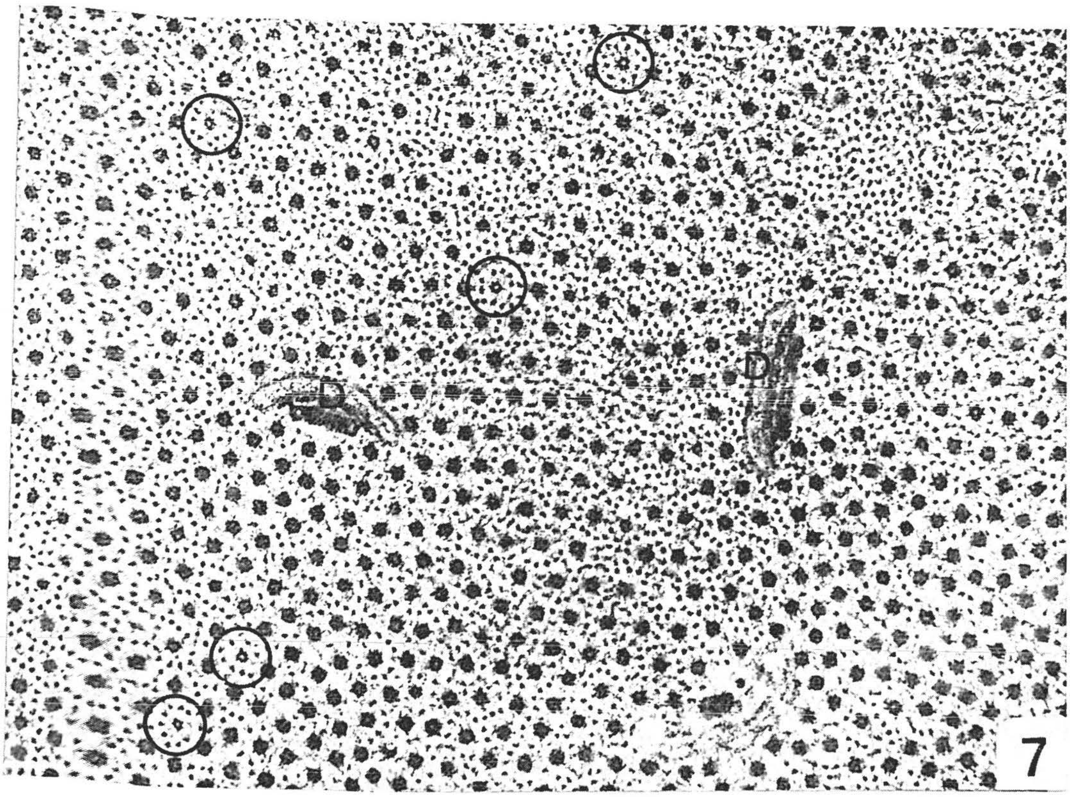
Although transverse sections show that thin filaments are found in an ordered array within the A band as in muslces of adult mites, no regular pattern of filaments is found within the I band. The density of thin filaments per unit area in the I band is about three times that in the A band (Figs. 8 and 9).

Transverse sections through Z lines suggest that they may be comprised of filaments embedded in or surrounded by a dense material (Fig. 8). These filaments, ca. 6 nm in diameter, resemble thin filaments of the I band. The density of these filaments in the Z line appears to be similar to the thin filaments in the I band.

