COMPARATIVE CYTOPHYSIOLOGY OF STRIATED MUSCLE WITH SPECIAL REFERENCE TO THE ROLE OF THE ENDOPLASMIC RETICULUM*, ‡

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INTRODUCTION

Previous investigations have shown differences in the fine structure of striated muscles that seem to be related to specific functions (for literature refer to Häggqvist, 1931; 1956). In insect muscles it has been confirmed by electron microscopy that high frequency flight and low frequency leg muscles differ considerably in their structure (Edwards and Ruska, 1955). Differences in metabolic (Gilmour and Calaby, 1953a; 1953b; Sacktor, 1953; 1955; Sacktor, Thomas, Moser, and Bloch, 1953; Pérez González and Edwards, 1954), structural (Edwards, Souza Santos, Souza Santos, and Sawaya, 1954a; 1954b), and nerve-muscle relationships (Roeder, 1951) exist between the essentially single type muscle of lower insects and the highly specialized muscles of higher insects.

We have now extended our studies to include representative animals from Crustacea up to man, choosing muscles whose specialized physiology is well documented. In such a general survey not all muscles and components could be included nor all problems clarified but the results to date have strengthened the thesis that specialized muscle function is associated with specialized fine structure. The present report presents working hypotheses of the role of the structural components and systems of the striated muscle cell.

Materials and Methods

The muscles studied were as follows:

High frequency: Indirect flight muscles of higher insects.

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Tympanal muscles of cicada.

Pectoralis major of a hummingbird.

Low frequency: Leg muscles of hummingbird, robin, turkey, mouse, frog, and insects.

Abdominal muscles of insects (nymphs, larvae, and adults), reptiles, birds, and man.

Diaphragm of rat.

Mandibular muscles from insects.

Claw adductor of crab.

Tail muscle of alligator.

Hyoglossus of snakes.

Flight muscles of lower insects, robin, and turkey.

Heart and alary muscles of *Periplaneta* and *Belostoma*.

Gut muscle of *Melipona*.

Small muscle bundles (5 to 20 fibers) were fixed *in situ*, in the desired stage of contraction, with 2 per cent osmium tetroxide buffered with veronal-acetate to pH 7.4 for 10 to 15 minutes. The fibers were then dissected out and placed in 1 per cent buffered osmium tetroxide for 4 hours in the cold. Thereafter the standard techniques of washing in distilled water, dehydrating in graded ethanols, and embedding in 80 parts butyl methacrylate and 20 parts methyl methacrylate were followed. Sections were cut with the glass knife in a Porter-Blum microtome. Observations were made in the Siemens, 100b, electron microscope at original magnifications of 970 to 10,000 at 60 to 80 kv.

RESULTS

Two general categories of muscle can be identified by gross inspection, the light microscope, and the electron microscope. By gross inspection one notes that the high frequency muscles of the insects are highly tracheolized, of a pink to reddish-brown color, and easily separated manually into fibers and fibrils. The hummingbird pectoralis is highly vascularized, and of an almost blackish red. The slow (low frequency) muscles in the insects have little tracheolization, are colorless or slightly yellow, and break rather than separate if one attempts to separate the fibers or fibrils manually. In the other invertebrates the slow muscles are colorless and not easily separable into individual fibers and fibrils. In the vertebrates, the low frequency muscles may be either colorless, slightly red, or deep red. They are less vascularized and not easily separable into individual components. Thus we shall call here high frequency muscle only the indirect flight muscles of the higher insects, the pectoralis of hummingbirds, and cicada tympanal muscles. Low frequency muscles include, in this paper, all the other muscles investigated, which differ among themselves in other morphological and physiological aspects.

For simplification of presentation, the results will be given according to structural components, beginning with the sarcolemma and working inward. Due to the amount of material covered in the study, not all points mentioned can be illustrated.

A. Sarcolemma.—The limiting membrane of the striated muscle fiber, the sarcolemma, consists of several layers and varies with species and fiber type.

In the insect fast muscles it is relatively thin (30 to 60 mμ) and smooth in outline. In the insect slow muscles the total sarcolemma is generally thick (50 to 90 mμ) with distinct layers, showing indentations in the region of the Z lines, thus giving a scalloped appearance to the fiber (Fig. 1 a). The scalloping has not been seen in high frequency muscles but was noted earlier with the light microscope in insect leg muscle (Cajal, 1933) and in cardiac and skeletal muscles of vertebrates. The scalloped condition is intensified during contraction, but may be quite distinct in stretched muscle, particularly in insect leg and intersegmental muscles. The sarcolemma in insects may be penetrated by other structures, e.g., nerves (Roeder, 1953) and tracheoles.

Separated from the outer layer of the sarcolemma and roughly parallel to it is a thinner membranous structure (15 mμ on the average) (Fig. 1 b). The two layers diverge widely around penetrating tracheoles (Fig. 1 c). Seen only in the slow muscles, but perhaps characteristic of all muscles, at the regions of the indentations of the sarcolemma opposite Z bands there are funnel-shaped invaginations of the inner membrane (Figs. 1 b and 5), leading directly to the endoplasmic reticulum, which in turn is attached directly to the Z line and the reticulum of the I region. The exact nature of the invagination can be determined in the total fiber only by serial sections. However, remembering the observations of Jones and Barer (1948) of regularly spaced dots on the inside of isolated sarcolemmae, one might consider a series or belt of funnels rather than a groove circling the fiber.

B. Mitochondria.—The mitochondria vary in size, form, internal structure, and distribution from one muscle to the next, and also with the activity of a given muscle. Three general types of mitochondria were seen in our preparations.

1. Small, roundish mitochondria showing internal cristae were found in crab claw muscle, flight muscles of lower insects, leg muscles of higher and some lower insects (Fig. 2), intersegmental muscles of all insects, and many vertebrate muscles (Fig. 6). Such mitochondria have an average diameter of 0.4 to 1.0 micron. In some preparations they are aligned, either singly or in pairs, in intimate relation to the reticulum between adjacent fibrils at the level of the Z line (mouse thigh muscles, cockroach heart muscle, Belostoma intersegmental muscle). They may extend transversely at this level (rat diaphragm).

