The Sarcoplasmic Reticulum of Skeletal and Cardiac Muscle

By Don W. Fawcett, M.D.

This paper traces the development of our present concept of the structural organization of the sarcoplasmic reticulum in striated muscle and reviews the physiologic evidence for its participation in intracellular impulse conduction. Comparative observations are presented showing that this system of membrane-limited tubules is particularly well developed in exceptionally fast-acting skeletal muscles. These findings are interpreted as evidence supporting the hypothesis that the reticulum is involved in the coupling of excitation to contraction, but it is considered likely that it also has other important functions in muscle metabolism. The sarcoplasmic reticulum of cardiac muscle is found to be much less extensive and less precisely arranged in relation to the cross-banded pattern of the myofibrils, than it is in skeletal muscle. It is believed, nevertheless, that it may prove to have a significant role in the physiology of the myocardium.

Among the most significant recent morphologic contributions to our understanding of muscle have been the demonstration by Huxley and Hanson1 that the actin and myosin of the myofibrils form two distinct sets of interdigitating filaments, and the description by Bennett2 and Porter and Palade3 of the sarcoplasmic reticulum, a submicroscopic plexiform system of membrane-bounded tubules that occupies the interfibrillar spaces throughout the muscle fiber. The first of these discoveries has formed the basis for a new, and now widely accepted, sliding-filament theory of muscle contraction, and the second has defined a new organelle in the sarcoplasm that may play an important role in the coupling of excitation to contraction.

We propose to review the evidence for the current belief that the sarcoplasmic reticulum may be involved in intracellular impulse conduction and then to present some comparative observations on the organization of this system of membranes in certain examples of skeletal and cardiac muscle that have unusual physiologic properties.

From the Department of Anatomy, Harvard Medical School, Boston, Massachusetts.

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Historical Considerations

Delicate intracellular networks surrounding the myofibrils were observed over half a century ago by Thin,4 Retzius,5 Veratti,6 and a few other able cytologists in preparations of muscle stained by special metal-impregnation methods. However, the membranous nature of this system, its continuity throughout the sarcoplasm, and its exact relationship to the contractile elements could not be fully appreciated with the light microscope. The reticulum therefore aroused the interest of very few morphologists and was quite unknown to physiologists until it was rediscovered a few years ago by Bennett and Porter,7 Andersson,8 and Porter and Palade9 in electron micrographs of skeletal muscle.

In these studies, electron micrographs of thin sections passing tangential to the myofibrils often revealed a plexus of smooth-surfaced tubules closely applied to their surface. From the examination of large numbers of micrographs of Ambystoma muscle, Porter and Palade arrived at an interpretation of the distribution of the sarcotubules that is presented diagrammatically in figure 1A. The tubules overlying the A band of each sarcomere are predominantly longitudinal in their orientation but communicate laterally with one another in the region of the H-band. At the ends of each sarcomere, the longitudi-
Figure 1

A. Diagrammatic interpretation of the organization of the sarcoplasmic reticulum in skeletal muscle of the salamander Amblystoma punctatum. Each myofibril is surrounded by a plexiform system of tubules. The tubules overlying the A-band are predominantly longitudinal in their orientation, but communicate freely in the region of the H-band. At the ends of each sarcomere the longitudinal tubules of the reticulum are confluent with dilated transverse channels called terminal cisternae. The complex oriented transversely at the Z-band, consisting of 2 terminal cisternae and an intermediate row of small vesicles or short tubules, is referred to as a triad. B. Diagram of the sarcoplasmic reticulum of rat cardiac muscle. The loose network of sarcotubes shows less regional differentiation in relation to the cross-banded pattern of the myofibrils. The terminal cisternae are small and typical triads are uncommon. (From Bennett, H. S.: In Muscle. vol. 1. New York, Academic Press Inc. Redrawn from Porter and Palade.)

