SOME MORPHOLOGICAL AND BIOCHEMICAL ASPECTS OF THE SARCOTUBULAR SYSTEM IN FROG SKELETAL MUSCLE CELL

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ONE of the main components of the skeletal muscle cell is a system of tubular and vesicular structures localized between the myofibrils[1,2]. The distribution and organization of this so called sarcotubular system can be seen in Fig. 1, which shows a longitudinal section of frog skeletal muscle cell. The myofibrils appear in a relaxed, or somewhat stretched position, with the characteristic bands of the myofibrils well recognizable.

At Z-band level of the myofibrils, the sarcotubular system forms a characteristic three-component structure, the triad, which appears in many other muscle cells, for example rat, amblyostoma larvae, and mouse. Components of the triad have been demonstrated, by means of serial sections, to be continuous transverse to the long axis of the myofibrils in the mouse muscle[1]. The triad structure also has an obvious contact with the surface membrane of the muscle cell. In Fig. 2, part of the triad can be observed continuously over a rather long distance transverse to the longitudinal axis of the myofibrils.

The triad is composed of two somewhat flattened vesicles placed next to each other with a rather constant distance of about 500Å between their membranes. In the interspace between the vesicles, small tubular elements with a diameter of about 300Å appear. The diameter of the big vesicles or tubules is about 500Å perpendicular to the Z-band.

In most preparations these vesicles contain a mass of small particles about 30Å in diameter. The rest of the sarcotubules connect the triads and run more or less parallel to the myofibrils (Figs. 1 and 2). Their diameter is about 300Å. At the H-band level they form a rather complicated pattern. The sarcotubules also show intimate contact with the mitochondria.

A. F. Huxley's experiments[3] with local stimulation showed in his frog preparation that the local contraction occurred only when the stimulus

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was applied at the Z-band level. The contraction was spread inward at this level transverse to the myofibrils.

From A. F. Huxley's experiments, and from the morphological distribution and characteristic organization of the sarcotubular system, the suggestion has been made that the sarcotubules could be responsible for the propagation of the stimulus from the cell membrane inward to the contractile material.

To elucidate further the function of the sarcotubular system in the muscle cell, a purified fraction of the system has been isolated by means of differential centrifugation from skeletal muscle homogenate, and some biochemical properties of the isolated fraction have been investigated recently[6].

TABLE I

Enzymatic properties of the mitochondrial and sarcotubular fractions from frog skeletal muscle homogenate

Fraction	Cytochrome oxidase activity (μ moles O_2/mg protein/hr)	ATPase activity (μ moles Pi/mg protein/hr)	
		with Mg^{++}	with Mg^{++} and Ca^{++}
Mitochondria	155·9	5·73	5·36
Sarcotubules	0	38·4	7·26

Experimental conditions: The mitochondria were isolated by differential centrifugation between 2000 g for 7 min and 15,000 g for 10 min and the sarcotubules betwen 75,000 g for 40 min and 105,000 g for 60 min. The homogenization medium used was 0·88 M sucrose. The cytochrome oxidase activity was measured according to the procedure described by Potter[9]. The ATPase activity was tested in a medium of the following composition in a final volume of 2 ml: 50 mM KCl; 25 mM Tris buffer (pH, 7·4); mM ATP, and where indicated 4 mM $MgCl_2$ and 0·8 mM $CaCl_2$ were added. Time of incubation: 20 min at 30°C. The inorganic phosphate was analysed according to the method described by Lindberg and Ernster[4].

Table I shows the distribution of enzymatic activities in different fractions of frog skeletal muscle homogenate. Since the cytochrome oxidase activity is localized only in the mitochondrial membranes[10], and the activity is not lost even after extensive fragmentation of the mitochondria[6], the presence or the absence of this activity has been assumed to be the indication of mitochondrial contamination.

As shown in Table I, it is possible to isolate a fraction, after the sedimentation of the mitochondria, with characteristic biochemical and also morphological properties. This fraction, obtained by high speed centrifugations, does not derive from the fragmentation of the mitochondria as shown by the absence of cytochrome oxidase activity.