2. Long, narrow, internal cristae type mitochondria are also characteristic in the leg (Fig. 2) and alary muscles of Periplaneta, leg muscles of the hummingbird, other bird muscles, and some mammalian muscles (Fig. 6). They generally are few in number and arranged between fibrils in scattered chains of 5, 10, or more mitochondria. They may be equal to or longer than the sarcomere in length (4 to 9 micra in insects, shorter in the vertebrates).

3. Large, rounded, or chunky mitochondria are found in the flight muscles of the hummingbird (Fig. 3) and higher insects (Fig. 4). They average 3 to 4

micra in diameter. These mitochondria form a continuum around the individual fibrils, *i.e.*, there may be 1 to 3 rows of mitochondria separating adjacent fibrils. They are generally tightly packed together, indeed fitted into each other. Their internal structure resembles, in some insects, piles of corrugated cardboards, inasmuch as the walls of the cristae are not parallel but alternately approaching and separating (*cf.* Edwards and Ruska, 1954; 1955; Chapman, 1954; Kisch and Philpott, 1955). In the hummingbird pectoralis, well separated cristae mitochondriales are predominant.

The mitochondria show a distinct outer membrane in all muscles examined. They are frequently in contact with, but not enclosed by, the endoplasmic reticulum. In all muscles the mitochondria appear in greater numbers in the belly than in the extremities of the muscle. Generally, in slow muscles, they are more numerous near the nucleus than between the fibrils (Fig. 8). In the slow muscles there are frequently concentrations of mitochondria just beneath the inner layer of the sarcolemma, more so than between the fibril bundles. In the midgut muscles of the bee we find outpocketings of the sarcolemma filled with large groups of small mitochondria (Fig. 5). Similar outpocketings have been observed in dog heart muscles and in certain flight muscles of birds,[1] particularly in the contracted state. We assume that the different positions of the mitochondria have an important bearing on their local function, depending upon the amount and kind of metabolites available in different cell regions.

Small, paired, dense mitochondria appear in mouse quadriceps and gastrocnemius muscles (Fig. 6) and in *Periplaneta* leg muscle (e.g., Fig. 2) at the Z line. They differ in shape and size from the more easily recognizable, larger mitochondria. In view of the fact that all sizes and stages of mitochondria (Figs. 2 and 6) have been observed in the same preparations we believe that the sarcosomes of some authors (Weinreb and Harman, 1955) are small mitochondria. We may thus have a mixed population of mitochondria, that is, long mitochondria between a number of fibrils, and small or intermediate sized mitochondria between the adjacent individual fibrils. Similar observations have been reported by others (Lindberg and Ernster, 1954).

In the flight muscles of higher insects mitochondria of two different densities are often observed. They are all of the large chunky or rounded type and show a distinct membrane, but some are densely laminar, some rarified appearing almost as "ghosts." These latter are apparently metabolically exhausted forms and not due to unequal fixation since they are statistically scattered among mitochondria of normal appearance (Edwards and Ruska, 1955). Also observable, particularly in *Hydrophilus* flight muscle and the hummingbird pectoralis, are apparent connections, or indeed openings, from one mitochondrion to the next when closely packed. We have observed apparent coalescence of mitochondria in both hummingbird and insect muscles. This could be expected

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[1] Moore and Ruska, unpublished data.

in view of the separation and fusion of mitochondria in the living cell (Biesele, 1955).

C. Contractile Material.—The fibers examined fall into two general categories according to the arrangement of contractile material.

1. The high frequency flight and tympanal muscle fibers have independent fibrils of large diameter, widely separated from each other by mitochondria (Figs. 3 and 4). These fibrils have never been seen to converge or split and apparently lack the *Grundmembran* of earlier authors.

2. (*a*) The majority of low frequency muscle fibers show closely packed, small diameter fibrils. These are separated, in general (Figs. 6 and 7), but splitting or unifications may occur. This type of fiber is the only one that shows the *Grundmembran*, *i.e.*, the well developed continuous reticular linkage between Z lines of adjacent fibrils. (*b*) Other low frequency muscle fibers, e.g., *some* of the fibers of *Belostoma* leg muscle (Fig. 1 *a*), mouse quadriceps and rat diaphragm, and *all* of the fibers of thrush and crow latissimus dorsi anterior, *Melipona* gut muscle (Fig. 5), and *Dytiscus* larva intersegmental muscle showed an afibrillar arrangement, indeed irregular continuous masses of contractile material. The first type has always been recognized as a distinctly separate type of muscle. The differentiation between *a* and *b* has been a matter of some controversy (refer to Krüger, 1952) but more recent electron microscope evidence shows that both fibrillar and afibrillar arrangement of contractile material exist in the different fibers of slow low frequency muscles of amphibia, birds, mammals, and insects (Ruska, 1954b).[2] These morphological differences are correlated with known physiological and pharmacological differences, which were previously related to nerve and neuromuscular junction only, rather than to nerve, neuromuscular junction, *and* muscle fiber.

The vertebrate fibrils show the classical regions and lines quite distinctly. In insect and crab muscles we have not found the N line claimed by several earlier authors but interpreted by others as a layer of granules. The M line does not appear in insect slow muscle. Measurements of insect (Edwards, Souza Santos, Souza Santos, and Sawaya, 1954a; 1954b; 1954c), reptile (Edwards, Souza Santos, Souza Santos, Hoge, Sawaya, and Vallejo-Freire, 1954), and mouse muscles show that 90 per cent of the shortening during normal contraction occurs in the I region. In violent contraction the I region practically disappears and the A region may shorten in addition. In the higher insect flight muscle the normal shortening during high frequency contraction is only 3 to 4 per cent, whereas vertebrate muscles normally shorten 25 to 60 per cent. In the vertebrates (Fig. 6), the N lines appear to be formed during contraction by a migration of substance from the A regions, forming at first sub N lines, which then unite to form the definitive N lines closely apposed to the Z, thus

[2] Edwards, Ruska, and Souza Santos, paper in preparation; Ruska, Moore, and Edwards, unpublished data.

forming the contraction band. During normal contraction the A region becomes less dense, and sub M lines are formed. Noteworthy from the physiological point of view, is that (a) during contraction (insects, reptiles, mice) the number of axial periods decreases although the period itself does not significantly decrease in length, and (b) a single contraction of the fibril is a sequence of sarcomere contractions. In violent contraction all sarcomeres participate by superposition of contraction waves and the M lines disappear.

