THE SARCOPLASMIC RETICULUM OF STRIATED MUSCLE OF A CYCLOPOID COPEPOD

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ABSTRACT

The fine structure of the abdominal musculature of the copepod *Macrocyclops albidus* was investigated by electron microscopy. Tubules penetrate into the muscle fibers from the sarcolemma, continuity between the wall of the tubules and the sarcolemma being clear. A dense network of tubules envelops the myofibrils, its interstices being occupied by cisternal elements. At the Z lines the tubules traverse the interior of myofibrils, giving off branches which course longitudinally within the substance of the myofibrils. These branches are also accompanied by elongate, non-intercommunicating cisternae. Comparison of this fast acting copepod muscle with other vertebrate and invertebrate muscles indicates that the complexity of the tubular system is a function of the myofibrillar geometry, whereas the degree of development of the cisternal system is related to the contraction speed of the muscle.

INTRODUCTION

The copepod *Macrocyclops albidus* (Jurine) 1820 (Arthropoda, Crustacea) is one of the most common North American copepods. The species is a freshwater dweller and measures between 1 and 2.5 mm in length. In pursuit of its prey or in escaping a predator, the animal is capable of brief, very rapid locomotion, produced by the synchronous, high frequency beating of all of its appendages. Both the appendicular and the general body musculature show an unusual elaboration of the sarcoplasmic reticulum, a feature commonly associated with fast acting muscles and, therefore, of considerable interest for comparative purposes. This reticulum is a more or less elaborate system of tubular and cisternal structures associated with myofibrils, which has become the subject of considerable attention in recent years. Its components are believed to mediate the process of excitation-contraction coupling.

In the present study the dorsal and ventral abdominal musculature was examined. The internal architecture of these muscles, which were used because of the greater ease of orientation, is identical to that of the appendicular musculature.

MATERIALS AND METHODS

Macrocyclops albidus cultures were obtained from Carolina Biological Supply Company, Elon, North Carolina. Adult animals were bisected in ice-cold, 1.3 per cent osmium tetroxide buffered to pH 7.4 with *s*-collidine, and fixed for 1 hour. They were dehydrated through several changes of 30, 50, 80, 95, and 100 per cent cold ethanol and allowed to come to room temperature. After two changes of propylene oxide, they were embedded in Epon. Sections showing silver interference colors were cut with glass knives on a Porter-Blum microtome and mounted on celloidin-coated grids. They were stained with lead-stains according to Reynolds (1) or Karnovsky (2) and subsequently stabilized with a layer of evaporated carbon. Electron micrographs were taken with an RCA EMU-3E microscope.

RESULTS

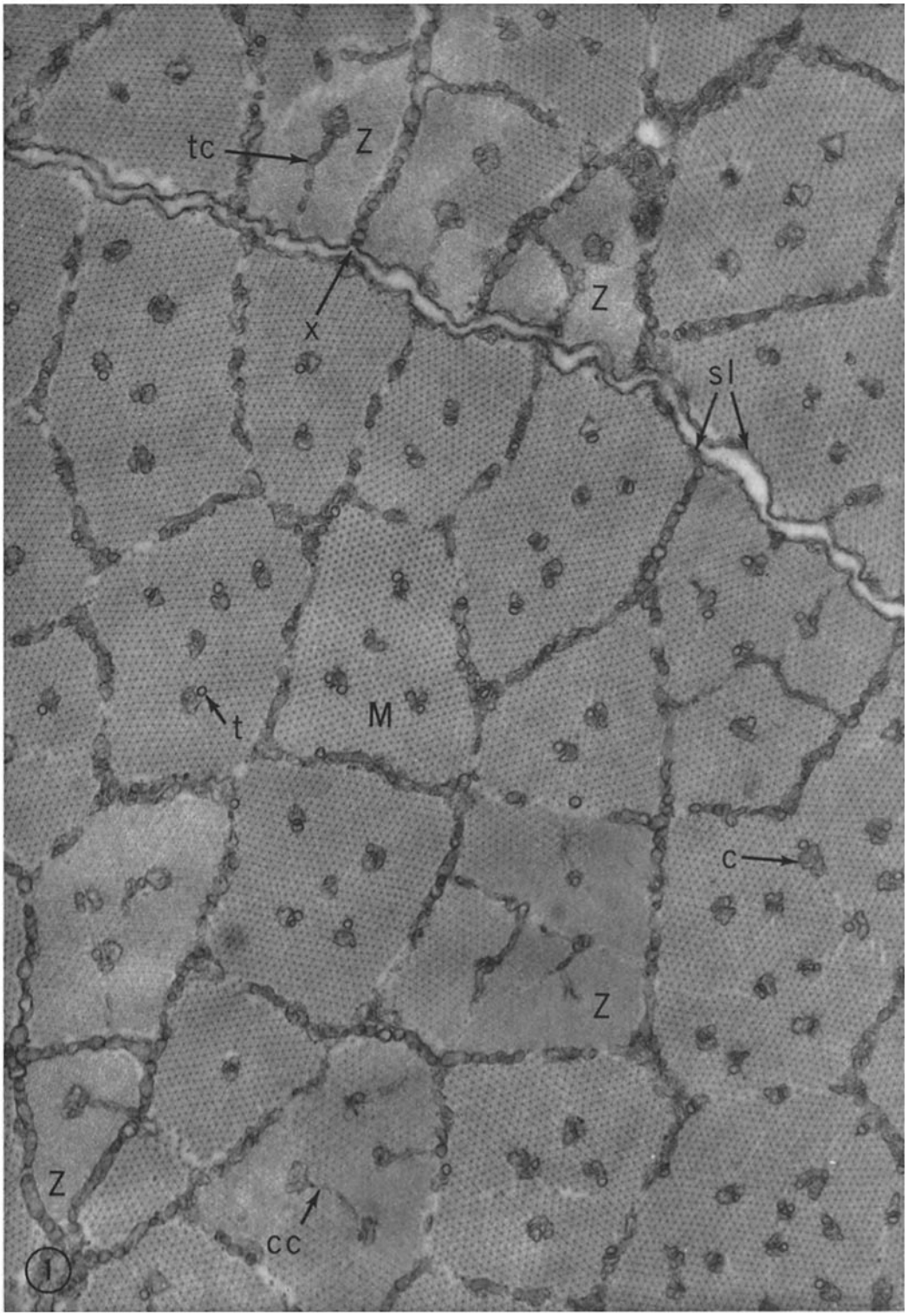
The dorsal and ventral abdominal musculature is composed of fibers ranging in diameter from 2 to 25 μ. The myofibrils have diameters between 0.5 and 12 μ, although these extremes are not generally encountered in any one fiber. The thick myosin filaments, which appear hollow and have a diameter of about 150 A, are each surrounded by six actin filaments forming the typical arthropodan hexagonal array in cross-section with a spacing of about 480 A between thick filaments (Fig. 2). While in vertebrate striated muscle each actin filament is located equidistantly between three myosin filaments, it is positioned between two myosin filaments in arthropod muscle as described by Huxley and Hanson (3) in the insect *Calliphora*. Nuclei of the fibers are located peripherally and usually at the surface of the muscle as a whole. It should be remembered that in an animal of this size even major muscles are composed of very few fibers, each of which reaches the surface of the muscle. Mitochondria tend to be concentrated in a dense layer or as large masses under the sarcolemma of each fiber, but only around the periphery of the muscle. They are almost absent from the interior of muscles, being rarely found under the intramuscular surfaces of the fibers and, even less frequently, between fibrils. The peripheral mitochondria often reach dimensions which rival or exceed those of adjacent nuclei. The maximum size recorded for a mitochondrion is 7 μ in diameter and 16 μ in length, as seen in a thick section. Innervating axons make contact with the sarcolemma at frequent intervals, forming synapses *en passant*. The neuromuscular junction is relatively unspecialized. There is a slight depression in the sarcolemma, but no corrugated footplate as occurs in the vertebrate neuromuscular junction. The contiguous cell membranes of nerve and muscle show increased electron opacity and are spaced 100 A apart.

