HANDBOOK OF ELECTROENCEPHALOGRAPHY AND CLINICAL NEUROPHYSIOLOGY

EDITOR-IN-CHIEF A. REMOND

VOLUME 2

Electrical Activity from the Neuron to the EEG and EMG

EDITOR: O. CREUTZFELDT

Max-Planck-Institute for Biophysical Chemistry, Göttingen-Nikolausberg (West Germany)

PART D

Basic Mechanisms of the EMG

EDITOR: A. STRUPPLER

Neurological Clinic of the Technical University, Munich (West Germany)

ELSEVIER

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Editor-in-Chief: **Antoine Rémond**
Centre National de la Recherche Scientifique, Paris (France)

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International Federation of Societies for EEG and Clinical Neurophysiology

A great need has long been felt for a Handbook givin complete picture of the present-day knowledge on the el trical activity of the nervous system.

The International Federation of Societies for EEG a Clinical Neurophysiology is happy to be able to present s a Handbook, of which this is a small part.

The decision to prepare this work was made formally the Federation at its VIIth International Congress. Si then nearly two hundred specialists from all over the wo have collaborated in writing the Handbook, each part be prepared jointly by a team of writers.

The Handbook begins with an appraisal of 40 years achievements by pioneers in these fields and an evaluat of the current use and future perspectives of EEG and EM The work subsequently progresses through a wide vari of topics—for example, an analysis of the basic principles the electrogenesis of the nervous system; a critical review techniques and methods, including data processing; description of the normal EEG from birth to death, w special consideration of the effect of physiological a metabolic variables and of the changes relative to br function and the individual's behaviour in his environme Finally, a large clinical section covering the electrical normalities in various diseases is introduced by a study electrographic semeiology and of the rules of diagnos interpretation.

The Handbook will be published in 16 volumes compris 40 parts (about 2500 pages altogether). For speed of pul cation most of the 40 parts will be published separately a in random order.

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* These parts have been published or are in press. The other parts are in preparation and will appear with the above or similar titles.

PART D

BASIC MECHANISMS OF THE EMG

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Preface

This Part treats three main aspects of motor function:

Section I deals with the basic mechanism of skeletal muscle fiber contraction, particularly excitatation–contraction, the effects of various ions on the membrane and with cell metabolism.

Section II is devoted to neuromuscular transmission, especially the importance of presynaptic factors. The basic pharmacology of motor nerve terminals including the role of trophic factors is discussed. Also, the action of ions, drugs, toxins and temperature on neurosecretory coupling is described. Depletion and replacement of transmitter stores is reviewed in relation to clinical diseases.

The central factors modulating motor function are discussed in Section III. The section is concerned with the analysis of reflex excitability under normal and pathological conditions.

The entire theme is directed to all interested in the physiology and pathophysiology of motor function. This includes clinicians, scientists and students interested in a better understanding of clinical neurological syndromes, and in applying the information as a basis for experimental study.

The basic electrophysiology of the excitable muscle membrane is treated partially in Part B of this Volume, as far as the general problems of membrane and action potentials are concerned, and partially in Vol. 16, where the emphasis is laid mainly on extracellular recordings of muscle action potentials such as done in clinical electromyography.

Section I. Sarcoplasmic Calcium Transport as Prerequisite for Electromechanical Coupling

A. INTRODUCTION

In recent years the study of the interaction of calcium with the isolated fibrillar muscle proteins in the muscle membrane has initiated a remarkable development in muscle physiology and biochemistry. Contributions from many laboratories have led to the formulation of the generally accepted working hypothesis that the mechanical activity of all contractile protein structures is controlled by the intracellular level of ionized calcium (*cf.* Hasselbach 1964; Ebashi and Endo 1968).

It is generally assumed that the electrical event occurring during excitation at the surface of the muscle fibers triggers a release of calcium which activates the contractile apparatus. The convincing but indirect evidence for this concept is based on the combination of the results of experiments performed with isolated muscle structures as well as with living muscles.