Reticulum of muscle

This system of sarcoplasmic tubules was interpreted by Porter and Palade as a special form of the endoplasmic reticulum, an organelle that they had described earlier in a wide variety of other cell types. In muscle, however, the reticulum lacked the ribonucleoprotein particles or ribosomes commonly associated with it in glandular cells and it was distributed in a very precise relation to the cross-banded structure of the myofibrils. This organization suggested to several investigators that the reticulum might have a special role in muscular contraction, possibly providing pathways for preferential diffusion of metabolites or intracellular spread of excitation.

Physiologic evidence tending to support this latter speculation was soon provided by the ingenious experiments of Andrew Huxley and Taylor. These investigators were concerned with the intracellular mechanisms whereby contraction of myofibrils deep in the interior of the muscle fiber is coupled to excitation of the surface membrane. In 1948, A. V. Hill had concluded, from consideration of the rates and distances involved, that
the latency of response in skeletal muscle is much too short to be accounted for by the inward diffusion of a hypothetical activating substance from the sarcolemma to the contractile elements. Approaching this problem with new methods, Huxley and Taylor applied a microelectrode to different points on the sarcolemma of single frog muscle fibers under direct observation with an interference microscope (fig. 2). When the tip of the micropipette was over the I-band (fig. 2A), passage of current was often followed by contraction of the adjacent half-sarcomeres (fig. 2B), but no response was obtained when the stimulus was applied over the A-band (figs. 2, C and D). These results suggested that some structural component located in the I-band was responsible for the inward spread of excitation. The possibility that it was the Z-band itself was considered, but this had to be abandoned when similar experiments on lizard muscle showed that the sensitive spots on the sarcolemma in this species were not at the level of the Z-band but over the A-band, near the A-I junction. Electron microscopic studies on the muscles of these two species revealed that the triads of the sarcoplasmic reticulum are situated at the Z-band in frog muscle but at the A-I junction in lizard muscle. The close correspondence between the position of the triads in the reticulum and the level in the sarcomere of spots sensitive to direct stimulation with a microelectrode strongly suggested that the impulse might be conducted inward by the membranes of the sarcoplasmic reticulum. This, then, is the historical background from which our own interest in the sarcoplasmic reticulum developed.

The Sarcoplasmic Reticulum of Fast-Acting Skeletal Muscles

If the sarcoplasmic reticulum is involved in the coupling of excitation to contraction,
SARCOPLASMIC RETICULUM OF MUSCLE

Figure 3

Longitudinal section of the sound-producing muscle in the swimbladder of the toadfish, Opsanus tau. In this unusually fast-acting muscle, the sarcoplasmic reticulum is exceptionally well-developed. There are 2 triads to each sarcomere length located over the A-band near the A-I junctions. Longitudinal tubules extend in either direction from each triad toward the H- and toward the Z-band respectively. The longitudinal elements of the reticulum are continuous over the H-band but often appear to be interrupted at the Z-band (see at arrows). (Electron micrograph by Dr. J. P. Revel.)

one might expect to find differences in its organization or its degree of development in muscles having different speeds of contraction. With this in mind, Dr. Jean Paul Revel and I have studied the reticulum of some particularly fast-acting muscles.

The first of these is a muscle that forms an equatorial band around the swim bladder of the common toadfish, Opsanus tau. Rapid contractions of this muscle set up vibrations in the taut gas-filled bladder that produce the audible sounds made by these fish when they are courting or when otherwise disturbed. This muscle is said to attain its peak contraction in only 5 to 8 msec.¹¹ and requires some 300 stimuli per second to tetanize.¹² Since the fine structure of this muscle has been described in detail in a separate publication,¹³ only a brief account of its salient features will be presented here. The fibers are of large diameter. Their myofibrils are flat ribbon-like structures arranged radially around a central core of sarcoplasm to form a thick-walled contractile cylinder. Mitochondria are seldom found between the myofibrils. Instead, they are located either in the core of the contractile cylinder or in the superficial layer of sarcoplasm around its periphery. The narrow clefts between the broad faces of the myofibrils are occupied by a highly developed