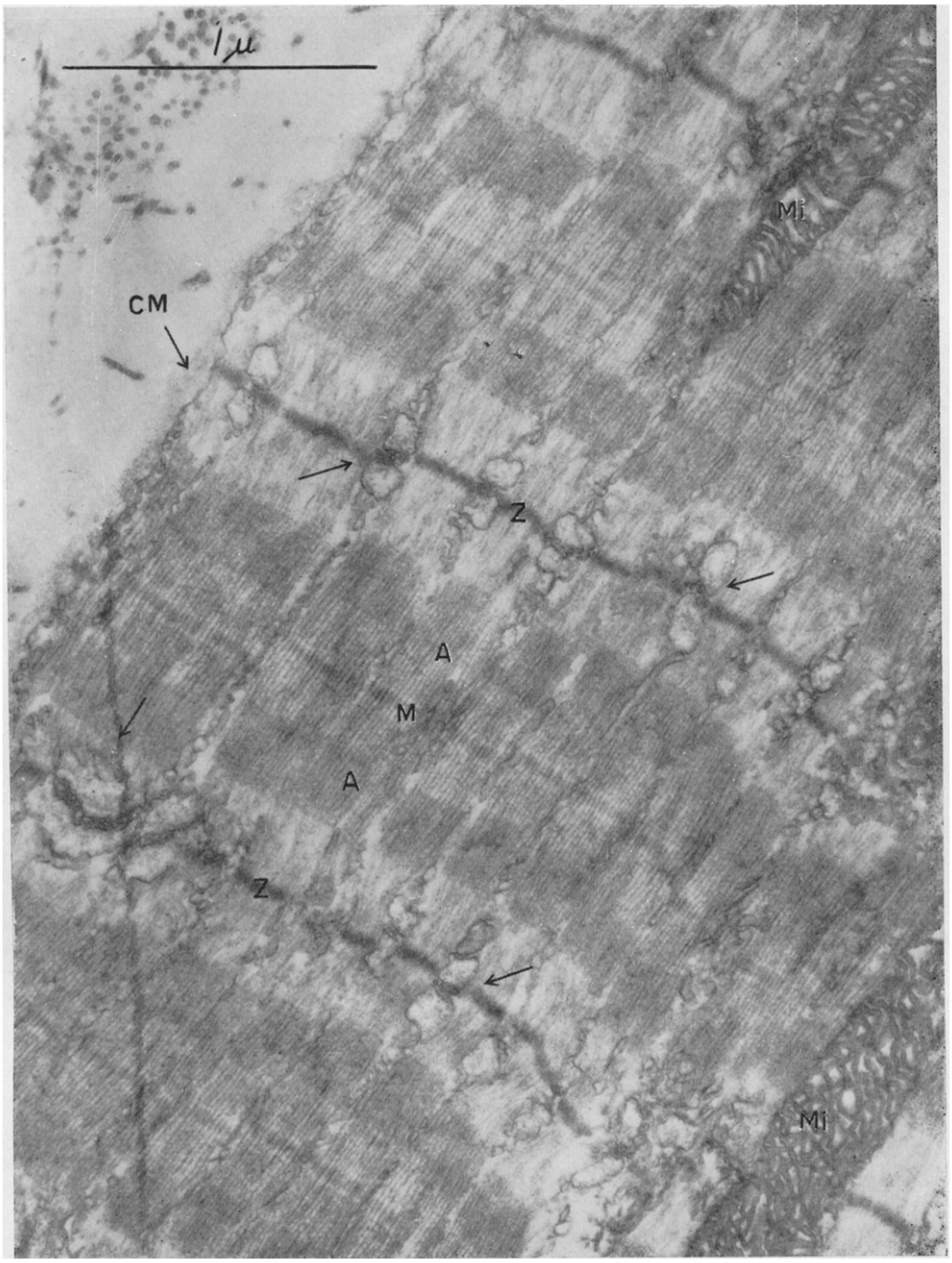

FIG. 1. Electron micrograph of a longitudinal section through a frog skeletal muscle cell. The myofibrils are seen in a relaxed position. The different bands of the myofibrils are indicated (Z band in the middle of the I band and M band in the middle of the light H zone, which appear in the A band). Arrows point to the three-structure component of the sarcotubular system, located at the level of the Z band. Mi indicates mitochondria and CM the cell membrane. The specimen is fixed in OsO_4, embedded in methacrylate, stained with uranylacetate and photographed in an RCA EMU 3 A microscope. Magnification × 50.000.