The sarcolemma is covered by a thin layer of basement membrane material which, however, tends to be absent in the regions between adjacent muscle fibers. Occasionally, the sarcolemma invaginates for a short distance into the fiber, carrying the basement membrane with it and forming a shallow, longitudinal partition. From the base and sides of these partitions, as well as from most parts of the surface membrane of the fiber, numerous tubules about 600 A in diameter and devoid of any basement membrane material extend into the interior and form a continuous, predominantly longitudinal network covering each fibril (Figs. 1 and 2). The points at which the tubules join the sarcolemma are not randomly scattered but are restricted to lines both transversely and longitudinally. These lines can be defined by the Z line level in a transverse plane and by intermyofibrillar regions directly beneath the sarcolemma in a longitudinal direction (Fig. 6). From its point of origin at the sarcolemma, the tubule is elaborately convoluted and usually cannot be followed for any considerable distance in cross-sections. However, in rare favorably oriented sections continuity of the limiting membrane of the tubules and the sarcolemma can be established beyond doubt. On entering the muscle fiber, the tubules branch extensively, forming a close meshed tortuous network which envelops the myofibrils (Fig. 4). This network is uninterrupted throughout the length of the fiber and is continuous with the sarcolemma both at the periphery and at the end of the fiber in the region of the myo-tendinous junction.

At each Z line the tubules enveloping the myofibril give off branches which penetrate it at the Z line and form a transverse network, more or less complicated, depending on the size of the myofibril (Fig. 3). The tubules of this transverse network branch further, giving rise to branches which run in a longitudinal direction within the substance of the myofibril, coursing between the myofilaments. In favorable longitudinal sections

FIGURE 1

A transverse section through part of two muscle fibers of copepod urosomal muscle. The sarcolemma (*sl*) gives off a tubule at *X*, and several close approaches of tubules to the sarcolemma are seen. Each smaller field surrounded by circular profiles constitutes a myofibril. Intrafibrillar tubules (*t*) and cisternae (*c*) are evident. Several fibrils have been cut at the level of the Z line (*Z*) and one at the M band (*M*). In the Z line regions both transverse connections of tubules (*tc*) and cisternal communicating strands (*cc*) are visible. × 27,000.


tc
Z
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the intrafibrillar tubules can be followed through several sarcomeres without interruption, giving off one or more side branches at each Z line level in passing (Fig. 5). At the level of the M band, the tubules frequently have short, blind side branches which account for the double profiles seen in cross-section in this region (Fig. 1). In contracted muscle the tubule is also folded back on itself at this same level, thus contributing to further complexity when the muscle is viewed in transverse section. The center-to-center spacing of the intrafibrillar tubules varies between 1000 and 4000 A, 2500 A being average. Hence, a fibril, depending upon its size, may contain from none to 60 or more longitudinal tubules, more or less regularly spaced within the myofibril.