B. THE ACTIVATION OF THE CONTRACTILE PROTEINS BY CALCIUM

1. The activation of the isolated contractile proteins

The isolated contractile proteins can be studied in gels of natural actomyosin, in isolated myofibrils and in muscle fibers whose membranes have been destroyed by glycerol water extraction. They react with their natural energy source magnesium-ATP only if the level of ionized calcium in the assay medium exceeds 10^{-7} *M* (Weber and Herz 1963a; Portzehl *et al.* 1964). Such extremely low levels of ionized calcium which are two orders of magnitude lower than the ubiquitous calcium traces in all reagents, can be achieved if calcium buffers are used. These are mixtures of calcium complexing agents with their calcium salts. When the concentration of the ionized calcium in the medium is raised, tension of the contractile proteins increases steeply. At a concentration of 10^{-6} *M* the tension reaches its maximal value. The calcium induced, tension producing interaction between Mg-ATP and the contractile protein requires the combination of calcium with one of the constituents of the contractile proteins (Weber and Herz 1963a). The amount of calcium bound when maximal activity is reached is in the order of 2 μmoles calcium per g of protein which corresponds to a concentration of total calcium of 0.1–0.2 mM/ml fiber water. As soon as the calcium is removed from the contractile protein complex by application of a calcium chelating agent the contractile system returns to the state of rest.

The protein component with which calcium combines is thought to be neither

myosin nor actin, the main constituents of the contractile protein. The complex of these two proteins—actomyosin—is able to interact with Mg-ATP whereby the structure shortens or produces tension. However, the activity of this system does not depend on the concentration of ionized calcium. The calcium sensitivity is brought about by the presence of an additional protein system, the tropomyosin–troponin complex (*cf.* Ebashi and Endo 1968). This calcium sensitizing complex is attached to the actin filaments from which it can be removed and isolated. The isolated calcium sensitizing complex has the same high affinity for calcium as the contractile complex. It is generally assumed that the calcium sensitizing complex inhibits contraction if it is calcium free. Relief of inhibition is assumed to occur if calcium combines with the protein complex.

2. *The activation of the contractile proteins in vivo*

The contractile system in the living muscle has the same high sensitivity to calcium as reported for the isolated contractile system. *In vivo*, this sensitivity has been established by microinjection of solutions containing calcium buffers.

Such injections induce threshold contractions at the same low level of $\gtrsim 10^{-7}$ *M* as found for the isolated protein (Portzehl *et al.* 1964). As a consequence, one must assume that in the resting living muscle the level of ionized calcium is below this value. Since the total concentration of muscle calcium is in the order of 2–3 μm/ml, practically all muscle calcium must be bound. This assumption is in agreement with the result of studies in which the soluble muscle calcium fraction has been determined.

The maximal activation of the contractile system in the living muscle likewise occurs at the same concentration of ionized calcium (10^{-6} *M*) which maximally activates the isolated contractile protein. Similar experiments have been performed with muscle fibers whose membranes have been stripped off and to which the calcium

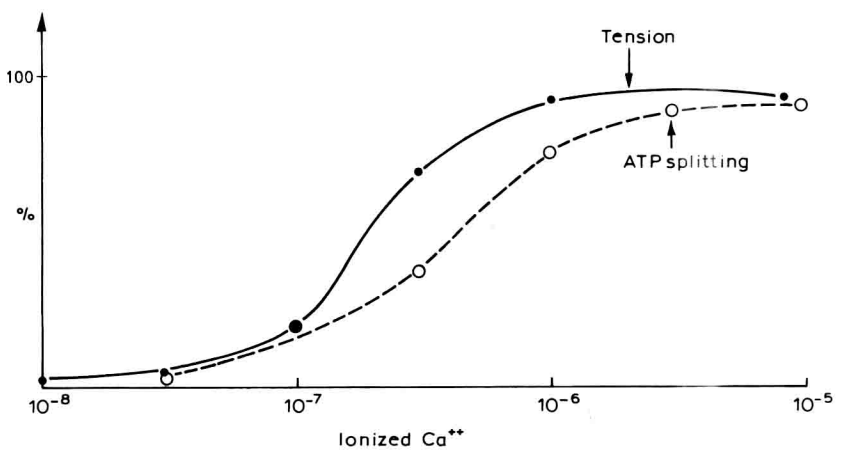

Fig. 1. The calcium sensitivity of isolated contractile structures (isolated myofibrils) and skinned muscle fibers. The myofibrils (broken line) get all substance required for contraction and ATP splitting from the bath. Tension development of the skinned fiber (continuous line) which is suspended in oil is induced by the addition of calcium only. Ordinate: tension development in relative values. (Compiled from Weber and Herz 1963a and Hellam and Podolski 1969)

has been delivered electrophoretically. The results of the latter experiments are in quantitative agreement with those performed with living fibers (Hellam and Podolski 1969) (Fig. 1). In contrast to the extracted contractile system, the living fibers of fiber bundles relax spontaneously if the application of calcium is interrupted. Hence, in the living fiber a calcium sink must be present.