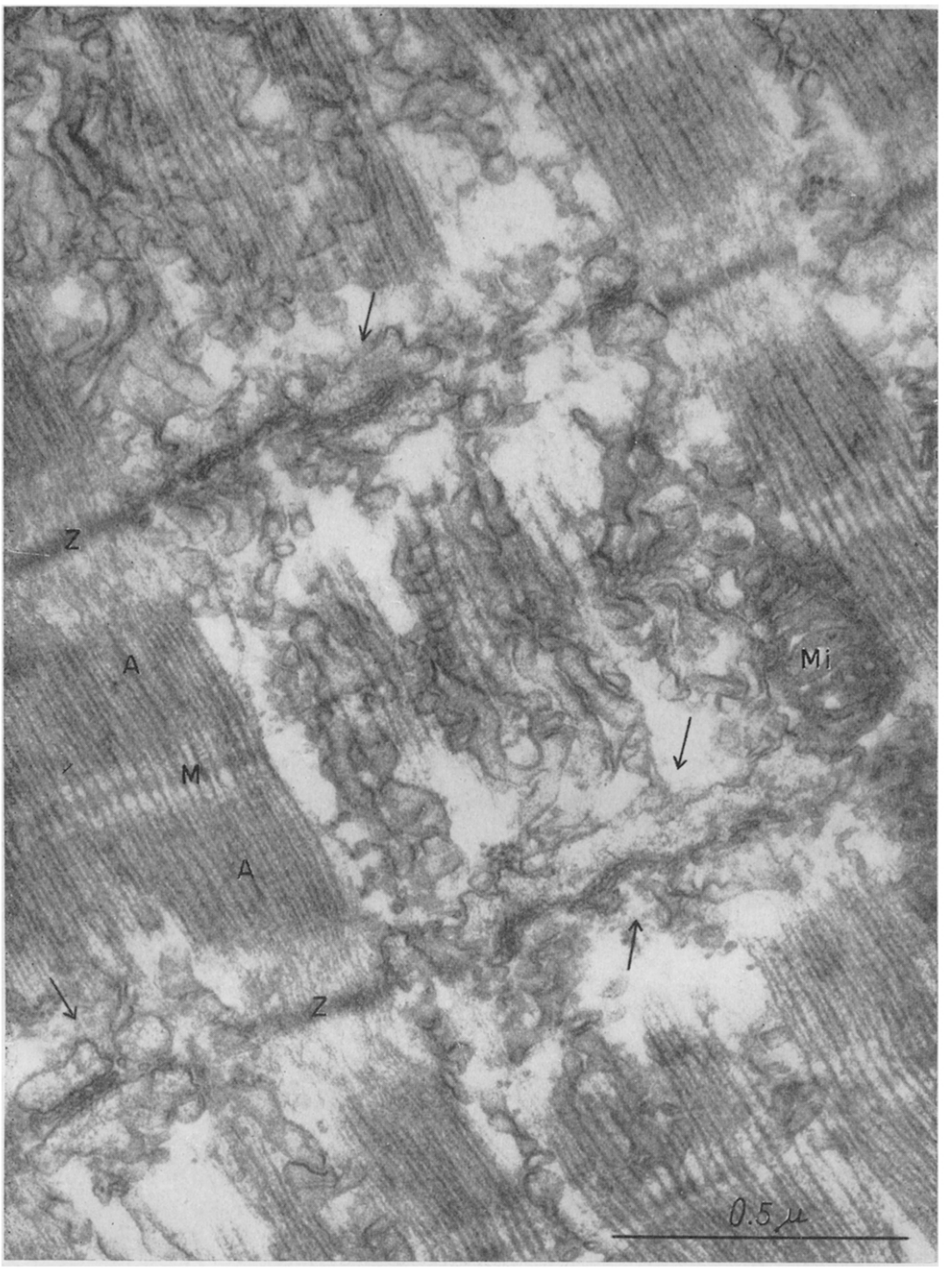

FIG. 2. A higher magnification of a longitudinal section through frog skeletal muscle cell. In the lower part of the picture part of the three-structure component of the sarcotubular system can be seen passing over a rather long distance in the direction transverse to the longitudinal axis of the myofibrils. The longitudinal part of the sarcotubular system forms a complicated pattern at the M band level. The sarcotubules have very intimate contact with the mitochondria. The specimen preparation the same as in Fig. 1. Magnification × 94,000.