The most constant feature of striated muscle is the Z line. Even this varies, however, particularly in alignment. The Z lines may be in phase across the fiber, may line up stepwise, giving a helical effect. The appearance of the Z line differs with the state of contraction. Contraction bands, especially in thick sections, appear homogeneous. In normal contraction the Z line comprises myofilaments, a dark "Z substance," and a finely vesicular structure. In the relaxed fibril all components are maintained but the continuous I filaments are less visible because of their small diameter.

As a rule the equilibrium length of the sarcomere increases with the amount of shortening the muscle is capable of and decreases with the frequency of contraction. For example, insect high frequency flight muscles have a period averaging 1.5 to 2 micra. Slow muscles commonly show an average period of 3 to 4 micra, but may attain 10 micra in the termite mandibular adductor and 15 micra in crab claw adductor muscles (Edwards, Souza Santos, Souza Santos, and Sawaya, 1954c). Similar findings have been reported by other investigators (Szekessy, 1946).

D. Endoplasmic Reticulum.—The high frequency muscles have little reticulum. In many preparations neither a longitudinal reticulum nor a *Grundmembran* is visible, although fragments may be seen adherent to the Z line (Fig. 3). The fibrillar low frequency fibers contain a variable quantity of fine tubes (50 mμ diameter) with dilatations (up to 150 mμ diameter) comprising the reticulum regularly distributed throughout the fiber. In practically all these fibers, e.g., *Periplaneta* flight (Fig. 7), cicada tympanal, alligator tail, robin latissimus dorsi posterior, and mouse gastrocnemius muscles, there appear two larger dilatations (150 mμ) between the fibrils at a regular distance from the Z line, actually at the boundary between A and I regions. The reticulum runs with the direction of the fibrils, but also forms a tubular ring (vertebrates) or an accumulation (insects) of tubules around the M and a greater quantity of tubules around the I regions, intimately associated with the Z line. In the afibrillar fibers the reticulum is distinctly less well developed.

In all muscle fibers, as in other cells (Watson, 1955), the endoplasmic reticulum is connected to the plasma membrane surrounding the nucleus and indeed formed by the evagination of this membrane. In the periphery it is attached to the inner layer of the sarcolemma. Thus the whole appears to constitute a correlation system between the nucleus, the contractile material, and the sarcolemma.

The reticular system in its distribution and varying size of dilatations resembles, on a lower size level, the system of air sacs along the tracheae of Orthoptera. The latter system aids O_2 and CO_2 diffusion by the connecting rigid tracheae acting as valves and the flexible sacs expanding until the intra-sac pressure is sufficient to overcome the tubular resistance to flow, thus passing the air to the next sac, etc. The endoplasmic reticulum, therefore, by analogy, could be a pressure wave and transport system with a series of dilatations just at the area of maximal shortening to overcome flow resistance, and to distribute the pressure wave. Since pressure develops with the contraction wave and the accompanying deformation of the fiber, a pressure wave could start from the contracted sarcomeres and initiate the process of contraction in its neighbors. In fact, pressure for a fraction of a second may cause a muscle twitch without an action potential (Brown, 1936). We have to consider, furthermore, the existence of membrane potentials in the reticulum and its possible role in impulse transmission to the fibrils. Conduction occurs on the sarcolemma surface by cell membrane depolarization and could occur internally by reticulum membrane depolarization.

At the nerve muscle junction (frog), the sarcolemma turns into the cell in a small number of lamellate indentations that may provide a larger contact surface between the endoplasmic reticulum and nerve ending for electrical and chemical transmission. During contraction the two halves of the sarcomere behave symmetrically, therefore excitation must start from symmetrical centers (Z and/or M). There is no continuity of alteration along the fibril that could serve for the conduction of excitation from sarcomere to sarcomere in the same fibril. The conduction must be saltatory from one sarcomere to the next, and the only morphological system present for such conduction to the centers of symmetry is the reticulum. The interfibrillar reticulum could provide the longitudinal conduction and the Z and M fibril-reticulum associations could serve to conduct to the symmetrical centers of sarcomere excitation. The nature of the mechanism is still unknown, but several possibilities exist. The mechanism of excitation may be essentially a release of an inhibition, e.g., the removal of Mg ions (for literature see Perry, 1956) through the membrane of the reticulum into its fluid phase. The morphological association of the reticulum with the nucleus and the biochemical association with basophilia and ribonucleic acid suggest a role in the exchange of adenylic acid compounds (Ruska, 1954a), which in turn are functionally correlated with the Mg^{++}. It is remarkable that the basophilic components are attached to the outer surface of the tubular reticulum which provides the conditions for ion exchange.

As noted above, the Z line is connected with the sarcolemma by way of the reticulum, and the sarcolemma becomes more scalloped during contraction. At the muscle-tendon junction of frog palmaris, we have observed the muscle fibrils terminating within finger-like projections, formed by indentation of the sarcolemma, between which run the tendon fibrils. These terminal indenta-

tions appear to be formed in the same manner as the lateral scalloping, *i.e.*, the reticular material from the last Z lines is again continuous with the sarcolemma, the difference being in this case that the linkage is longitudinal as well as transverse (Text-fig. 1). Thus the myotendon junction is essentially a splicing of the two different and separate materials. The splice is fastened further by circular connective tissue fibrils around the junction (Ruska, 1954a).