C. EXTRACELLULAR CALCIUM AND MUSCLE ACTIVATION

The analysis of muscle contraction *in vivo* induced by the depolarization of the surface membrane furnished the earliest evidence for the involvement of calcium in the activation process. The experiments revealed that depolarization is a necessary but not sufficient requirement for the activation of the contractile process.

When muscles are kept for a longer period of time in solutions free of calcium ions, membrane potential drops slowly and depolarization becomes ineffective, even if the membrane potential has been reduced by only a few millivolts. If the membrane potential is shifted to its resting value by the application of a hyperpolarizing current, contractions can be induced again by depolarization. Obviously calcium depletion has affected the fiber membrane but not the contractile system. Also in heart muscles, the well-known antagonistic influence of calcium and sodium on tension development takes place at the fiber membrane (*cf.* Lüttgau 1965). If contractions are induced by depolarizing the muscle with isotonic KCl, changes of the sodium or calcium content of the medium immediately influence tension development. Calcium effects occurring at the contractile system develop more slowly since calcium has to diffuse into the fiber.

Further indications for the role of calcium were provided by studies dealing with the movement of calcium across the plasma membrane during rest and activity. In all muscles the exchange between extra- and intracellular calcium increases when the muscles are depolarized. In twitch muscles, calcium exchange is enhanced only when depolarization sets in. In tonic muscles the calcium movements remain accelerated as long as the muscle is depolarized and a net uptake of calcium occurs. While the twitch muscle relaxes again even if depolarization continues, the tonic muscle remains contracted. In none of the experiments calcium enters the fiber in such quantities that are comparable to those necessary for the activation of the contractile system (Bianchi and Shanes 1959). The role of calcium for excitation–contraction coupling in these experiments on intact muscle fibers is obviously confined to processes in the outer membrane of the fibers. The link between these calcium dependent processes and the activation of the contractile system by calcium has remained a matter of speculation for a long time.

D. THE STRUCTURAL ORGANIZATION OF THE MYOPLASMIC MEMBRANE SYSTEM

At the time when the calcium sensitivity of the contractile protein *in vitro* and *in vivo* was established, the existence of two complicated membrane systems inside the muscle

fiber was confirmed: The sarcoplasmic reticulum and the transverse tubular system. These systems have been studied in the test-tube and in the living muscles respectively. Conclusive evidence has been presented that the gap between membrane excitation and calcium activation of the contractile system is bridged by these structures.

A network of membranes in the myoplasma of different muscles has been discovered and accurately described already in 1902 by Veratti (*cf.* Porter 1961). However, the delicate structures were considered to be artifacts and therefore set aside by most physiologists. When in 1957 the structures were rediscovered in the electronmicroscope (Porter and Pallade 1957) intense interest arose. The discovery that the elaborated network was composed of two different membrane systems was the structural basis for the understanding of the function of the internal membranes (Fig. 2).

