The Accumulation of Calcium Ions by Sarcotubular Vesicles

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ABSTRACT The accumulation of Ca^{++} by microsomal (sarcotubular) preparations of rabbit skeletal muscle in the presence of oxalate, and the concurrent splitting of nucleoside triphosphate, displayed moderate nucleotide specificity in the sequence ATP > GTP, CTP, ITP > UTP > (ADP) > ATetraP for the former, ATP > (ADP) > ITP > GTP > CTP > UTP > ATetraP for the latter process. The "calcium pump" was weakly inhibited by caffeine, and was inhibited together with the ATPase by pyridoxalphosphate. Carnosine had no effect as such nor in the presence of pyridoxalphosphate except at high concentration; thiourea and *p*-chloromercuribenzoate were inhibiting while iodoacetate was inactive. Ca^{++} accumulation and ATPase were inhibited by atabrine (not tested on ATPase), dinitrophenol, and amytal. High concentrations of oligomycin and rutamycin inhibited Ca^{++} uptake while slightly stimulating ATPase. Antimycin A stimulated the Ca^{++} uptake. These results are discussed in the light of their possible relation to partial reactions in oxidative phosphorylation. The Ca^{++} uptake and relaxing factor activities did not behave identically throughout. This is in part ascribed to changes in reactivity of actomyosin in the relaxation test, in part to the participation of relaxing substances other than the calcium pump.

INTRODUCTION

An important advance in the study of the relaxing factor activity of the microsomal, particulate, or vesicular sarcotubular fraction of muscle was the discovery of its ability to accumulate calcium ions while splitting ATP (1–4). While evidence continues to be mentioned indicating the existence of a relaxing substance (5, 6), there is no doubt that the Ca^{++} concentrating activity represents an important part of the relaxing effect, in view of the dependence of actomyosin contractility upon calcium (2, 7) and in keeping with physiological knowledge about excitation-contraction coupling (8–12). In fact, the additional role of relaxing substances might consist in modifying the calcium pumping action of the granules, or in altering the Ca^{++} requirement of actomyosin.

The relaxing activity tested upon myofibrils or glycerol-extracted muscle fibers shows a specific nucleotide requirement (13) and is modified by certain metabolic inhibitors (14). It was the main purpose of this investigation to establish to what extent these properties are due to the Ca^{++} concentrating phenomenon itself, and in addition, to gain some additional insight into the biochemical mechanism of the latter.

MATERIALS AND METHODS

Preparation of Microsomes A rabbit was anesthetized with nembutal and bled to death. The back muscles were taken out, chilled on ice, and minced in the cold; 50 gm of minced muscle were homogenized with 3 volumes of a solution of 0.08 M KCl, 0.005 M oxalate for 30 seconds in a Waring blendor. The homogenate was centrifuged for 20 minutes in a Lourdes centrifuge at 10,000 RPM (12,000 × *g*) to remove tissue fragments, myofibrils, and mitochondria. The supernatant was filtered through gauze and centrifuged in a Spinco model L ultracentrifuge at 25,000 RPM (40,000 × *g*) for 90 minutes (15). The pellets were dispersed in KCl-oxalate, and the protein concentration determined according to Lowry *et al.* (16) using bovine serum albumin as standard. This fraction contained the Ca^{++} concentrating and the relaxing activity. Further centrifugation of the supernatant at 150,000 × *g* yielded some protein, approximately one-fourth of the first fraction; this was devoid of calcium-pumping activity under our conditions (*cf.* also reference 17) and hence was discarded.