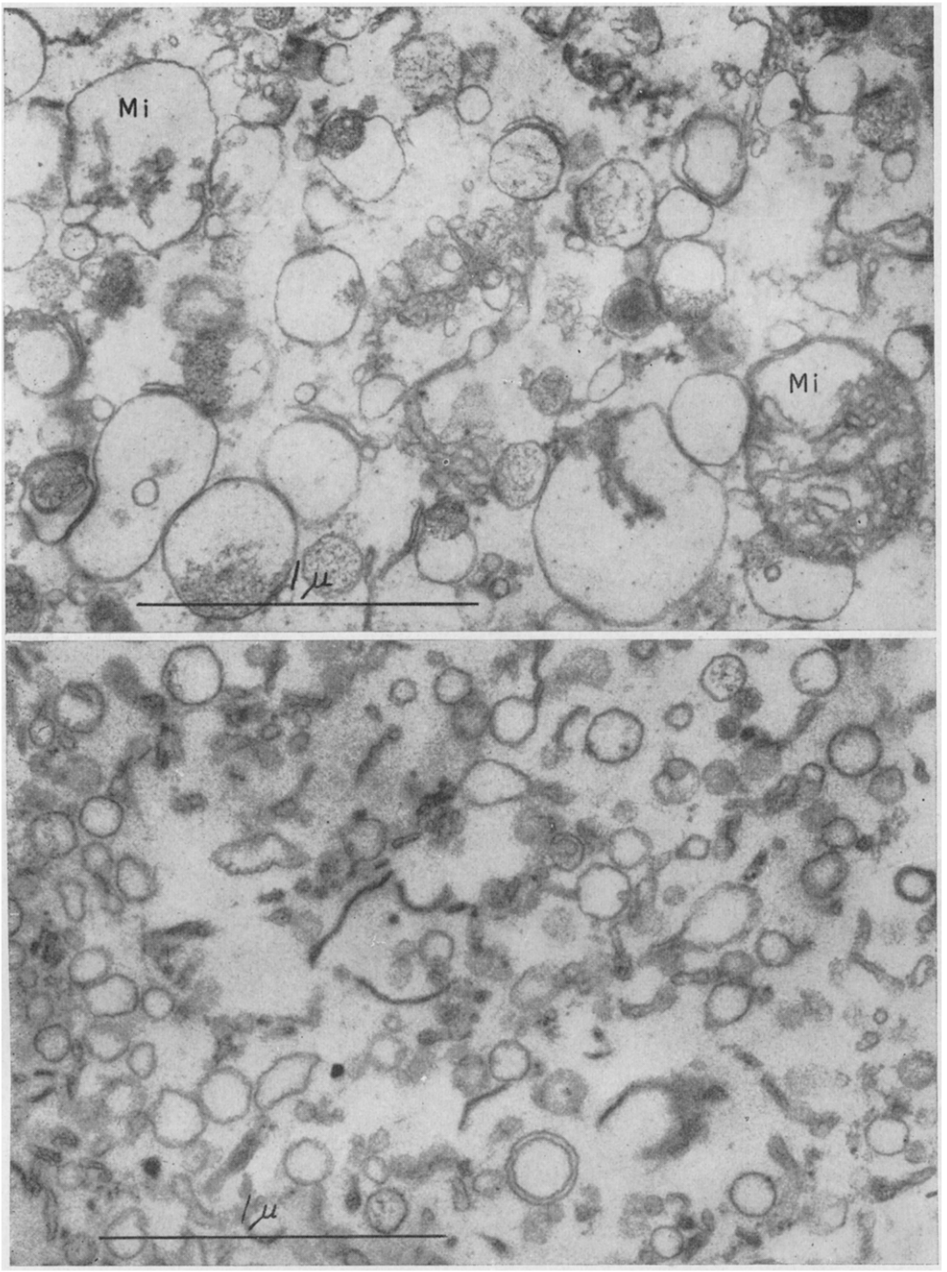

FIG. 3. Electron micrograph of a mitochondrial fraction. The mitochondria were isolated from frog skeletal muscle homogenate by differential centrifugation between 1,000 g for 7 min and 30,000 g for 10 min. The homogenization medium was 0·88 M sucrose. Swollen mitochondria with still visible double inner and outer membranes appear. Single lined vesicles are seen, containing fragments of double membranes or masses of small granules. The pellet was fixed in formalin and OsO_4 and embedded in Epon. The section is stained with uranylacetate and photographed by an RCA EMU 3 A electron microscope. Magnification × 55,000.