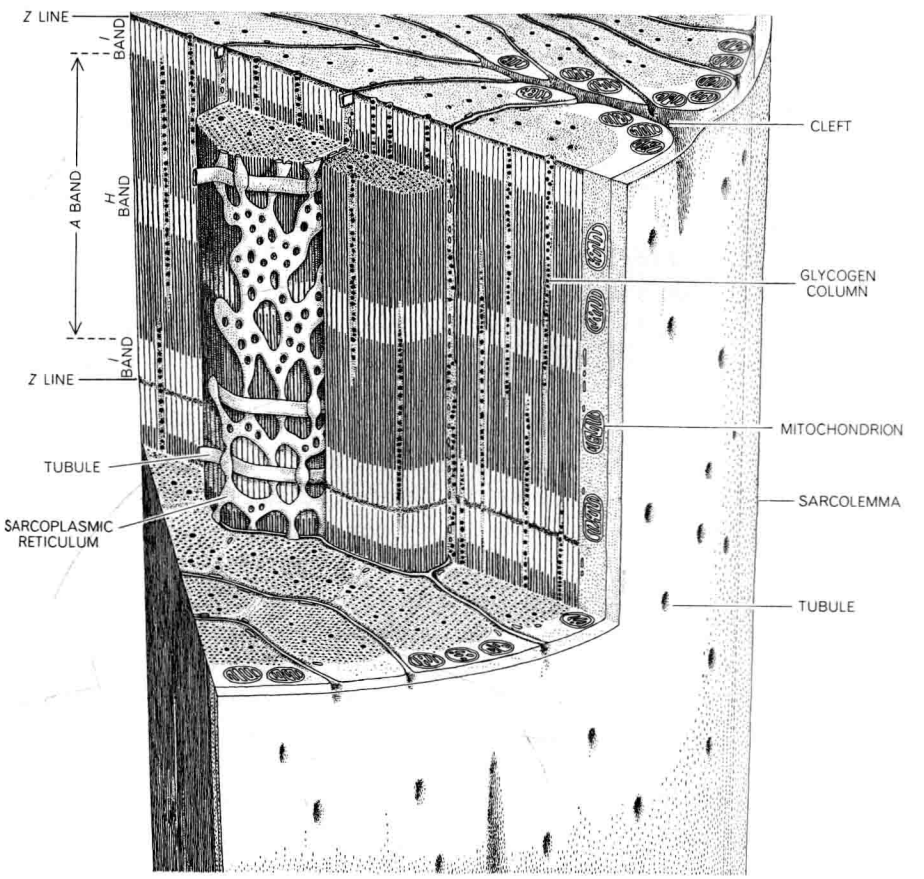

Fig. 2. The organization of the sarcoplasmic reticulum and the transverse tubular system in a muscle fiber. (G. Hoyle, *Sc. American*, 1970, *222*: 4–87)

In fast contracting muscles, the transverse tubular system consists of narrow channels which enter the fiber as processes of the plasma membrane in close contact with specific sarcomere structures (Z-line or A–I junction). In these muscles each sarcomere

has its own transverse tubular (T) system. In slow muscles the transverse tubular system is less developed and several sarcomeres are usually interconnected by it. In both muscle types the T-channels connect the extracellular space with the innermost myofibrils. The continuity between the extracellular space and the tubular lumen has been demonstrated first by Huxley (1964) showing that ferritin particles added to the extracellular fluid diffuse along the T-channels. In muscles composed of very thin fibers or in slowly contracting muscles the system is only weakly developed or even lacking. In twitch muscles the total volume of the system comprises less than 0.5% of the muscle volume, its surface, however, is 5–10 times larger than the surface membrane (Peachey 1965). At the edges of the A-band (*e.g.*, in human muscle) or adjacent to the Z-lines (*e.g.*, in frog muscles) the T-channels are flanked by vesicular enlargement of the second internal membrane system, the sarcoplasmic reticulum. The structural units formed by the two membranes have been called triades. The role of the transverse tubular system as an intracellular excitation conducting system was verified in experiments with living muscle (Huxley and Taylor 1958).

The sarcoplasmic reticulum is composed of a complicated network of channels whose diameter is larger than that of the T-channels. They surround each myofibril running mainly longitudinally. If the transverse tubules encircle the myofibrils, the system forms vesicular-like structures, cisternae, which are connected by special areas with the transverse tubular system. The channels arising from the cisternae flow together at the so-called area fenestrata. In fast contracting muscles each sarcomere has its own isolated sarcoplasmic system, in most slow muscles the system extends over several adjacent sarcomeres. In muscles which have no T-system the rudiments of the sarcoplasmic membranes are found as vesicles beneath the surface membrane. In muscles with a well developed sarcoplasmic reticulum, this comprises approximately 5–10% of the fiber volume and its surface has been estimated to exceed the surface membrane by a factor of at least 1000.

E. SARCOPLASMIC CALCIUM TRANSPORT

1. Calcium storage in situ

As to the function of the sarcoplasmic membranes, the main contributions were obtained by *in vitro* studies.

The involvement of the longitudinal system in compartmentalization and transport of calcium has been substantiated by numerous observations (Hasselbach 1964, 1965; Costantin *et al.* 1965; Zebe and Hasselbach 1966). One of the most plastic demonstrations of this function is the electronmicroscopic visualization of calcium precipitates in the cavities of the membranes. If isolated muscles from the crayfish are kept for some hours in Ringer solution their calcium content increases considerably (Grundfest 1966). In spite of the rising total calcium these muscles remain in the state of relaxation. Electronmicroscopic pictures show that calcium deposits have been formed in the channels of the sarcoplasmic reticulum between the myofibrils. (In these muscles the sarcoplasmic reticulum only consists of narrow channels.)