Materials Crystalline Na salts[1] of ATP, CTP, GTP, ITP, UTP, AtetraP, de-ATP, ADP, and FAD were obtained from Sigma Chemical Company. Most of the Ca^{++} was removed from the ATP by leading a solution containing about 1 gm ATP through a 1 × 5 cm column of Dowex 50 x 8 H^+ (18). The pH of the ATP-containing effluent was adjusted to 7.0 and the concentration determined spectrophotometrically. After suitable dilution the solution was kept frozen until used. The nucleotides were freed of phosphate by precipitation with alkaline $CaCl_2$ (19), after which the supernatants were led through a column of Dowex 50 × 8 H^+ and treated as described for ATP. The nucleotide solutions were found to be chromatographically pure, with the exception of adenosinetetraphosphate, which showed a trace of ATP. Pyridoxalphosphate was a gift from California Foundation for Biochemical Research. Antibiotics and inhibitors were obtained from commercial sources and private laboratories.[2]

Experimental Procedure Granules were incubated at room temperature in a medium containing 6×10^{-3} M potassium oxalate, 5×10^{-3} M $MgCl_2$, 1×10^{-3} M ATP or other nucleotide, 0.1 M KCl, 0.02 M NaCl, and histidine buffer (0.016 M

[1] Abbreviations: ATP, CTP, GTP, ITP, UTP, the 5′-triphosphates of ribosyl adenine, cytosine, guanine, hypoxanthine, uracil; AtetraP, adenosinetetraphosphate; de-ATP, deoxy ATP; ADP, adenosinediphosphate; FAD, flavin-adenine dinucleotide; NTP, nucleoside triphosphate; PCMB, *p*-chloromercuribenzoate; amytal, 5-ethyl-5-isoamyl barbituric acid; atabrine, quinacrine HCl.

[2] We wish to thank Professors Paul D. Boyer and Albert L. Lehninger for generous gifts of rutamycin and of oligomycin.

KCl, 0.004 M histidine, pH 7.2), and 1×10^{-4} M $CaCl_2$ in a total volume of 4 ml. The microsome concentration usually was 0.08 mg/ml. Calcium uptake was measured using Ca^{45}, the reaction mixture containing approximately 0.1 μc Ca^{45} ml. In order to measure the time course of the reaction, a series of test solutions was set up and at given time intervals from the time of addition of the microsome suspension, the reaction mixtures were filtered by suction through Millipore filters (0.45 μ diameter) (20).[3] Counting can be done on the filters fitted into planchets, or upon the filtrate. We have usually employed the latter procedure. It is believed that the filter retains the particulate matter entirely because recounting of the filtrate after an additional filtration 1 hour later yielded the same number of counts.

We have preferred to work at 10^{-3} M ATP so as to be in a concentration range where the calcium uptake is not dependent on the ATP concentration (1). It is clear from the data of Hasselbach and Makinose (1) that all calcium remains soluble under these conditions. Since, however, later calculation by these authors (4) leaves uncertainty about the allowable reactant concentrations, we have experimentally tested whether in media of the composition employed there was any precipitation of calcium oxalate. None was found, even when the Ca^{++} concentration was doubled. Adsorption of 2.5 to 3 per cent of the counts to the filter papers, found under all conditions even in the absence of oxalate, was corrected for by setting up controls as routine.

The pH of all additions was adjusted to 7.2. At the termination of the experiments the pH was measured and found to be constant. Experiments under nitrogen or helium were carried out in Warburg flasks. The granule suspension was in the side arm while the gas was bubbled through the reaction mixture for 10 minutes. The granule suspension was then tipped in and the reaction allowed to take place for specified time intervals (up to 16 minutes) while the gas was continuously bubbled through the reaction mixture.

Adenosinetriphosphatase activity was investigated by determining the phosphate liberated during the same runs used to measure the calcium uptake. The Fiske-SubbaRow method was used (21).

RESULTS

In the experiments with 0.08 mg microsomal protein per ml, with an initial amount of 1.25 μmoles of Ca^{++} per mg protein and at 1×10^{-3} M ATP, the largest part of the Ca^{++} uptake was complete after 8 minutes of incubation, and continued slowly thereafter. The activity was somewhat variable from one preparation to the next, and was always lower in older preparations. Because of this variability, a control experiment with ATP, without variations or additions, was run simultaneously with each set of observations. Results of at least three separate experiments were averaged. Most observations were done within 24 hours, otherwise within 48 hours after the animal had been killed.