FIG. 4. Electron micrograph of a sarcotubular fraction. The fraction was prepared from frog skeletal muscle homogenate. The homogenization medium was 0·88 M sucrose. The fraction was obtained by differential centrifugation between 75,000 g for 30 min and 105,000 g for 60 min. Single and double lined vesicles are seen. Their mean diameter is much smaller than the vesicles appearing in the mitochondria fraction in Fig. 3. Small tubular element with a diameter of about 300 Å are also seen. The electron microscopical preparation as in Fig. 3. Magnification × 55,000

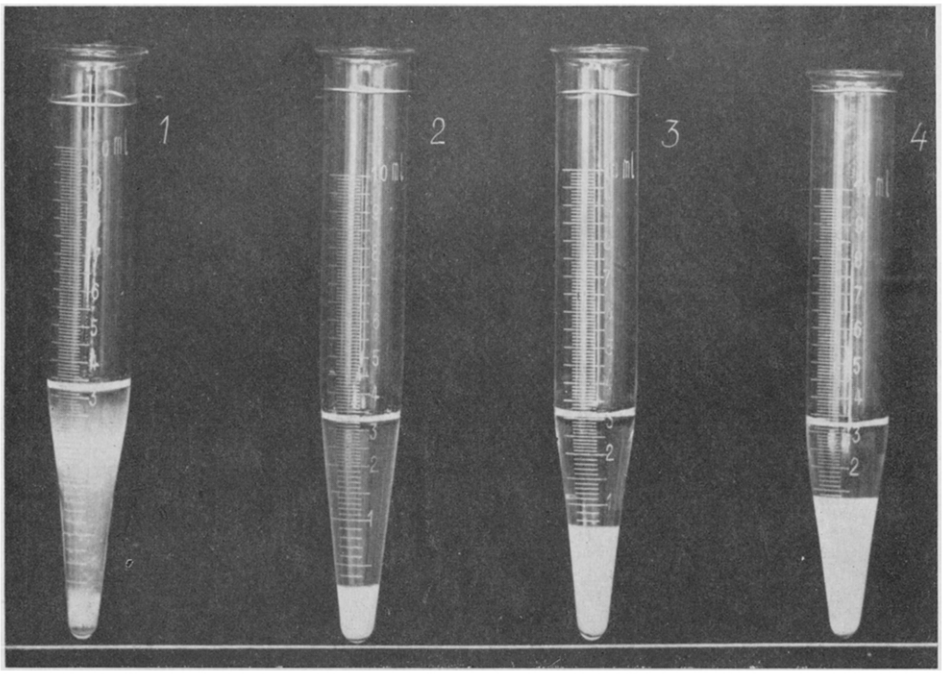

FIG. 5. Effect of the sarcotubules on the superprecipitation of the myofibrils induced by ATP. Each tube contained in a final volume of 3 ml: 25 mM KCl-39 mM borate buffer (pH, 7·1); 13·85 mg of myofibrillar protein, and, when indicated, 3 mM ATP. The myofibrils were prepared according the procedure described by Perry and Grey[7]. The sarcotubules were isolated from skeletal muscle homogenate as described previously[6]. The muscle homogenate in 0·88 M sucrose was centrifuged between 75,000 g for 30 min and 105,000 g for 60 min. 3 mM ATP was added to the tubes no. 2, 3, and 4. Sarcotubules, corresponding to 0·164 and 0·326 mg protein, were added to the tubes no. 3 and 4, respectively. The reaction was started by adding ATP. Immediately after addition of ATP, all the four tubes were centrifuged at about 100 *g* for 2 min.

Like mitochondria, the sarcotubules have a Mg-stimulated ATPase activity; but, as shown in Table I, the ATPase activities of the sarcotubules and of the mitochondria differ in their properties. In the presence of Mg^{++}, the ATPase of the sarcotubules is 4–5 times higher on a protein basis than the ATPase of the mitochondria. Furthermore Ca^{++} in low concentrations strongly inhibits the Mg-activated ATPase of the sarcotubules, but the same concentrations are without any effect on the mitochondrial ATPase. The fragmentation of the mitochondria does not change the features of the enzymatic activities.

In agreement with the biochemical data, the mitochondrial and sarcotubular fractions show different morphological structures. In Fig. 3 is shown a representative section through a mitochondrial fraction. Swollen mitochondria appear with the typical double inner membranes still visible. Single lined vesicles with a diameter up to 1/2 μ are observed to be empty or to contain masses of small particles. Fig. 4 represents the sarcotubular fraction. The structures visible are small vesicles, single or double lined, with a much smaller mean diameter than the vesicles appearing in the mitochondrial fraction. Small tubules with a diameter of about 300Å are also seen. Very few particles can be observed, and they are not attached to the membrane.

The only main components in the skeletal muscle cell which can be expected to be the origin of similar structures in the procedure used, are the mitochondria and the sarcotubular system. As the fraction was obtained without any mitochondrial contamination, the fraction is called the sarcotubular fraction to denote its origin.