■ *The Sarcotubular System.*

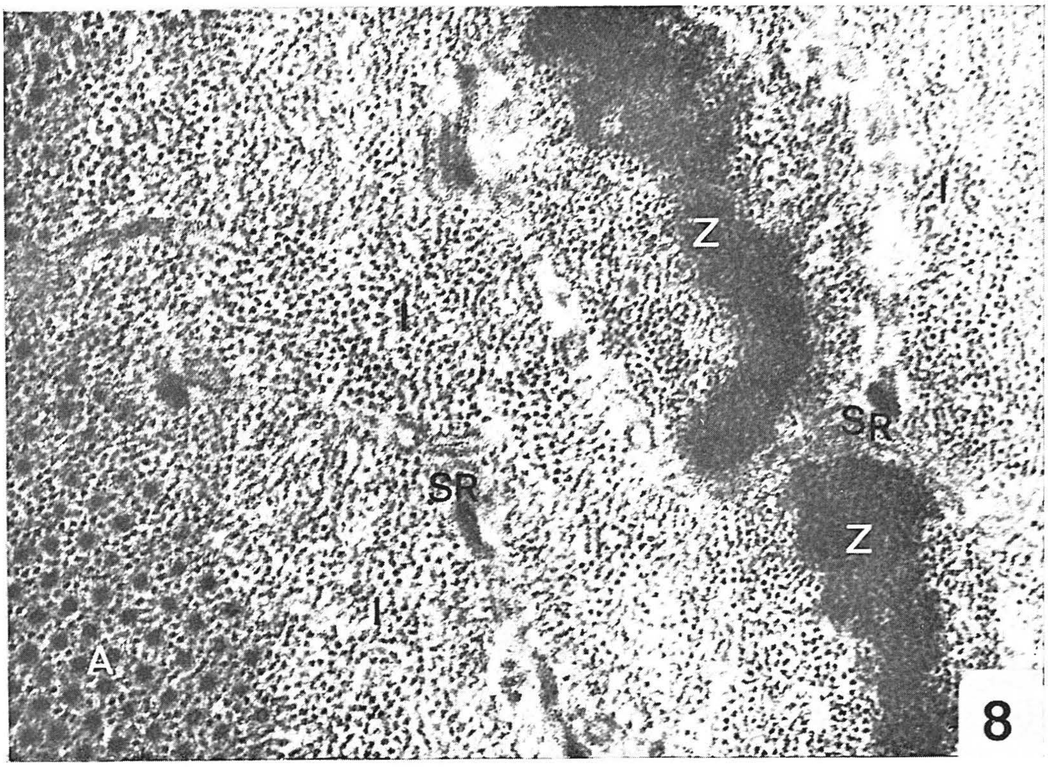
Both the T system and the sarcoplasmic reticulum are poorly developed compared to those in striated muscles of other animals (Fig. 2). How-