If the reticulum is the system for excitation, the question arises why there is so little reticulum in the high frequency muscle fibers. The answer may be
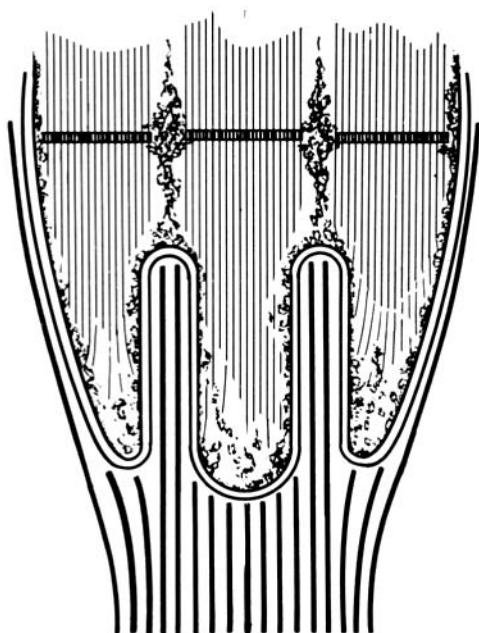
TEXT-FIG. 1. Diagram of muscle-tendon junction in frog. Three contracted myofibrils are shown in which the Z band is connected to the finger-like projections of sarcolemma by way of endoplasmic reticulum. The sarcolemma terminates as a glove around the finger-like muscle fibrils. The tendon fibrils are longitudinally attached to the increased surface of the terminal sarcolemmal sheath.

found in the work of Pringle (1949) and of Roeder and Weiant (1950) on insect flight. They have shown that the higher insect flight muscle is of the asynchronous type, *i.e.*, a single nerve impulse is sufficient to cause several muscle contractions, the contractions being essentially a series of damped oscillations. As the first muscle contracts, it stretches its antagonist, which responds similarly, etc. Using average figures, we can calculate as follows. The high frequency muscle shortens 4 per cent, the slow muscle 40 per cent, giving a ratio of 1/10. The high frequency muscle contracts (partial tetanus)

at a frequency of 400 per second (Diptera), the low frequency muscle at 40 per second (cockroach), again a ratio of 1/10. Therefore the velocity of contraction is no greater in the high frequency muscle than in any other muscle, but the number of shortenings in time, *i.e.*, frequency, differs. Thus the high frequency is not due to the neuromuscular relationship but is an intrinsic property of the high frequency muscle fiber which has sacrificed amount of shortening in order to attain high frequency of shortening. We do not know the amount of shortening in the hummingbird flight muscle fibers (pectoralis), but if we assume that they contract more, as vertebrate fibers do in general, we can account for the fact that the hummingbird fiber has slightly more reticulum than the insect high frequency fiber.

In the low frequency muscle fiber (high fibril-sarcoplasm ratio, and 25 to 60 per cent shortening) a large quantity of reticulum is present. The system reaches its peak development in the fibrillar type of fiber where it is concentrated around the regions of maximum shortening, that is, the I region and to a lesser extent the H region. The reticulum, and very likely with it the Mg^{++}-adenylic acid system, is therefore not only related to ion exchange and conduction of excitation within the cell, but also functionally linked to the amount and region of shortening of the sarcomere.

E. Nucleus.—The nuclei vary in form, size, and distribution in various muscles. There are varying numbers of nuclei per cell, and they may be peripheral, central, or scattered. In general, the nucleus (Figs. 5 and 8) appears ovoid, but often with an irregular outline.

Within the nucleus the chromatin is generally located peripherally in dense aggregates, although smaller, dense particles of chromatin material may be distributed throughout the nuclear matrix. In addition, small particles of various densities are scattered throughout the nuclear interior. In some preparations a nucleolus is visible as a dense body of irregular outline. At times nucleolar fragments of various sizes are visible. The close relationship between tracheoles and nucleus is characteristic of higher insect flight muscle. Tracheolar branches pass close to, or in contact with, the nucleus.

One can distinguish two membranes in most preparations. The inner, more dense membrane is that of the nucleus proper; the outer, less dense membrane that of the cytoplasm. Both are connected by short channels or pores leading between nucleoplasm and cytoplasm (Watson, 1955). The cytoplasmic membrane is continuous with that of the tubular endoplasmic reticulum, as noted before, particularly in those regions where the *Grundmembran* of the fibrils approaches the nucleus. This may imply that the nucleus, in contrast to the mitochondria, is actually outside the cytoplasmic matrix, but connected with it by pores. Since the endoplasmic reticulum by its semipermeable membrane separates a more fluid phase from the cytoplasmic matrix, the nucleus becomes a mediating element between the cytoplasmic phases. The endoplasmic retic-

ulum is the correlating system of the cell, the organelle for nuclear-cytoplasmic exchange. Each muscle nucleus is the center of the system for a given area.

F. Metabolism and Function.—In order to interpret better the fine structure, we may briefly consider the known biochemistry and physiology of the various muscles concerned. Most of our more recent comparative knowledge comes from the insects.

The insects are divided according to their evolutionary position into lower and higher forms. All the muscles of a lower insect appear to be in the low frequency category, are colorless or pale, have similar rates of oxygen consumption and enzymatic activity, little tracheolization (Pérez González and Edwards, 1954), and a wing rate of less than 40 beats per second with a 1:1 ratio between wing beat and spike potential (Roeder, 1951). In *Periplaneta* (representative lower insect), flight and leg muscles have QO_2 values of 7.3 and 6.2, respectively, in male and 4.5 and 4.4 in female. The flight muscle reduces methylene blue (endogenous dehydrogenase activity) in 14 minutes, the leg muscle in 18 minutes. It is found upon separation of mitochondrial and fibril residues that the oxidative activity resides in both fractions, although relatively more in the mitochondrial fraction. The muscle of lower insects has an oxygen consumption higher than vertebrate muscle, with the exception of pigeon breast muscle, and higher than the slow muscle of higher insects. The metabolism appears to follow the Krebs and Szent-Györgyi cycle (Barron and Tahmisian, 1948). The cockroach ATPase more closely resembles the housefly fibrillar ATPase than the mitochondrial enzyme (Sacktor, 1953; Sacktor, Thomas, Moser, and Bloch, 1953).