The ability to identify the origin of fractions obtained from muscle homogenate by means of correlated morphological and biochemical investigations created the opportunity to decide the origin of Portzehls muscle "granules"[8]. These were shown by her to have the so-called relaxing activity. The relaxing effect, described by Marsh in 1951[5], is the capacity of a fraction of muscle homogenate to induce muscle fibre relaxation, to inhibit myofibrillar ATPase, and to inhibit the ATP-induced superprecipitation of isolated myofibrils.

Table II shows the distribution of the relaxing effect in different fractions of frog skeletal muscle homogenate. The relaxing activity, measured by the capacity to inhibit the ATPase of myofibrils, is localized in the sarcotubular fraction. The mitochondria and the sarcotubular supernatant are without any effect on the myofibrillar ATPase activity.

The localization of the relaxing effect in the sarcotubular system was confirmed by showing the capacity of the sarcotubules to inhibit the superprecipitation of the myofibrils. The superprecipitation, or synaeresis, is the contraction induced by ATP of the muscle protein gel in connexion with expulsion of water. Fig. 5 shows the effect of the sarcotubules on the superprecipitation of isolated myofibrils. The tubes

TABLE II

Distribution of the relaxing effect in different fractions of frog skeletal muscle homogenate

Addition to the myofibrils	ATPase activity (μ Moles Pi/mg protein/5 min.)	Relaxing effect (ATPase inhibition, %)
None	0·579	/
Mitochondria	0·658	none
Sarcotubules	0·269	53·6
Final supernatant	0·548	5·4

Experimental conditions: Each tube contained in a final volume of 2 ml: 50 mM Tris buffer (pH 7·2); 12·5 mM KCl — 19·5 mM borate buffer (pH, 7·1); 4 mM $MgCl_2$; 5 mM ATP, and 5·54 mg of myofibrillar protein. Time of incubation: 5 min at 25°C. The mitochondria were obtained by differential centrifugation between 2000 g for 7 min and 25,000 g for 10 min. The sarcotubular fraction was prepared by centrifuging the mitochondrial supernatant between 25,000 g for 10 min and 105,000 g for 180 min. The muscle homogenate was prepared according the procedure described by Muscatello *et al.*[6]. The fractions were resuspended in 0·25 M sucrose in a final concentration equivalent to 100 mg of fresh tissue. Mitochondria or sarcotubules equivalent to 10 mg of muscle were added where indicated. The inorganic phosphate was measured as in Table I.

contained the same amount of myofibrils isolated from rabbit skeletal muscle and suspended in 25 mM KCl 39 mM borate buffer, pH 7·1.

Increasing amounts of the sarcotubules were added to the tubes Nos. 3 and 4. Immediately after addition of 3 mM ATP to the tubes Nos. 2–4, all four tubes were centrifuged for two min at about 100 *g*. In the absence of added ATP, the myofibrils do not contract (tube No. 1); that is, they do not show any superprecipitation phenomenon, and they cannot be sedimented at low gravitational speed. The addition of ATP induces the superprecipitation of the myofibrils and they sediment even at this low speed (tube No. 2), but the synaeresis is inhibited by the addition of the sarcotubules (tubes No. 3 and 4). This inhibition or relaxing effect seems to be proportional to the increasing amount of the sarcotubules.

Because of the role of acetylcholine in conduction phenomena, it was of some interest to investigate the influence of acetylcholine, or analogous substances, on the relaxing activity of the sarcotubules. In physiological concentrations, acetylcholine and physiostigmine did not show any effect on the myofibrillar ATPase or any modifications of the relaxing activity of the sarcotubules.

DISCUSSION

From the results presented above, it is possible to conclude that the relaxing activity is localized in the sarcotubular system. Thus the sarco-

tubules seem to be involved in the mechanisms controlling the production of energy and its utilization.

In agreement with the biochemical results, the sarcotubular system shows morphologically in the intact muscle cell a very intimate contact with the mitochondria and with the myofibrils.

However, the very peculiar organization of the sarcotubules forming the three-component structure, the triad, might not be explained completely by these biochemical data. The morphological and electrophysiological relationship of the triad to the cell membrane could be interpreted more satisfactorily by localization of the excitation–contraction coupling mechanism at the level of these structures. Further, the results showing that the acetylcholine has no effect on the sarcotubular activity seem to support the conclusion that the coupling mechanism cannot be identified with the relaxing activity.

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