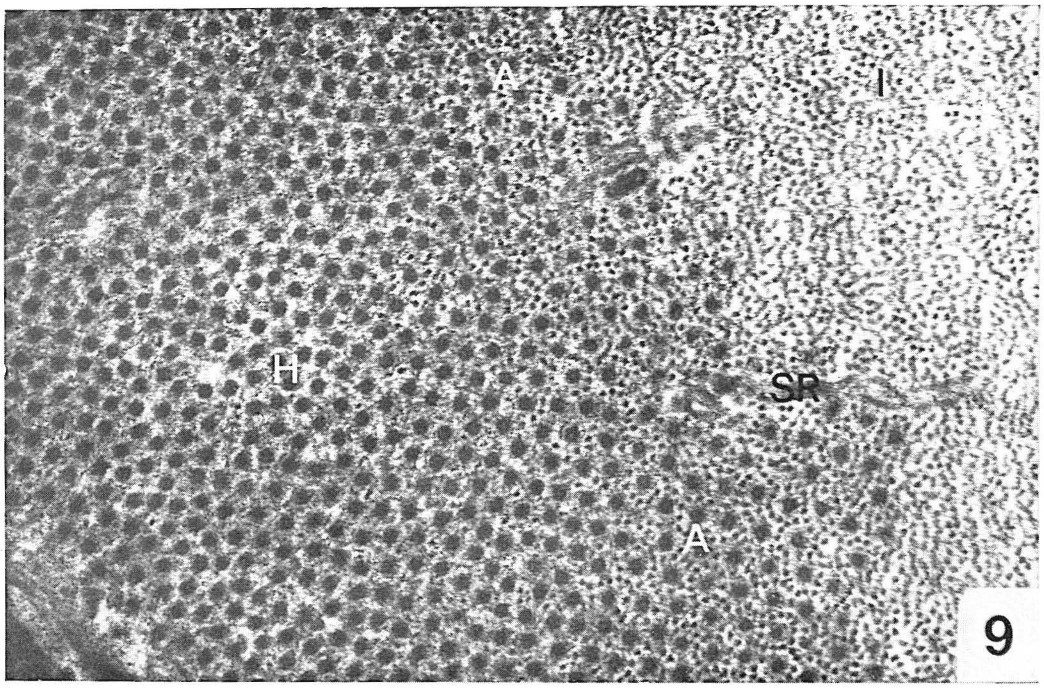




FIGS. 6-7 : A skeletal muscle fibre of hypopus. — 6) High magnification of a transverse section showing the thick-thin filament relationship in the A band. Note that the thick filaments are electron transparent in their centre. These thick filaments are arranged in a hexagonal pattern with one thick filament in the centre. Each thick filament is surrounded by eleven to twelve thin filaments. The cross bridges also can be seen between the thick and thin filaments. A dyad is a region where the sarcoplasmic reticulum (SR) and the T system tubules (T) come close together. × 150,000.

7) The third type of filament (circles) present in the ordinary thick filament position. These filaments are also hollow but only ca. 10 nm in diameter. This type of thick filament is also surrounded by eleven to twelve thin filaments. D, dyadic structure. × 95,000.





FIGS. 8-9 : A skeletal muscle fibre of a hypopus. — 8) The I bands (I) containing only the thin filaments which appear to have changed their arrangements from that in the A band (A) (cp. with Fig. 7). Among the electron dense Z line materials (Z) there are numerous filament-like structures which appear similar in diameter and electron density to thin filaments of other regions of the sarcomere. The sarcoplasmic reticulum (SR) is extended into both the I band and Z line regions. × 110,900.

9) In the A band (A) thick and thin filaments occur together, while in the H band (H) only thick filaments are present. The I band (I) contains only thin filaments. Portions of the sarcoplasmic reticulum (SR) also can be seen. × 92,400.

ever, the sarcoplasmic reticulum of the hypopus muscle appears more extensive than in the adult muscle. As well as developing in the A and I bands, this system is also seen in the region of the Z line (Fig. 8). This arrangement is not seen in the adult muscles. Another difference between adult and hypopus muscle is seen in the structure of the dyadic junctions (Fig. 6). The width of the T tubule in the dyad in the hypopus is only 7 nm compared to 15 nm in the adult. Furthermore, the electron dense area between the T system and the sarcoplasmic reticulum is only about 7 nm in the hypopus compared to 14 nm in the adult muscle. However, the distribution and numbers of dyadic structures seen in the cross sections of sarcomeres appear to be similar in both adult and hypopus muscles.

DISCUSSION

Table 1 summarizes some relevant data available on the fine structure of skeletal muscles in various groups of the animal kingdom. While in general the fine structure of the *Caloglypjus* muscles resembles that of striated muscles of other animals, there are several distinct differences. The most outstanding of these differences is the large number of thin filaments which orbit each thick filament. While up to twelve filaments per orbit have been reported in the slow acting muscles of members of the phyla Plathyhelminthes, Annelida, Nematoda, Mollusca and Arthropoda (see references in HAGOPIAN, 1966 ; ANDERSON and ELLIS, 1967), orbits containing as many as thirteen filaments are frequently seen in the *Calopglyphus* adult skeletal muscles (Fig. 5). Furthermore, if one makes total counts of thin and thick filaments over a considerable area of micrographs of good cross sections of mite skeletal muscles, there is constantly a thin : thick ratio of 8.5 : 1 in the adult and 6 : 1 in the hypopus. A ratio of 7 : 1 is the highest reported for skeletal muscles of any other animal (HOYLE and MCNEILL, 1967), though the ratio for smooth muscle in mammals is ca. 50 : 1 (RICE, et *al.*, 1970).