Similar calcium deposits have been produced under more controlled conditions in a variety of muscles. In these experiments the plasma membranes of the muscles have been made more permeable for calcium by a short glycerol treatment, so that calcium can enter the fibers from outside more rapidly. Subsequently thin fiber bundles were immersed in a bathing solution which imitated the intracellular milieu of the muscle as far as the concentration of magnesium ATP and ionized calcium are concerned, and which contained calcium precipitating anions. Under these conditions the fibers could store tremendous amounts of calcium. In muscles in which the reticulum forms triad structures, the calcium precipitates were found mostly in the sarcoplasmic cisternae. In muscles where the reticulum forms dyads beneath the outer membrane, the calcium was found in these structures. If the reticulum forms neither dyads nor triads structures the calcium precipitated in the longitudinal channels between the myofibrils. In contrast, however, calcium never precipitated in the transverse tubular channels.

2. Calcium storage in the isolated sarcoplasmic vesicles

When a muscle is homogenized, its contractile structure and its membranes are fragmented. Sarcoplasmic membranes intermixed with the small fraction of the membranes derived from the T-system can be separated from all other structures by relative simple procedures (Hasselbach and Makinose 1963) (Fig. 3). On electronmicroscopic pictures the membranes appear as closed, mostly spherical vesicles with a mean diameter of ~800Å (Nagai *et al.* 1960; Martonosi 1964; Hasselbach and Elfvin 1967; Deamer and Baskin 1969). The healing over process by which the fragmented mem-

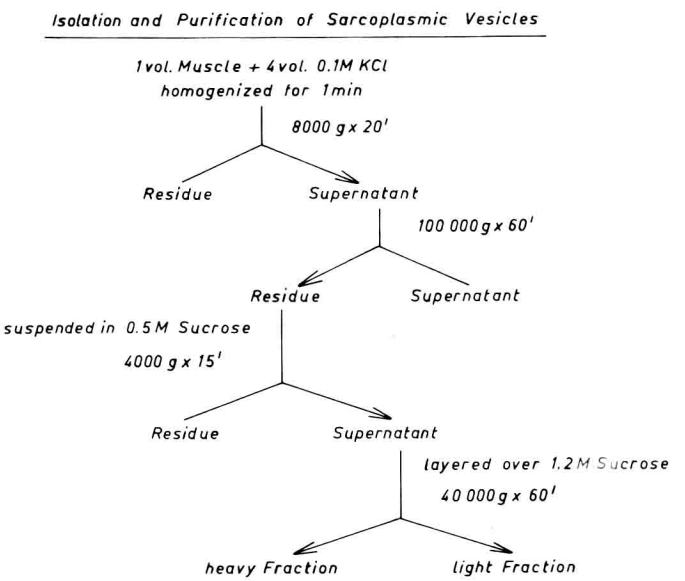

Fig. 3. Isolation and purification of sarcoplasmic vesicles. As an additional purification step, the light fraction can be washed with a solution containing 0.6 M KCl and 2 mM Mg ATP. This procedure which removes actomyosin traces can be omitted for most purposes since only very little actomyosin is extracted together with the membranes by 0.10 M KCl. (Hasselbach 1972)

branes are transformed into vesicles is most remarkable. In contrast to many other membranes whose fragments also form vesicular particles, the sarcoplasmic vesicles become astonishingly tight. This is the reason why the transport of calcium across these membranes results in the accumulation of calcium as observed in the intact structures. Due to this unique property, muscular calcium transport could be analyzed in great detail.

(a) Active calcium transport

When the sarcoplasmic vesicles are incubated in an assay medium as described in Table I, large amounts of calcium oxalate or, if phosphate is used instead of oxalate, calcium phosphate are precipitated inside the vesicles (10 μM Ca/mg prot.) (Hasselbach and Makinose 1961; Weber *et al.* 1963b). This accumulation process does not occur spontaneously as can be concluded from the composition of the assay medium. In the medium the ion product of calcium and oxalate is 20 times smaller than the solubility product of calcium oxalate. If a precipitation of calcium oxalate occurs inside the vesicles suspended in the medium, the ion product must have been increased by a factor of at least 20 inside the particle. This can be brought about either by an active accumulation of calcium or an active accumulation of oxalate ions, if it is assumed that the membrane is permeable to the respective passive ion. An active transport of oxalate and phosphate has been excluded (Hasselbach and Makinose 1963). An additional argument for the assumption that calcium is the ion which is actively translocated will be given when the transport ATPase activity of the membranes is discussed.