In the higher insects there is a definite differentiation of musculature. Their high frequency flight muscles are reddish in color, have a heavy tracheolization and high oxidative activity. The higher insects have a wing beat usually above 100 per second, attaining 1,000 in some Diptera. The ratio between spike potential and wing beat is from 1:5 to 1:16. Thus the higher forms have a specialized motor mechanism in the flight muscles (Roeder, 1951). This is reflected also in the fact that the higher insect flight muscle only shortens 3 to 4 per cent in contraction, whereas the leg muscle or lower insect muscle shortens from 8 to 15 per cent normally. The dehydrogenase activity is 20 times greater in flight than in leg muscle in the higher insects. In *Hydrophilus* (higher insect) the QO_2 of flight muscle is 5.7 and of coxal muscle 1.9 (Pérez González and Edwards, 1954). All oxidative activity occurs in the mitochondrial fraction of high frequency flight muscle (Sacktor, 1955), whereas in the slow muscle both fractions show oxidative activity (Pérez González and Edwards, 1954).

In the insects, at least, there occurs therefore a physiological and biochemical differentiation between low and high frequency muscles, this differentiation being related to the evolutionary specialization of muscles for flight. Related to this specialization is the differentiation of the fine structure shown above, *i.e.*, the low frequency muscles being essentially a fibril-reticulum continuum

with few mitochondria, and the high frequency muscle a tracheole-mitochondria continuum with relatively few fibrils.

We have less comparative information concerning the physiology and biochemistry of vertebrate muscle. The rate of oxygen consumption of some vertebrate muscles may be equal to, or greater than, that of insect flight muscle. The rates of oxygen consumption differ among various vertebrate muscles as do the total rates among various organisms, according to their size and activity, e.g, the Allen hummingbird shows a metabolism 15 times that of a pigeon (Pearson, 1955). Vertebrate low frequency muscles may be either red or colorless, indicative of more or less mitochondria and Fe compounds. Among the non-flight vertebrate muscles the orbicularis oculi shows the highest frequency of contraction (20 times per second) before entering a smooth tetanus. The hummingbird flight muscle, principally the pectoralis major, has a frequency of 55 per second. Thus the latter is more comparable to insect high frequency muscle.

It is interesting to note, on a comparative basis, that in the insects the high frequency muscles are reddish and the low frequency muscles colorless or pale. On separation of fibril and mitochondrial fractions, one finds the color only in the mitochondrial fraction. In the vertebrates, on the other hand, the so called white, or colorless, muscles are usually faster than the red, e.g., turkey flight and leg muscles. In this case, both muscles are low frequency muscles by the definition of this paper, but the red muscles, like the heart, the diaphragm, and some skeletal muscles of mammals are capable of continuous activity and have a short recovery period, whereas the colorless muscles are capable of sporadic, rapid bursts with more absolute power but have a long recovery time. The hummingbirds, as in the case of the higher insects, represent a special phylogenetic development. The less highly developed fliers, especially after domestication, e.g., turkeys and other Gallinaceae, have light pectoralis muscles. The faster and more continuous fliers, e.g., robin, have darker flight muscles, and the hummingbird has the darkest as well as a frequency of beat and fine structure resembling that of the higher insect flight muscle. Thus the color is associated with the oxidative system, i.e., the mitochondria-tracheole continuum in insect flight muscle, and the mitochondria-myoglobin system in the bird flight muscle. The latter system is developed for long period high frequencies, and in other vertebrate muscles for continuous action at low frequency.

SUMMARY AND CONCLUSIONS

1. The structure and distribution of the components of striated muscle cells vary with the species and with the specialization of muscle fiber function.

2. There appear to be two, easily distinguishable, general categories of striated muscle structure.

A. High frequency muscle (represented by flight muscle of higher insects

and hummingbird, and cicada tympanal muscle) is characterized by widely spaced, non-branching fibrils of large diameter and short period, little endoplasmic reticulum, and large quantities of large mitochondria (low fibril-sarcoplasm ratio). This structure is correlated with heavy tracheolization or vascularization, high oxidative activity, and dark color as compared with other muscles of the same species.

B. Low frequency muscle is characterized, in general, by high fibril-sarcoplasm ratio, relatively long period, few mitochondria increasing with activity and decreasing with absolute power of the fiber. Oxidative capacity and color are proportional to the quantity of mitochondria. These fibers are further differentiated into (a) fibrillar arrangement of contractile material which permits a regular pattern of interfibrillar and segmental reticulum, and (b) afibrillar arrangement of contractile material leading to an unsystematic distribution of reticulum.

3. The endoplasmic reticulum appears as a complex coordination system in the muscle fiber. Peripherally, it links the Z and M lines of the fibrils to the sarcolemma and between the fibrils it links the cross-bands, forming the *Grundmembran* of earlier authors. By longitudinal linkage, it connects with the sarcolemma at the muscle extremity to form a digital arrangement into which the tendon fibrils are spliced. The extent of its development and its position have a definite relationship to the degree and site of fiber shortening. At present the reticulum is the only structure that one can consider to be an internal conducting system. It may distribute the excitation transversely from fibril to fibril, and lengthwise saltatorially to the symmetry centers of the sarcomeres.

4. The nucleus is the mediating element between the cytoplasmic phases within and without the tubular system of the endoplasmic reticulum. A possible mechanism which correlates nucleus, adenylic acid system, ion exchange, and reticulum with the initiation of contraction is postulated.