Associated with this continuous interconnected network of tubules is a system of cisternae. Those that are distributed between the meshes of the perifibrillar network of tubules are relatively large and flat vesicles, reaching maximal dimensions of about 2500 A (Fig. 5). They do not have so sharply defined a wall as the tubules and their interior is filled with a diffuse, granular appearing material. Glycogen particles measuring 200 to 400 A are commonly associated with the surface of the cisternae (Fig. 2). The cisternae which are oriented parallel to the intrafibrillar tubules assume a more elongate shape than the perifibrillar cisternae, but rarely exceed 1500 A in diameter (Fig. 5). They can generally only be traced continuously for the length of a sarcomere, although occasionally they traverse the Z line and show a break in continuity after a short distance in the next sarcomere. These cisternae also tend to have glycogen scattered along their surfaces.

Vicinal tubules and cisternae show local specializations of contiguous areas of their limiting membranes. These regions are irregularly distributed in the perifibrillar network, whereas they are more regularly located in the intrafibrillar units, lying on either side of the M band but stopping short of the Z band (Fig. 5). In these places the contiguous membranes of the tubule and cisterna are closely coapted, maintaining a relatively constant interspace of about 100 A. Frequently it can be seen in cross-section that a tubule occupies a shallow depression in its accompanying cisterna. The opposing membranes show a distinct increase in electron opacity. These differentiated regions consisting of a tubule closely associated with a cisterna can be referred to as *dyads*. The term dyad was coined by Smith (4) in describing a similar association between infoldings of the sarcolemma and adjacent vesicles of the sarcoplasmic reticulum in the flight muscle of the beetle *Tenebrio*. The association between the members of a dyad is a mechanically firm one, for in muscle which has been slightly disrupted by faulty preparatory technique the dyads remain in their normal paired condition even though the adjacent unspecialized areas of reticulum are greatly disorganized. A certain amount of continuity between cisternae can be traced by way of thin, filamentous strands representing a continuation of their closed ends. These strands can be seen to cross the Z line to make contact with the cisternae in the next sarcomere or to parallel the transverse course of tubules at the Z line to adjacent intrafibrillar or perifibrillar cisternae (Fig. 1). However, these interconnections are not sufficiently consistent or frequent enough to warrant the assumption that all or even most cisternae are connected with each other. There are, moreover, frequent breaks in the continuity of intrafibrillar cisternae within the same sarcomere.

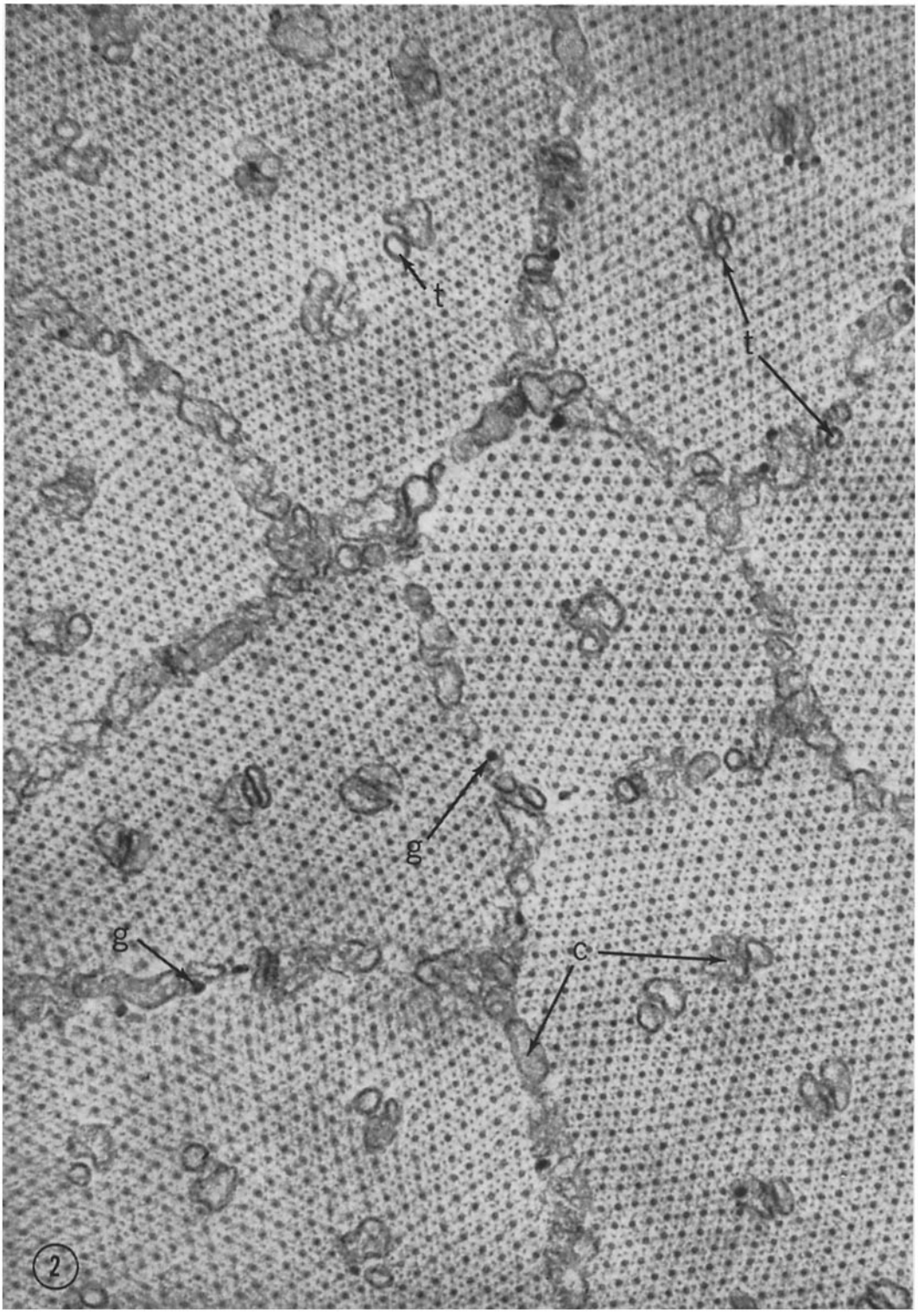
Near the myo-tendinous or myo-cuticular junction the internal architecture of the muscle changes somewhat. The tangle of perifibrillar sarcotubules and cisternae which prevails elsewhere in the muscle fiber becomes reduced to rather uniformly spaced pairs of a tubule and a cisterna, indistinguishable from the intrafibrillar units. The tubules become continuous with the sarcolemma at the myo-tendinous junction, while the cisternae end blindly.