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Electron micrograph of a thin section passing through toadfish muscle, parallel to the broad face of a myofibril. The triads are seen running across the myofilaments parallel to each other and to the Z-band. Crowding and superimposition tend to obscure the plexiform nature of the reticulum between the successive triads. The middle element of the triad, which appears here as a row of small vesicles, is actually a continuous slender tubule.

sections passing through the interfibrillar clefts, in which the triads are cut longitudinally as they traverse the broad face of the underlying myofibril, provide a more extensive view of the reticulum (fig. 4.). In this view, the intermediate element often appears as a row of vesicles, but in the best preserved specimens it seems to be a continuous tubule. In the intervals between triads, the plexiform nature of the longitudinal elements of the reticulum is often obscured by superimposition.
An electron micrograph of a section passing tangential to a myofibril in the cricothyroid muscle of the bat, Myotis lucifugus. In this fast-acting mammalian muscle too, the sarcoplasmic reticulum is far more elaborately developed than in slower muscles. The triads are located at the A-I junctions and the longitudinal sarcotubules seem to be continuous across the Z-band as well as across the H-band. (Micrograph by Dr. J. P. Revel.)

Physiologic measurements of the time course of contraction in this muscle have not been made, but one can infer from its normal function that it is very fast. In its sonic navigation, the bat uses pulses of supersonic sound of the order of 5 to 10 msec. in duration and within this brief period it is capable of modulating the frequency over a considerable range. To accomplish this, the cricothyroid must be able to change its state of contraction very rapidly. When examined in electron micrographs of low magnification, the appearance of this muscle does not differ greatly from other mammalian muscles. The mitochondria are numerous and arranged in rows between myofibrils of the usual rounded or polygonal cross-sectional shape. At higher magnification, it is evident that the sarcoplasmic reticulum is exceptionally well developed (fig. 5). The transverse triads near the A-I junctions appear to encircle the myo-
Figure 6

Longitudinal section of a peripheral portion of a cardiac muscle fiber from the bat heart. Many large mitochondria (MT) are located immediately beneath the sarcolemma (Scl) and between the myofibrils (MB). Numerous lipid droplets (Lp) among the mitochondria are evidently used as an energy source. At several places indicated by arrows, transverse elements of the sarcoplasmic reticulum corresponding to the triads of skeletal muscle are seen between the mitochondria at the level of the Z-band.

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The finding of an unusually extensive and highly ordered sarcoplasmic reticulum in these 2 exceptionally fast-acting skeletal muscles is consistent with the hypothesis\cite{18} that this system of membrane-bounded channels is involved in intracellular conduction of the impulse that activates the myofibrils.

**Sarcoplasmic Reticulum of Cardiac Muscle**

Several considerations would lead one to expect that the sarcoplasmic reticulum might be less well developed in cardiac than in skeletal muscle. Some of these are: the smaller fiber diameter; the central position of the nucleus, which brings the contractile elements nearer to the surface; the myogenic nature of the contraction; the presence, at frequent intervals along the cardiac muscle fibers, of specialized cell-to-cell junctions (intercalated discs) that may offer pathways of inward conduction from the sarcolemma, not present.
Another longitudinal section of the interior of a cardiac muscle fiber from the bat heart showing contracted myofibrils alternating with rows of mitochondria, each about the length of one shortened sarcomere. Again the arrows point out transverse elements of the reticulum located at the Z-band instead of at the A-I junction, which is the usual location of the triads in mammalian skeletal muscle.

in the syncytial fibers of skeletal muscle; and finally the slower rate of contraction of heart muscle. Nevertheless, there is sufficient correlation between the rate of heart beat in various animal species and the degree of development of the sarcoplasmic reticulum to suggest that this system has a significant function in cardiac as well as in skeletal muscle. In a previous study of the fine structure of the turtle atrium, the reticulum was found to be rudimentary. Evidently it is not essential in the slow-beating heart of this cold-blooded species. In the rat, which has a rather rapid heart rate, Porter and Palade found a loose network of sarcotubes with relatively little differentiation in relation to the cross-banded pattern of the myofibrils (fig. 1B). Although small terminal cisternae were identifiable on either side of the Z-band, these did not form typical triads nor did they extend laterally for any considerable distance.