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EXPLANATION OF PLATES

PLATE 49

FIG. 1. (a) *Belostoma* leg muscle afibrillar fiber showing thick sarcolemma indented at the level of endoplasmic reticulum connections. × 20,000. (b) *Belostoma* leg muscle fibrillar fiber showing distinct sarcolemma layers and reticulum (er) connecting with inner layer and Z lines. × 16,000. (c) *Belostoma* abdominal intersegmental muscle. Note divergence of sarcolemma layers around penetrating tracheole (tr). × 24,000.

FIG. 2. *Periplaneta* leg muscle fibrillar fiber with narrow, regular fibrils (fi), mixed population of mitochondria (m), and reticulum (er). × 17,000.

(Edwards *et al.*: Cytophysiology of striated muscle)
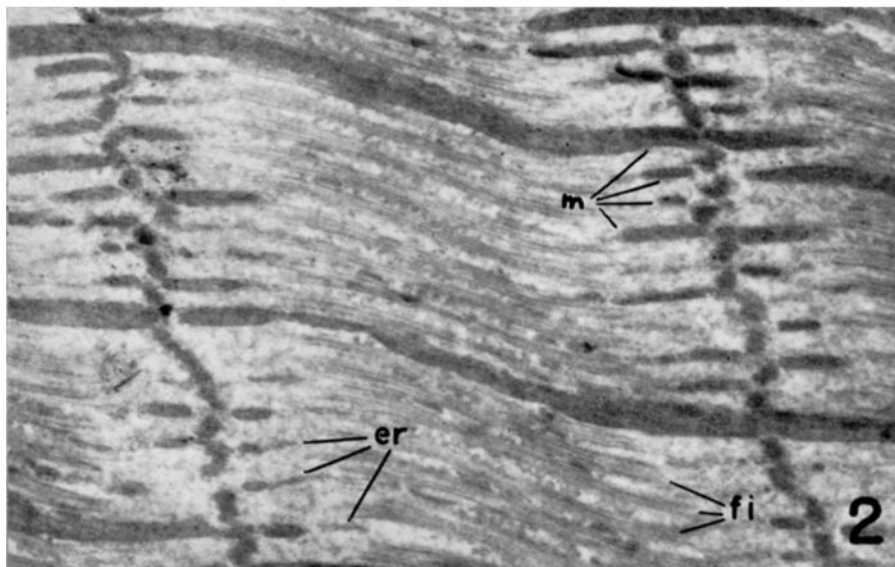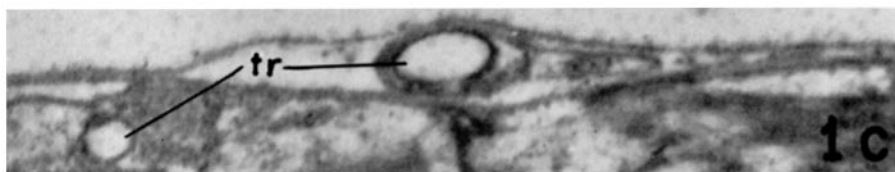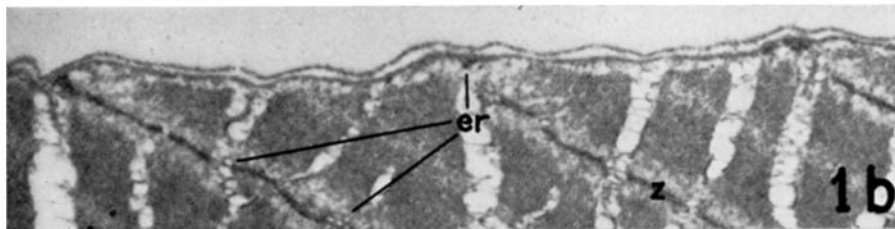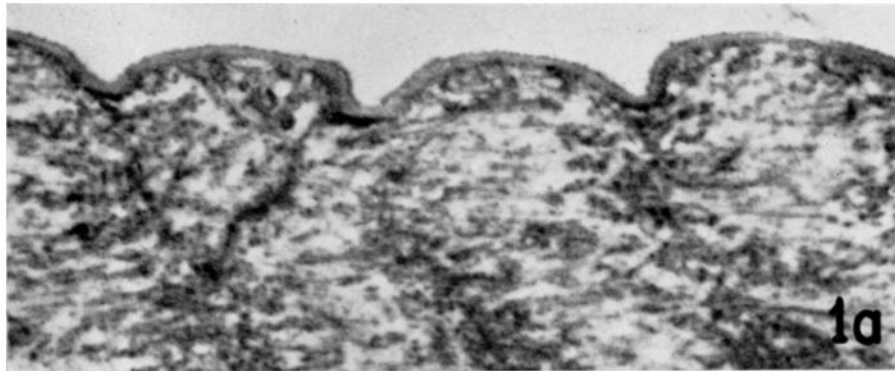
PLATE 50

FIG. 3. Hummingbird pectoralis major (high frequency) fiber. Continuum of large mitochondria surrounding widely spaced fibrils. × 17,500.