DISCUSSION

It would be highly desirable to have some information concerning the physiological characteristics of this muscle to correlate with its unusual fine

FIGURE 2

A higher power electron micrograph of a transverse section of copepod muscle, showing the hexagonal array of myofilaments, perifibrillar and intrafibrillar tubules (*t*), cisternae (*c*), and scattered glycogen granules (*g*). × 62,000.


t
t
g
g
c
2

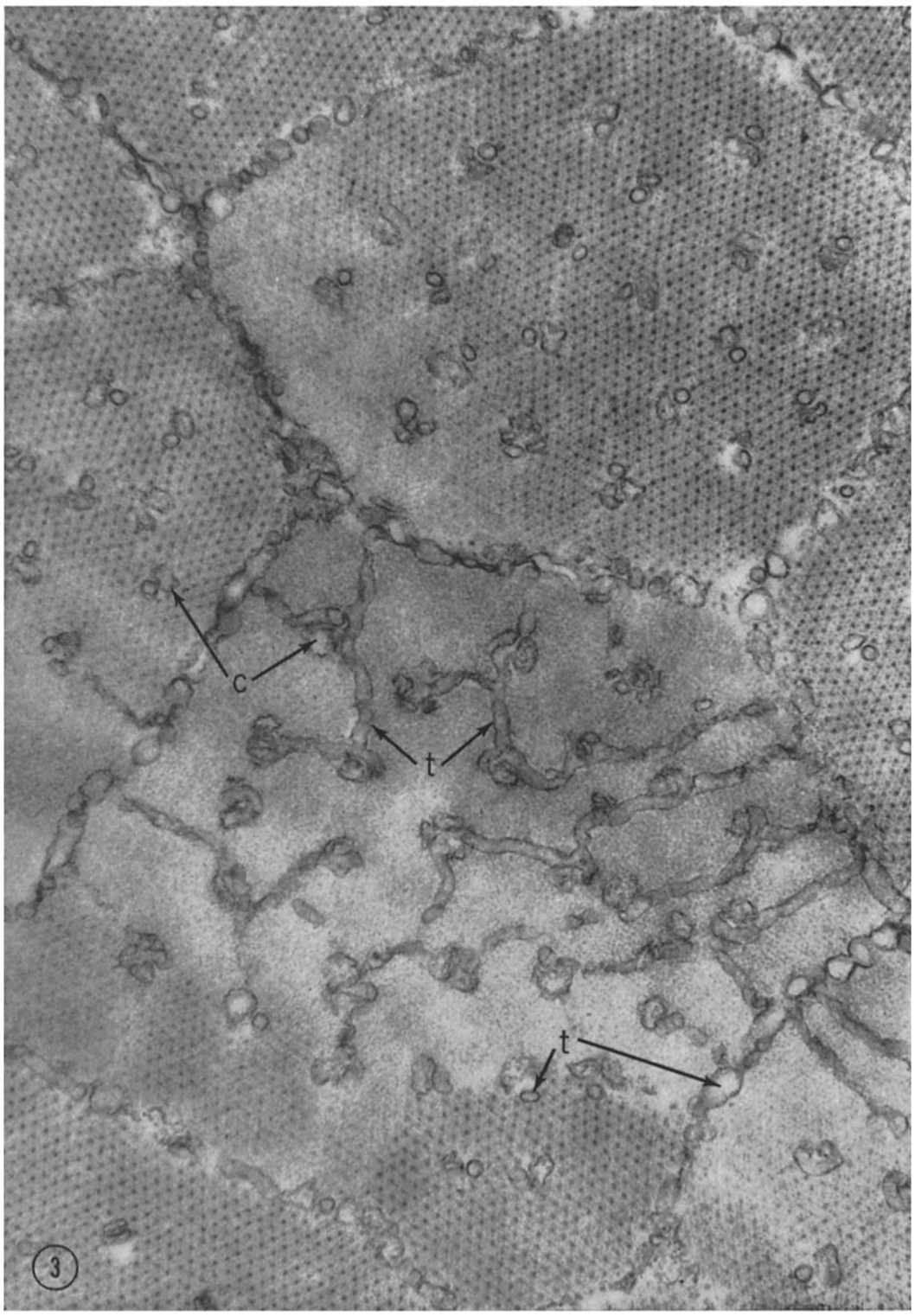
structural organization. Because the small size of *Macrocyclops albidus* virtually precludes direct physiological experimentation to this end, it is necessary to resort to observations of the live animal in its normal environment. In its high speed jumps a copepod 2 mm long can move at an estimated rate of about 10 cm per second. Since the viscosity and resistance of the water in relation to the animal's size are very high, this speed has to be maintained by uninterrupted beating of its appendages. The frequency of the beating of the antenna has been determined by Lowndes for a number of calanoid copepods by stroboscopic means (5). He recorded such values as 20 beats per second for *Calanus finmarchicus*, 22 to 27 per second for *Diaptomus gracilis*, and 45 per second for *Eurytemora velox*. If one assumes that *Macrocyclops albidus* may move a distance of its own body length per beat, a frequency of 45 to 50 beats per second is required to propel it at the observed rate, an apparently not unreasonable assumption. The consequent twitch duration amounts to about 20 milliseconds, a value that compares favorably with that for synchronous insect wing muscles, dragonfly wing muscle contracting in about 40 milliseconds (6), but is exceeded by that for such exceptionally fast vertebrate muscles as the bat cricothyroid (15) or the toadfish swimbladder muscle (14).