We have recently studied the myocardium of the bat, Myotis lucifugus. These small mammals normally have a heart rate of the order of 500 to 600 per minute but, under some physiologic conditions, it may reach as high as 1,000 per minute. Electron micrographs of longitudinal sections reveal an extraordinary number of large mitochondria of complex internal structure located in the clefts between myofibrils, at the poles of the centrally placed nucleus, and immediately beneath the sarcolemma.

The mitochondria are often about the length
A low power electron micrograph of portions of 4 cardiac muscle fibers of the bat in transverse section. Observe the centrally placed nucleus (Ncl) and the fact that the large dense mitochondria occupy fully half the cross-sectional area of the fiber. The clear areas that appear to be holes in the section are in fact lipid droplets whose content has been largely extracted during specimen preparation. The area enclosed in rectangle A is shown at higher magnification in figure 9 and that in rectangle B constitutes figure 10.

of a sarcomere. The periphery of a partially contracted fiber frequently shows a characteristic scalloped or corrugated appearance owing to the fact that the sarcolemma is closely adherent to each Z-band of the outermost myofibrils but is separated from the myofibrils elsewhere by mitochondria. The mitochondria immediately beneath the sarcolemma would thus seem to be confined within relatively stable compartments, bounded by the lines of adhesion of the sarcolemma to the Z-band of successive sarcomeres (fig. 6). The structural basis for this close binding of the surface membrane to the Z-band is not clear from the micrographs. Numerous lipid droplets are interspersed among the mitochondria that are more deeply situated in the fibers (fig. 6) and are evidently an important energy source in the rapidly beating hearts of this and other small mammals. Since bats hibernate, it would be of interest to know whether there are seasonal variations in the abundance of the myocardial lipid, but, thus far, our studies do not extend over a large enough span of time to throw any light on this subject.

Also located between the ends of the mitochondria at the level of the Z-band are transversely oriented tubular elements of the sarcoplasmic reticulum, indicated by arrows on figures 6 and 7. These evidently correspond to the triads of the reticulum of skeletal muscle but tend to be single or at most double and
are placed at the Z-band instead of in the A-band near the A-I junction. One tends to underestimate the extent of the sarcoplasmic reticulum in cardiac muscle because of the peculiar geometry of its myofibrils. They are not discrete fibrils uniformly round or polygonal in cross section, as in skeletal muscle, but instead exhibit a greater degree of confluence and branching so that, in transverse sections, the size of the myofibrils is variable and their shape highly irregular. In consequence of the inconsistency of their tridimensional form and the prevalence of curving surfaces, one rarely encounters such extended surface views of the sarcoplasmic reticulum in longitudinal sections as one sees overlying the more regular faces of the myofibrils in skeletal muscle. It is necessary therefore to rely mainly on cross sections in attempting to construct a mental image of the 3-dimensional organization of the reticulum.

In transverse sections of bat heart muscle viewed at low magnification (fig. 8), one is struck by the irregular shape of the myofibrils around the centrally placed nucleus and by the great number of large mitochondria that take up nearly half of the cross-sectional area of the fiber and occupy nearly all of the interfibrillar sarcoplasm. In micrographs of higher magnification (figs. 9 and 10) the mitochondria are found to conform very closely to the irregular contours of the surrounding myofibrils; however, in the narrow interstices between the two, there are numerous circular profiles, 400 to 500 Å in diameter (see at arrows), which are cross sections of the longitudinally oriented tubules of the sarcoplasmic reticulum. Owing to the paucity of interfibrillar sarcoplasm, these profiles are easily overlooked in low-power micrographs but, from the large numbers visible in micrographs of higher magnification, it is clear that the sarcoplasmic reticulum is quite well developed in cardiac muscle of this animal species.