FIG. 4. *Belostoma* indirect flight muscle (high frequency) fiber showing wide spacing between large diametered fibrils, large mitochondria, and tracheole (*tr*). × 20,000.
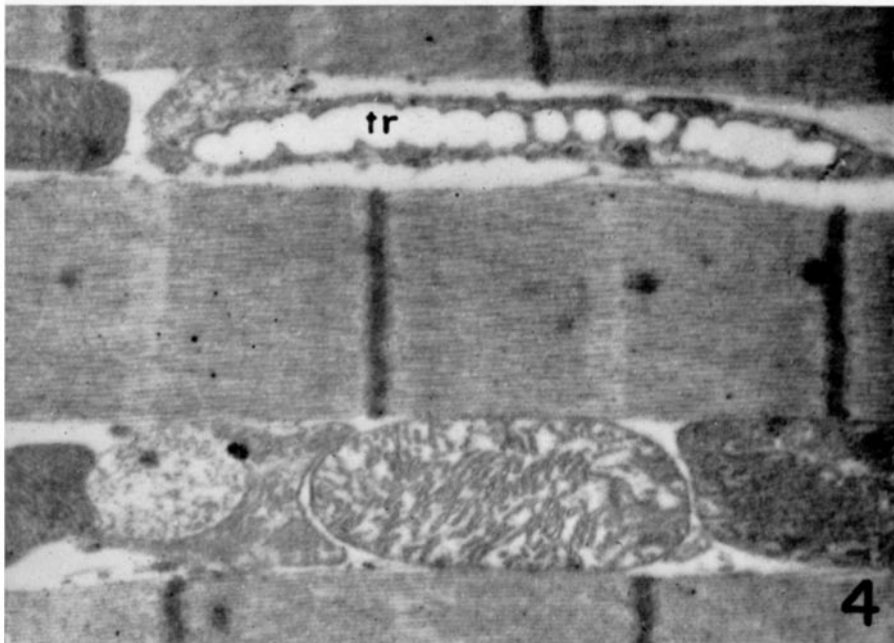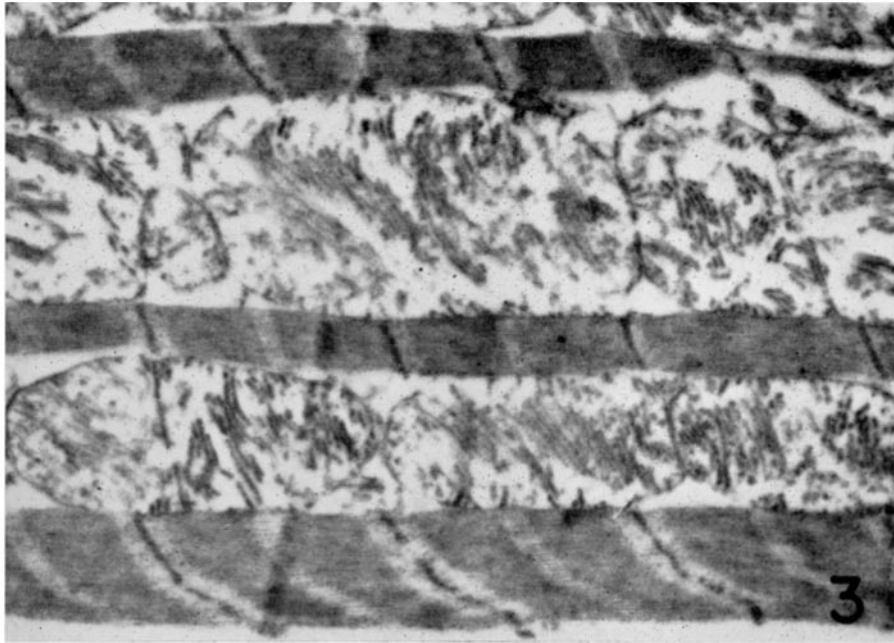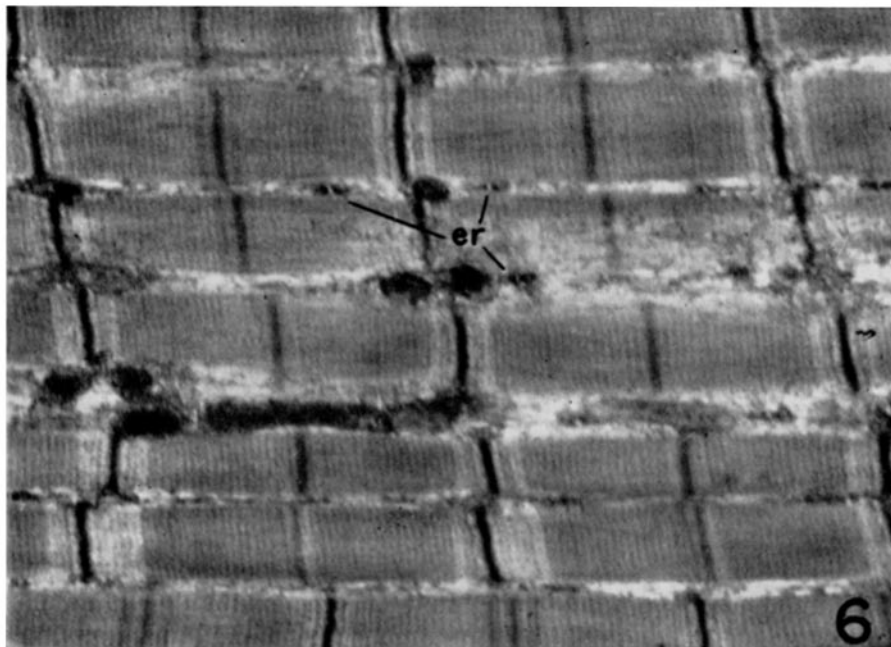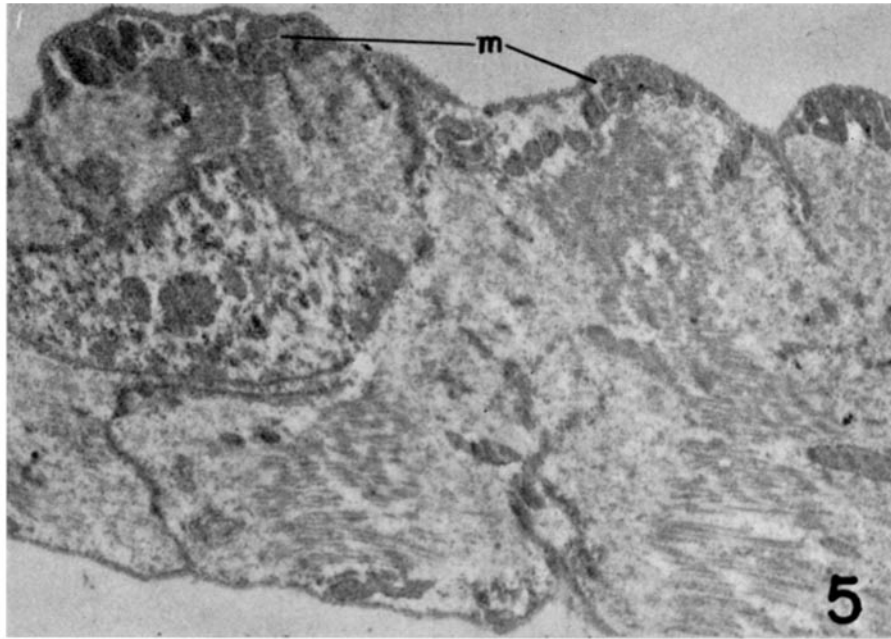
(Edwards *et al.*: Cytophysiology of striated muscle)