These high contraction rates necessitate an equally fast acting excitation-contraction coupling mechanism in muscles other than asynchronous insect flight muscles. Since this process in its final stages may well be chemically mediated by way of a diffusible transmitter substance, the distance between the nearest electrically active surface, which passes an action or local potential in direct response to stimulation, and the contractile elements is of prime importance. The electrophysiological investigations of Huxley (7), Huxley and Taylor (8, 9), and Huxley and Straub (10) on crab, frog, and lizard muscle demonstrated that the excitation is carried into the muscle fiber preferentially at those levels at which the triad is located. This structure is composed of a tubule—the intermediate element—and flanking cisternae running transversely across the surface of myofibrils. In the vertebrate the intermediate element routinely approaches the sarcolemma, but only in rare cases appears to be in continuity with it. The only clearly documented instance is that of the sheep heart muscle (11) in which, however, the intermediate tubule does not correspond to the usual appearance of this element in other vertebrates. In the arthropods, on the other hand, the intermediate tubule or its homologue joins the sarcolemma in all muscles that have been investigated. Hence, it can be assumed that the chemical sequence of events of the excitation-contraction coupling process originates in the immediate proximity of the myofibrils by virtue of the transverse (intermediate) tubule. With these considerations in mind, it becomes particularly noteworthy that in most fast muscles the distance that has to be traversed by a transmitter substance from the nearest potential impulse-conducting element to the center of the myofibril is kept to a value of less than 1 μ. Representative values amount to 0.3 to 0.35 μ in the dragonfly *Aeshna* (12), 0.2 μ in *Periplaneta* (13), 0.18 to 0.2 μ in the toadfish *Opsanus* (14), and 0.15 to 0.25 μ in the bat *Eptesicus* (15). In *Macrocyclops*, 92 per cent of all myofilaments lie within a radius of 0.2 μ of the nearest tubule. In many fast muscles the myofibrils are disposed in thin sheets, thus bringing the enveloping reticulum in close proximity to all parts of the fibril. In *Macrocyclops*, on the other hand, in which the myofibrils are frequently of large cross-sectional diameter, the diffusion distance is reduced by modification of the tubular rather than of the myofibrillar geometry.

Various observations indicate that the asynchronous flight muscles of insects do not fit into a general scheme that tries to relate contraction rate to degree of development of the reticulum. These muscles are relatively well supplied with invaginations of the sarcolemma but are very deficient in the cisternal component. Further, the diffusion distance from the nearest membranous component is generally quite high and out of proportion to the high frequency of the

FIGURE 3

A transverse section encompassing most of the Z line of a myofibril. Tubules (*t*) pass into the fibril from the perifibrillar network to contact the intrafibrillar tubules. Adjacent cisternae (*c*) can also be seen. × 36,000.


c
t
t
3

muscle, while the muscle fibers are supplied with an extraordinary quantity of mitochondria. As it is known that the high contraction rates in the indirect flight muscles of insects are produced by sustained, but low frequency, asynchronous nerve impulses (16) (a fundamentally different mechanism as compared to other muscles), it may be bladder muscle (14), and bat cricothyroid (15). In the bat cricothyroid muscle the transverse tubule occasionally forks to form a doubled structure which, together with its associated cisternae, constitutes the pentad. This peculiarity, however, need not be considered a specialization for rapid contraction since the same feature is found in the