TABLE I

COMPOSITION OF ASSAYS FOR CALCIUM UPTAKE BY SARCOPLASMIC VESICLES IN THE ABSENCE (LEFT COLUMNS) AND THE PRESENCE OF CALCIUM PRECIPITATING (RIGHT COLUMNS) AGENTS
5 mM oxalate can be replaced by ~20 mM phosphate. In addition to the agents listed in the table all assays contain tris or histidine buffer 2.0 mM and KCl or NaCl ~50–100 mM. (Hasselbach 1972)

	Concentration total (M)	Concentration free (M)	Concentration total (M)	Concentration free (M)
ATP	$5 \cdot 10^{-3}$	$7 \cdot 10^{-4}$	$5 \cdot 10^{-3}$	10^{-3}
Mg	$5 \cdot 10^{-3}$	$7 \cdot 10^{-4}$	$5 \cdot 10^{-3}$	$4 \cdot 10^{-4}$
EGTA*	—	—	$2 \cdot 10^{-4}$	10^{-4}
Oxalate	—	—	$5 \cdot 10^{-3}$	$4 \cdot 10^{-3}$
Ca^{++}	10^{-4}	$2.7 \cdot 10^{-5}$	10^{-4}	$2 \cdot 10^{-7}$
Calcium storage (binding?)	0.1–0.2 μmoles/mg		10 μmoles/mg	
Initial concentration ratio $\frac{Ca_i}{Ca_o}$	~0?		25	

* Ca^{++}-binding agent

(b) Calcium transport energetics

The energy requirement of the calcium transport can be estimated any time if the ion activities of calcium and oxalate in the solution and inside the vesicles are known.

In the presence of oxalate as calcium precipitating anion, the product of calcium × oxalate inside the vesicles is identical with the solubility product of calcium oxalate as soon as the first calcium oxalate crystal is formed. In the solution outside the vesicles the ion product of calcium × oxalate can be determined experimentally at every moment. The minimal reversible energy requirement is thus obtained from the ratio

$$\frac{(\text{calcium} \times \text{oxalate})\ \text{inside}}{(\text{calcium} \times \text{oxalate})\ \text{outside}} = \frac{\text{solubility product}}{(\text{calcium} \times \text{oxalate})\ \text{outside}}$$

This ratio reaches its maximum when at the termination of net calcium uptake the influx of calcium is balanced by the efflux. This balance is reached if the ratio approaches 3000, corresponding to an energy requirement for the net uptake of 1 *M* of calcium of 5000 calories. During the initial phase of net calcium uptake where transport occurs at lower activity ratios, the energy needed for concentrating calcium is correspondingly low. An additional energy quantity is required to maintain the high net flux rate. It is interesting that in this case the total energy equals the energy requirement when the maximal gradient is established (Hasselbach and Makinose 1963; Hasselbach 1969) (Table II).

TABLE II

THE PERFORMANCE OF THE SARCOPLASMIC CALCIUM PUMP WITH ATP AND UTP AS ENERGY DONATORS

The rates are related to the surface of the vesicles 3000 cm^2/mg ves. protein. For the estimate of the initial energy requirement the energy needed to maintain the efflux has been taken into account. (Hasselbach 1972)

Performance of the calcium pump			ATP	UTP
Initial	Rates (20°C)	Ca influx [pmoles/cm^2 · min]	250	70
		Ca efflux	15	4
	Concentration ratio	$\left[\frac{Ca_iOx_i}{Ca_oOx_o}\right]$	25	25
	Energy requirement	cal/mol	~4000	~4000
Steady state	Rates (20°C)	pmoles/cm^2 · min	0.12	0.04
	concentration ratio	$\left[\frac{Ca_iOx_i}{Ca_oOx_o}\right]$	2500	2100
	Energy requirement	cal/mol	~5000	~5000

It should be emphasized that every kind of calcium accumulation including the accumulation of calcium in solutions containing no calcium precipitating anions depends on the tightness of the vesicular membranes. When the vesicles of the sarcoplasmic membranes are mechanically or chemically opened or made more permeable for calcium, calcium accumulation is abolished simultaneously in the presence as well as in the absence of calcium precipitating anions. This observation indicates that the uptake of calcium in the absence of calcium precipitating anions is in fact an accumula-