PLATE 51

FIG. 5. *Melipona* gut muscle afibrillar fiber. Observe aggregations of small mitochondria (*m*) in sarcolemma outpocketings, sarcolemmal invaginations, reticular connection between sarcolemma and nucleus by way of Z line. × 21,000.

FIG. 6. Thick section of fibrillar mouse gastrocnemius fiber in partial contraction. Note variations in the state of contraction from sarcomere to sarcomere. Formation of cross-lines, long mitochondria, and small mitochondria at Z line level. Paired, small, interfibrillar, dark bodies at regular distance either side of Z are reticular dilatations (*er*). × 27,000.
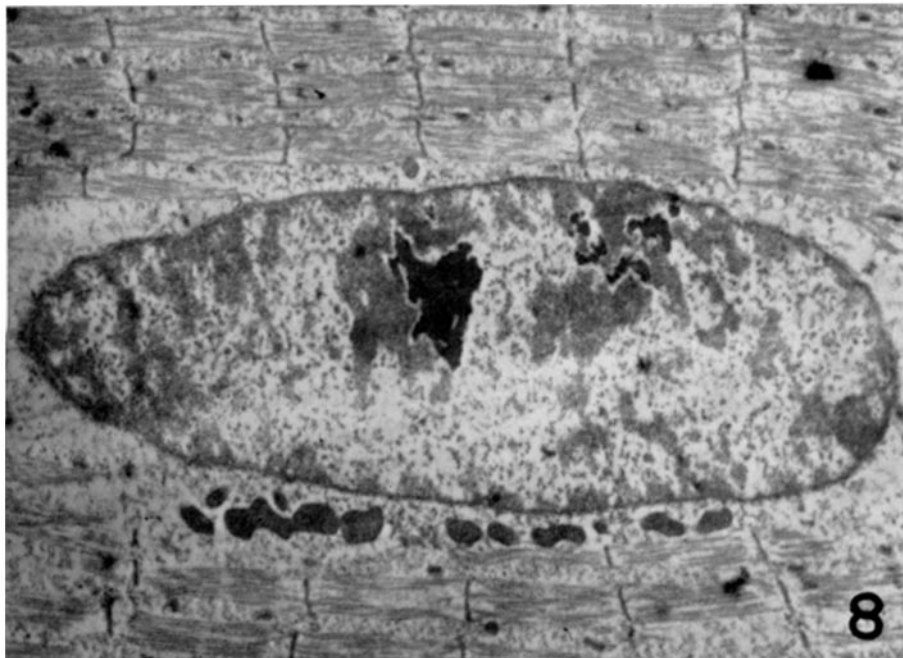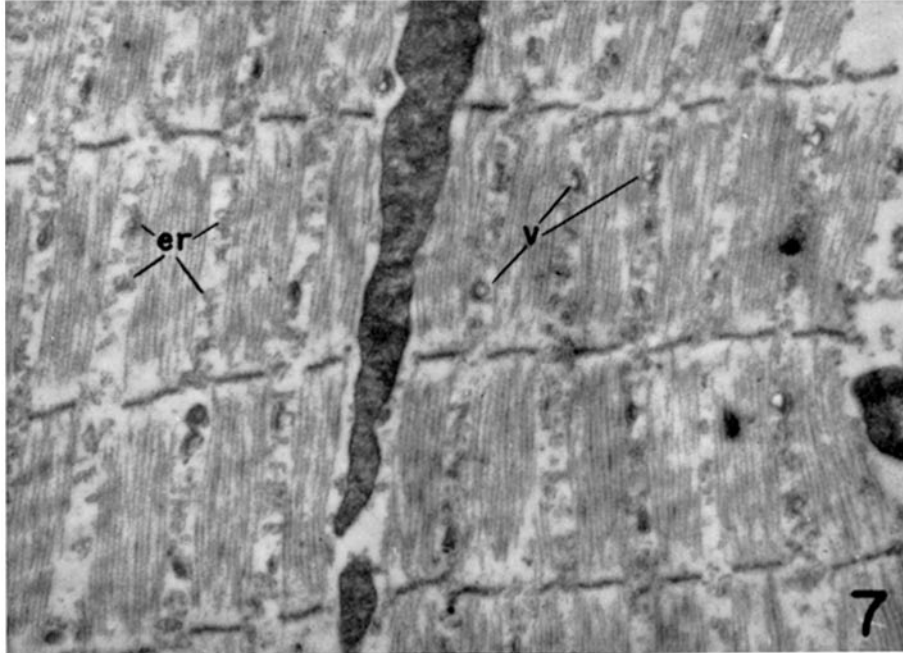
(Edwards *et al.*: Cytophysiology of striated muscle)

PLATE 52

FIG. 7. *Periplaneta* flight muscle fibrillar fiber with elongated mitochondria, well developed reticulum (*er*) with vesicular paired dilatations (*v*). × 21,000.

FIG. 8. *Periplaneta* flight muscle fibrillar fiber showing well developed reticulum with paired dilatations and aggregations of mitochondria near nucleus. × 16,000.

(Edwards *et al.*: Cytophysiology of striated muscle)