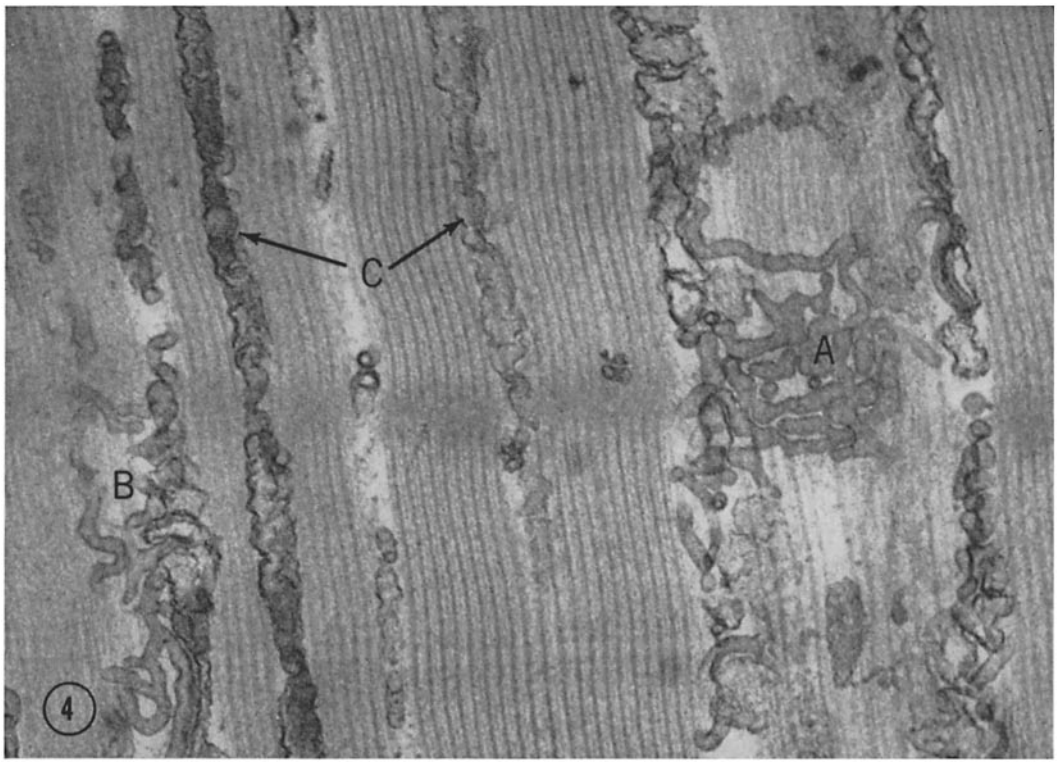


FIGURE 4

Longitudinal section of copepod muscle. Any array of tubules having any lateral extent belongs to the perifibrillar network. At *A* this network is composed almost entirely of tubules, at *B* of tubules and cisternae. *C* denotes intrafibrillar tubules and cisternae. × 39,000.

permissible to exclude these muscles from the following discussion.

If various fast muscles are viewed with respect to the distribution of the tubular element alone, it is found that these tubules traverse the myofibrillar surface at the level of the A-I junction in a manner that is no different from the distribution of the corresponding elements in more average and slower muscles, such as those of the rat sartorius muscle (17). The arrangement of the tubule paralleling the A-I junction applies to the wing muscle of *Aeshna* (a synchronous flight muscle) (12), leg muscle of *Periplaneta* (13), toadfish swim rat levator ani (18), most probably not a fast muscle.

The tubular network of *Macrocyclops* deviates from this scheme in enveloping the fibrils uniformly and, further, penetrating into the fibrils. The latter feature is correlated with the large dimensions of the myofibrils which in other fast acting muscles are either sheet-like (*Aeshna, Periplaneta, Opsanus*) or irregular as in *Epiesicus*. In both of these cases the maximal center-to-surface radius is kept at 0.3 μ or less. A perifibrillar network may be a common crustacean characteristic. Some evidence for this may be