Effect of Inorganic Cations Sodium-stimulated ATPases have been found

[3] We are indebted to Dr. A. Martonosi for demonstrating this method to us prior to publication.

in tissues displaying active transport, such as nerve (22), brain (23, 24), muscle (24), and erythrocytes (25). It has been suggested that such ATPases represent a mechanism for active Na^+ transport across cell membranes. In view of possible interrelations among different ion transport processes, it was of interest to see whether the calcium pump activity of the microsomes displayed a similar cation requirement. Experiments in that direction have led to variable

TABLE I

EFFECT OF NA^+ AND K^+ ON CA^{++} PUMPING AND ON ATP SPLITTING OF SARCOTUBULAR VESICLES

	μmoles Ca^{++}/mg protein		μmoles P_i/mg protein	
	2 min.	*8 min.*	*2 min.*	*8 min.*
Granules 5 hrs. old				
Prepared in K^+				
All K^+, no Na^+ during experiment	0.285	0.825	0.457	1.40
All K^+, no Na^+, no Mg^{++}	0	0	—*	—*
K^+:Na^+, 6.2:1, during experiment	0.400	0.825	0.466	1.44
K^+:Na^+, 6.2:1, no Mg^{++}	0	0	—*	—*
Prepared in Na^+				
Na^+:K^+, 6.2:1 during experiment	0.970	1.50	0.491	1.45
Na^+:K^+, 6.2:1, no Mg^{++}	0	0	—*	—*
All Na^+, no K^+ during experiment	1.14	1.68	0.525	1.42
All Na^+, no K^+, no Mg^{++}	0.541	0.641	0.203	0.584
Granules 24 hrs. old				
Prepared in K^+				
All K^+, no Na^+ during experiment	0.155	0.800	0.466	1.22
All K^+, no Na^+, no Mg^{++}	0	0	—*	—*
K^+:Na^+, 6.2:1 during experiment	0.313	0.779	0.398	1.27
K^+:Na^+, 6.2:1, no Mg^{++}	0	0	—*	—*
Prepared in Na^+				
Na^+:K^+, 6.2:1 during experiment	0.440	0.829	0.466	1.27
Na^+:K^+, 6.2:1, no Mg^{++}	0	0	—*	—*
All Na^+, no K^+ during experiment	0.383	0.957	0.441	1.31
All Na^+, no K^+, no Mg^{++}	0.100	0.100	0.229	0.550

* P_i not measured in this experiment; P_i was generally ⅓ to ½ of that produced in the presence of Mg^{++}. In the absence of Ca^{++}, ATP splitting was also about ½ of that obtained in the presence of Ca^{++}.

results. In certain series, such as that represented in Table I, it was found that very fresh granules prepared and studied in Na media were more effective with respect to Ca^{++} transport than were granules in the described standard K medium; also, the usual requirement for Mg^{++} ions was not absolute in the Na solutions. Such results were not always obtained, however, and it is not clear as yet which factors determine this behavior.

Nucleotide Specificity Results obtained with different nucleotide triphosphates and other nucleotides are given in Figs. 1 *a* and 1 *b*, which show the

time course of Ca^{++} uptake and phosphate liberation, respectively. ATP was the most active in both instances, followed by other triphosphates which in diminishing order, still allowed sizable reaction rates. To allow a comparison between the two phenomena, we plotted in Fig. 2 the initial rates of Ca^{++} ac-