To what extent is this tubular system developed in the human heart? Although there have been several brief reports on the fine structure of the human myocardium, none has devoted particular attention to the sarcoplasmic reticulum. Our own studies on man are as yet too fragmentary to permit us to do more than to affirm its presence and to record some preliminary impressions on the degree of its development as compared to other animal species.

In electron micrographs of human atrial muscle, the myofibrils show the same orderly arrangement of two interdigitating sets of filaments that has been described in other striated muscles (fig. 11). The myofibrils vary considerably in size and in cross-sectional shape but are, on the whole, less pleomorphic than those described here for the bat. The mitochondria, which have a dense matrix and a complex internal membrane structure, are numerous and are distributed singly or in sizeable clusters among the myofibrils. The interfibrillar sarcoplasm is more abundant than in the bat myocardium. The mitochondria, being less crowded, show less tendency to adopt unusual shapes conforming to the spaces between the myofibrils. Among the mitochondria and in the clefts between adjacent myofibrils are tubular elements of a sparse sarcoplasmic reticulum (see arrows, fig. 11). Definite triads have not been identified at the level of the Z-bands in our material, nor have any clear connections been demonstrated between the loose meshes of the reticulum and the sarcolemma. Although it is probably basically similar in its distribution and organization, the reticulum in the human myocardium is evidently far less elaborately developed than is that of smaller mammals with a more rapid heart beat.

Comment

The history of the discovery of the sarcoplasmic reticulum of striated muscle has been traced and the physiologic evidence for its participation in intracellular impulse conduction has been reviewed. Our own comparative observations indicate that this system of membrane-limited tubules is particularly well developed in certain exceptionally fast-acting skeletal muscles. These findings
Figure 11

An electron micrograph of a small area at the periphery of a human cardiac muscle fiber. The sarcolemma (Scl) is at the top of the figure and shows the usual coating of basement-membrane material. The mitochondria (Mt) are fairly large and rich in internal structure. The myofibrils (Mf) show the usual precise hexagonal pattern of myofilaments. The sarcoplasmic reticulum is less well developed than in the hearts of small mammals with rapid heart beats, but several profiles of sarcotubules in cross section can be seen between the myofibrils in this figure (see at arrows).

are offered as further indirect evidence for the hypothesis that the reticulum is involved in the coupling of excitation to contraction. It is recognized, however, that this canalicular system may function in other ways besides the conduction of an impulse by its limiting membrane. It may prove to be important in the synthetic activities of the muscle cell, or its lumen may provide a continuous pathway for distribution of energy-rich compounds or other essential metabolites to the myofibrils.

The sarcoplasmic reticulum has been shown to be less highly developed in cardiac than in skeletal muscle but it is so organized in

Figures 9 and 10

Higher magnification electron micrographs of two small areas of the cardiac muscle fiber shown in figure 8. The myofibrils (Mf) are highly irregular in shape and their surface closely conforms to the shape of the mitochondria (MT), which occupy nearly all of the interfibrillar sarcoplasm. Between the myofibrils and the mitochondria are numerous small profiles of membrane-bounded tubules in cross section (see at arrows). These are the longitudinal components of the sarcoplasmic reticulum.
relation to the cross-banded pattern of the myofibrils as to suggest that it may have a similar function in both. The reticulum is rudimentary in the slow-beating heart of the turtle but reaches a rather high degree of complexity in the very rapidly beating heart of the bat. The reticulum of the human myocardium has not been adequately studied but appears to be intermediate between these extremes. It is not possible now to state how important a role the sarcoplasmic reticulum plays in the physiology of the human heart, but new findings in research often turn out to have far more significance than at first seems likely. It may not be too fanciful to imagine that a few decades hence the cardiologist may be concerned with functional disturbances of this intracellular communication system just as he is concerned today with defects of conduction at a grosser tissue level.

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Charles E. Kossmann and DON W. FAWCETT

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