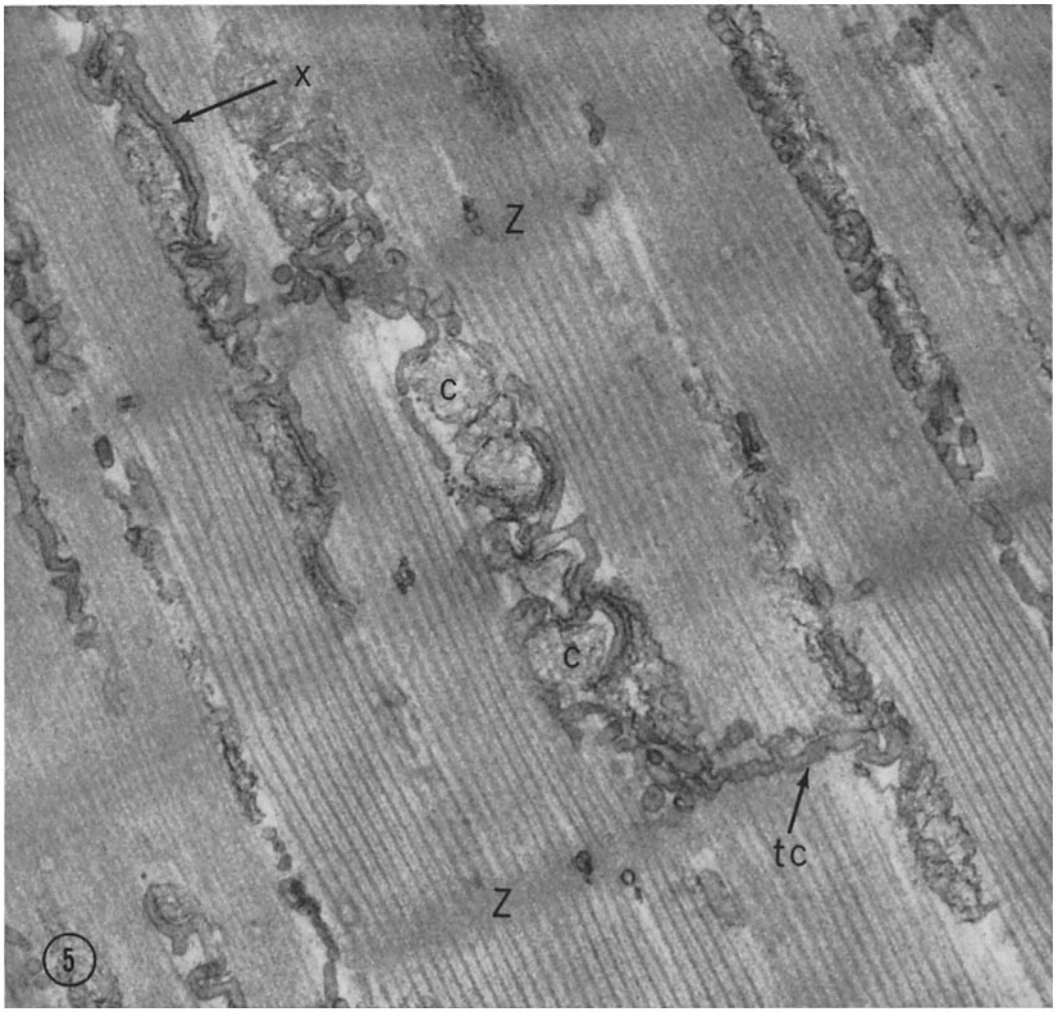


FIGURE 5

This longitudinal section shows in the center a number of large perifibrillar cisternae (*c*) and tubules (*t*). At one of the visible Z lines (*Z*) an uninterrupted connection (*tc*) can be observed between the perifibrillar network and an intrafibrillar tubule. The intrafibrillar tubule at *X* shows both its association with the adjacent cisterna and its characteristic folding at the M band × 37,000.

found in several papers dealing with various crayfish muscles (19, 20). The direct continuity of the tubules with the sarcolemma seems to be the rule for arthropods, this condition having been found in the insects *Periplaneta*, *Calliphora*, *Apis* (13), *Aeshna* (12), as well as the crustaceans *Artemia* (21) and *Orconectes* (19). Also, the tubules of arthropods are generally of a larger diameter, 600 A in *Macrocyclops*, up to 200 by 1000 A in *Aeshna*, than the intermediate element of the triads of vertebrate muscle which measures between 100 and 300 A in diameter. It may be pointed out here that the transverse tubules in sheep heart muscle (11), measuring an exceptional 1000 A or more in diameter, appear to be of a different generic variety than other such tubules in that they carry the basement membrane with them into the interior of the muscle fiber, a condition not found in other muscles. Also, the extrinsic innervation serves to modify the rate of contraction, its function being somewhat analogous to that of the nerves supplying asynchronous