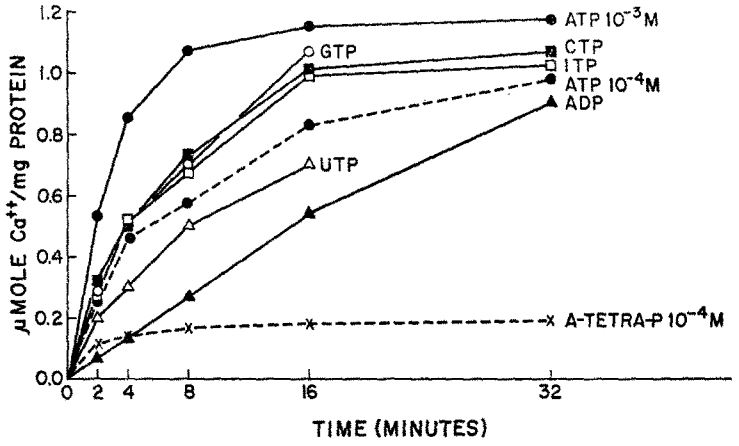

FIGURE 1*a*

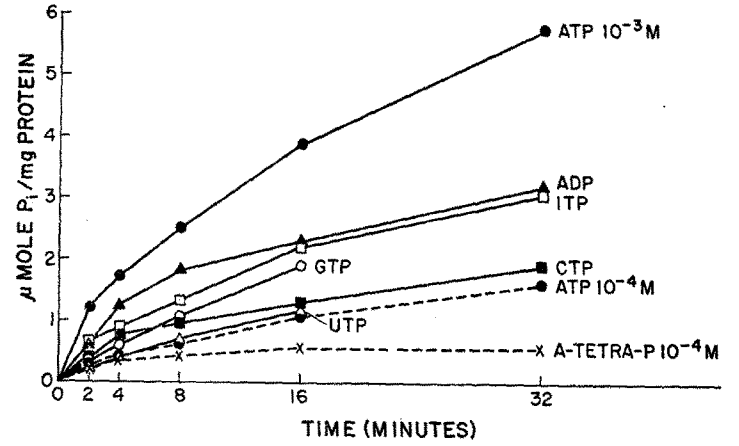

FIGURE 1*b*

FIGURE 1. (*a*) Effect of various nucleotides on Ca^{++} uptake. Nucleotide concentration was 10^{-3} M, except for ATetraP 10^{-4} M. (*b*) Splitting of various nucleotides by the sarcotubular nucleoside triphosphatase.

cumulation against those for the phosphate liberation. These estimated rates are not very accurate, but show that there were no gross deviations from a curvilinear dependence between the two phenomena except that ITP seems to be split somewhat faster. This would indicate that the limiting steps for Ca^{++} uptake and for NTP-splitting vary in the same sense when the absolute rates

of these processes are changed by the choice of different triphosphates. AtetraP had little activity in either respect; it was used at 10^{-4} M concentration to suppress the effect of the few per cent of admixed ATP, and corresponding curves for 10^{-4} M ATP are provided in Fig. 1 for comparison. On the other hand, ADP (Fig. 2) was rapidly split, but showed a considerably lower rate of Ca^{++} uptake than the other nucleotides.

We consider that the ADP splitting proceeds indirectly *via* the myokinase reaction, and suppose either that the ATP so formed is not readily available to the calcium pump mechanism, that the ADP acts as an inhibitor for this process, or that the high ADP/ATP ratio is energetically unfavorable.

No Ca^{++} accumulation was observed when inorganic phosphate, pyrophos-

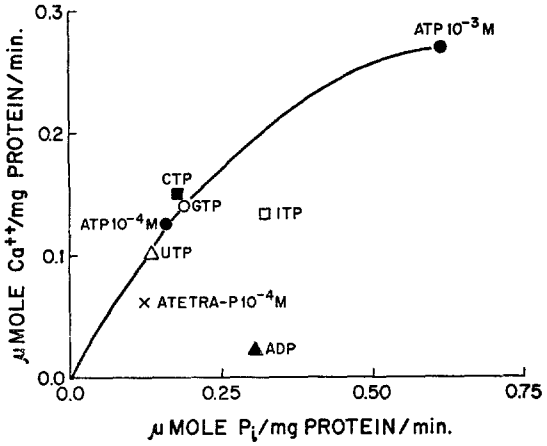

FIGURE 2. Ca^{++} uptake of sarcotubular granules plotted against splitting of nucleotide at 2 minutes' incubation.

phate, pyridoxalphosphate, cyclic adenosine-3′,5′-phosphate, or deoxy-ATP was substituted for the nucleotide phosphate.