TABLE 1 : The relationship of the thick and thin filaments in various muscles

Type of muscle and references	Sarcomere Length (μm)	Thick filament length (μm)	Thick filament diameter (nm)	Numbers of thin filaments orbit each thick filament	Ratio of thin filaments to thick filaments
Human flexor carpi radialis *	—	1.5	10-12	4	2 : 1
Cat heart *	—	1.5	10-12	4	2 : 1
Dragonfly flight (Smith, 1966 *a*)	2.3	2.2	—	6	3 : 1
Moth flight (*Agro-(tis, Phytometra*) (Auber, 1967)	—	—	14	6	3 : 1
Butterfly flight (*Pieris, Vanessa*) (Auber, 1967)	—	—	14	7-9	4 : 1
Cockroach femoral (Hagopian, 1966)	5.3-8.2	4.5	18.5-20.5	10-12	4 : 1
Musca domestica femoral (Pasquali-Roachetti, 1970)	4.5	—	20	10-12	5 : 1
Cockroach inter-segmental (Smith, 1966 *b*)	7.8-8.3	3.6-4.1	16-18	12	6 : 1
Insect visceral (Smith *et al.*, 1966)	7-8	—	16-18	12	6 : 1
Insect abdominal air-guide retractor (Walcott & Burrows, 1969)	7	3.5	16-20	11-12	6 : 1
Spider leg (Kawaguti & Kamishima, 1969)	5.3-5.5	3.5	16	12	6 : 1
Caloglyphus hypopus skeletal	—	—	20	11-12	6 : 1
Crab eyestalk withe levator (Hoyle & McNeill, 1967)	7.9	—	—	not in orbit	7 : 1
Caloglyphus adult skeletal	5	4	23	11-13	8.5 : 1
Mammalian smooth (Rice *et al.*, 1970)	—	—	15-20	not in orbit	50 : 1

* From Hagopian & Spiro (1968).

The muscle of the hypopus is also unique amont striated muscles described to date in having the two types of thick filaments which are apparent in good cross sections of the muscles (e. g. Fig. 7). The nature of the smaller thick filaments is not clear. The electron transparent core of this type of filament is the same diameter as the core of a normal thick filament in the hypopus, and it is possible that these small filaments represent an intermediate stage between the normal thick filament of the adult muscle and the thick filament predominating in the hypopus. There is no evidence that the small thick filaments are not functional beause each is surrounded by the normal orbit of thin filaments, and cross bridges between the thin filaments and the small thick filaments can be seen and appear similar to those between thin filaments and normal thick filaments. Of course, the possibilities of fixation, dehydration and embedding causing the appearance of these filaments cannot be eliminated.

It is interesting that the H lines which are seen in longitudinal sections of vertebrate skeletal and insect flight muscles (e. g. SMITH, 1966 *a*) are absent from similar sections of the mite muscles. At first appearance, this seems to fit with the reported absence of H lines non-flight skeletal muscles of insects (SMITH, *et al.*, 1966 ; WALCOTT and BURROWS, 1969 ; ODHIAMBO, 1970). However, when cross sections cut serially through mite muscle are examined it is possible to find areas in the centre of the sarcomere where thin filaments are absent (Fig. 9). Thus this confirms the presence of at least a narow H band. It is possible that serial cross sections would show the presence of similar zones in the insect muscles from which these bands are though to be absent.

There are definite differences between the fine structure of the muscles of the adult and the hypopus. The most noticeable difference seen at low magnification is the very electron dense material that fills much of the intercellular spaces between adjacent muscle cells in the hypopus, but is absent from adult muscles though the intercellular spaces approximately the same width in each case. The second difference is a considerably increased number of mitochondria in the hypopus muscle and their distribution among the fibres as well as around the periphery of the cells. Thirdly, the ratio of thin : thick filaments is lower in the hypopus (6 : 1) than that of the adult (8.5 : 1). Furthermore, the thick filaments of the adult appear to be significantly bigger in diameter (20 nm compared to 23 nm) than those of hypopus muscle. Also, there is the appearance of the " hollow " second type of thick filament which is confined to the hypopus muscle.

The higher number of mitochondria, better developed sarcotubular system and lower thin to thick filament ratio all suggest that hypopial skeletal muscles are faster acting than those of the adult. The hypopus in culture is more active than the adult and thus it seems reasonable that they would be faster acting muscles. Other differences in structure between adult and hypopial muscles such as the changes in the surface of the muscle cells and the changes in substructure of the thick filaments in the hypopus cannot be clearly linked to changes in function.

It would be of great interest to follow the changes which must occur in the muscles as the hypopus becomes an adult. It is obvious that these changes are not only at the relatively macro level involving redistribution and changes in the number of mitochondria and changes in the intercellular material between muscle cells, but also in the arrangement of thick and thin filaments relative to each other and at the level of macromolecular configuration of the thick filaments.

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