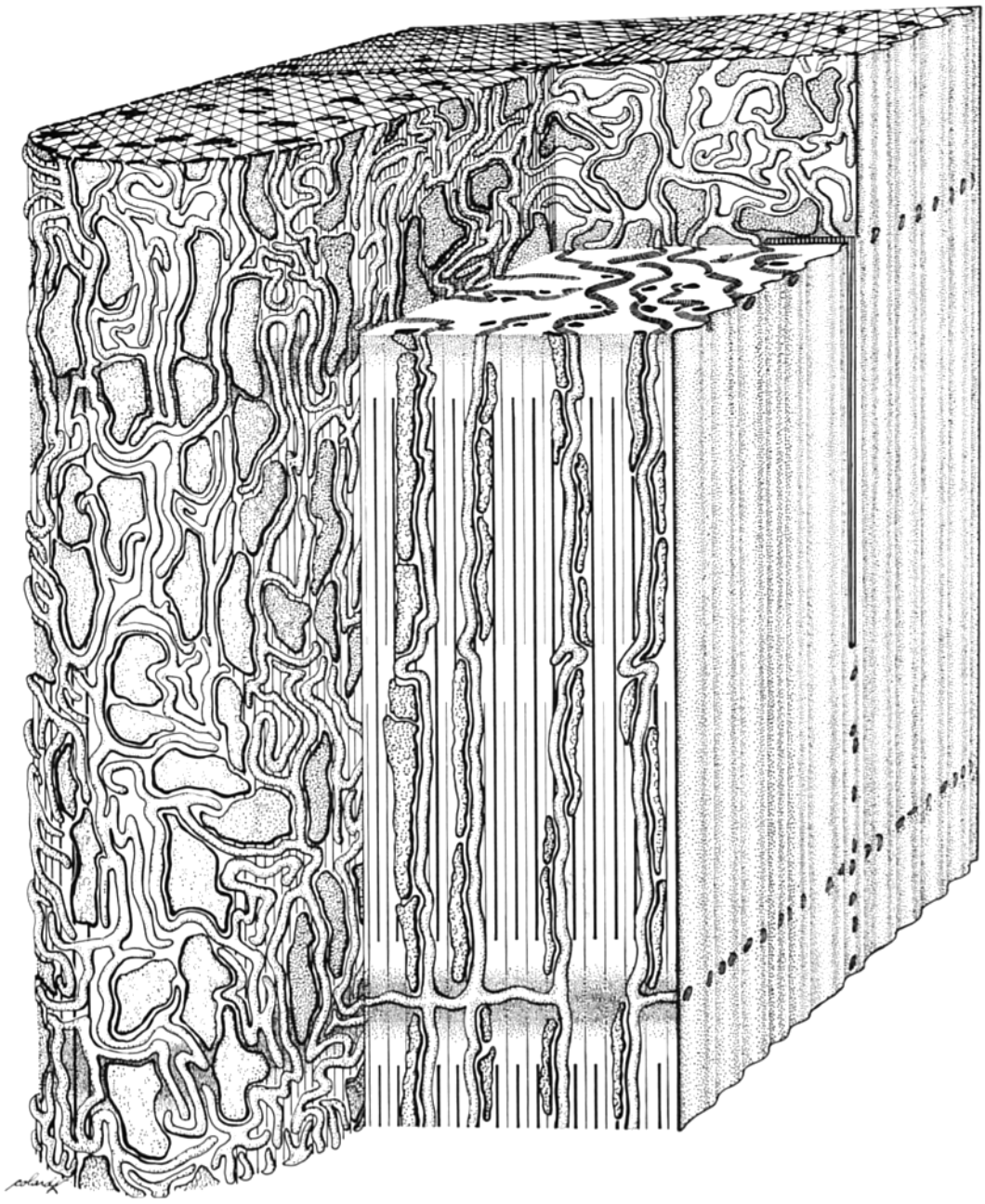


FIGURE 6

Diagrammatic representation of the sarcoplasmic reticulum of *Macrocyclops albidus*. From the wavy surface of the sarcolemma at the right a shallow partition invaginates between two myofibrils. In addition, numerous tubules enter between myofibrils and into them at the Z line. The cut myofibril shows two Z lines and the intrafibrillar tubules and cisternae. At the Z lines the tubules are connected to the perifibrillar network which consists of a continuous maze of tubules and interspersed cisternae.

insect flight muscles, rather than supplying one action potential for each contraction.

The cisternal elements of fast acting muscles show a much more pronounced development than those of slow muscles, as has previously been pointed out by Porter (22). In the bat cricothyroid muscle (15) the terminal cisternae of two neighboring triads are interconnected by a single layer of densely packed channels, a slight elaboration beyond the arrangement in slow muscles in which these channels form an open net at the surface of the fibril. In the toadfish swim bladder muscle (14) a much broader connection exists between terminal cisternae, amounting to a doubled or tripled layer of longitudinal intercisternal channels. In both of these muscles the relationship of the intermediate element to the cisternae, to be considered below, conforms to the pattern described for the rat sartorius muscle (17). In *Aeshna* (12) the cisternae form a continuous fenestrated sheet enveloping the myofibrils, interposed between the tubule invaginated from the sarcolemma and the myofibril. Hence, the association between tubule and cisterna is a side-by-side one in respect to the long axis of the muscle, rather than end-to-side as in vertebrate muscle. A related condition seems to exist in *Periplaneta*, although the geometry of the tangled cisternal network enveloping the myofibrils has not been described in this species and may be dyadic rather than triadic as in *Aeshna*. In *Macrocyclops* the cisternae are not interconnected to any extent and their association with the tubules occurs in a predominantly longitudinal direction, this arrangement differing from that in all the previously mentioned cases, including the insects, but showing some similarity to that in the thoracic limb muscles of *Artemia salina* (21).

Throughout these greatly differing arrangements one feature remains quite constant, namely, the differentiation of the area of close approximation between cisterna and tubule. Its appearance consists of a greater electron opacity of the opposing membranes, particularly striking in the cisterna, while between the tubule and the cisterna a uniform space of about 100 A is maintained. These characteristics apply in whatever orientation the mutual association is established. A purely conjectural point may be brought up here. The membrane differentiation as well as the 100 A spacing is reminiscent of the appearance of the electrical synapses of the crayfish giant nerve fibers (23), which were shown to pass an electrotonic potential directly across the intervening gap (24). It is conceivable that a propagated wave of depolarization along the tubule triggers the cisternae into metabolic activity by direct electrical transmission, a process which, if analogous to that in the crayfish synapse, would have a delay of about 0.1 millisecond.

The evidence presented here indicates that with increasing contraction speeds the cisternal portion of the system becomes more extensive while the potentially impulse-conducting system of tubules remains essentially constant in its degree of development. The conducting, intermediate tubular element may, therefore, be thought of principally as the seat of the triggering mechanism for the complex sequence of chemical events leading to contraction and relaxation. That the cisternae may have a more active part in the elaboration, storage, and release of metabolites is suggested by their common association with glycogen and by their apparent connection with the calcium ion–inhibited ATPase (25), which acts as a relaxing factor. The close and constant pairing between tubules and cisternae may favor an activation of the cisternae by an impulse conducted along the tubule. This would provide a more positive and temporally coordinated mechanism than diffusion alone.

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Note:

A recent paper, Les ultrastructures du muscle strié et de ses attaches au squelette chez les cyclops, by Yves Bouligand (*J. Micr.*, 1962, **1**, 377) deals briefly with the sarcoplasmic reticulum of copepod muscle. Although the illustrations show it to be identical to the sarcoplasmic reticulum of the muscle described in the present paper, it has been given a different interpretation.

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