Effect of Phosphate and Arsenate Addition of inorganic phosphate or of arsenate at 0.1 to 5×10^{-3} M concentration had no significant effect.

Effect of Cyclic Adenylate and of Caffeine These observations are grouped together, because caffeine is known to inhibit the diesterase which breaks down the cyclic nucleotide (26). Cyclic adenosine-3′,5′-phosphate was tested extensively and with some variation of the composition of the medium, without showing any effect upon the calcium pump. Caffeine, which inhibits the relaxation of glycerol-extracted fibers induced by microsomes (27), gave a small inhibition (Fig. 3). This is not likely to explain its effect upon the complete relaxation system, and the possibility that it might be variable and dependent on the condition of the particles would explain the previous negative finding (28) in this regard. Some indication was obtained that cyclic adenylate (6×10^{-7} M) overcame the caffeine inhibition.

Sulfhydryl Reagents Addition of PCMB inhibited Ca^{++} uptake completely and ATPase activity partially. The inhibition was overcome by cysteine (Fig. 4) in agreement with the inhibition by mersalyl (salyrgan) described by

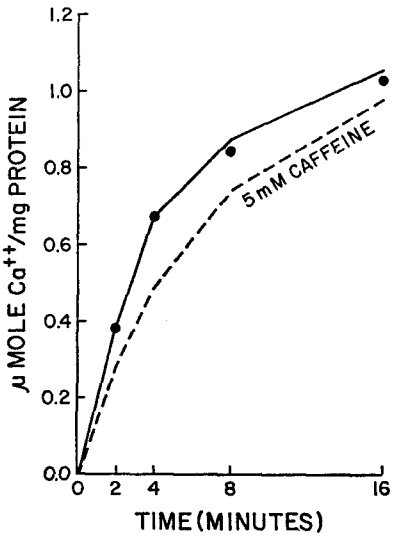

FIGURE 3. Effect of caffeine on Ca^{++} uptake of the sarcotubular system. Solid line, no addition; broken line, 5 mM caffeine added; filled circles, 5 mM caffeine + 6 × 10^{-7} M cyclic adenosine-3′, 5′-phosphate.

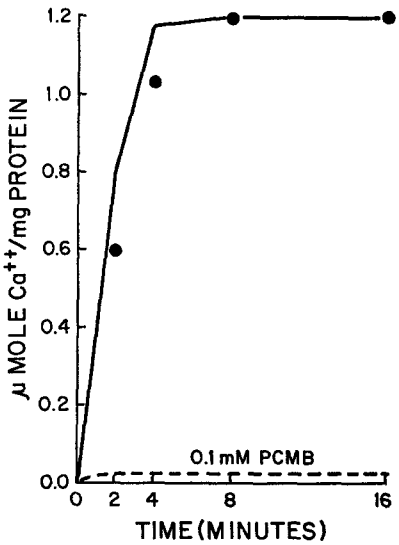

FIGURE 4. Inhibition of Ca^{++} uptake of the sarcotubular vesicles by PCMB. Solid line, no addition; broken line, 0.1 mM PCMB; filled circles, 0.1 mM PCMB + 5 mM cysteine.

Hasselbach and Makinose (1, 29). No effect was exerted by 0.5 or 2.0 mM iodoacetate, added to the microsomes 2 to 16 minutes before the assay. This is, of course, not in contradiction in view of the generally different reactivities of different sulfhydryl reagents. The latter observation is in keeping with the fact that the iodoacetate-poisoned frog muscle still contracts (30) in a way which

does not suggest any direct influence upon the excitation-contraction coupling mechanism.

Effects of Pyridoxalphosphate and Carnosine These are also presented together, in view of the observations (31) showing an inhibition of the contrac-

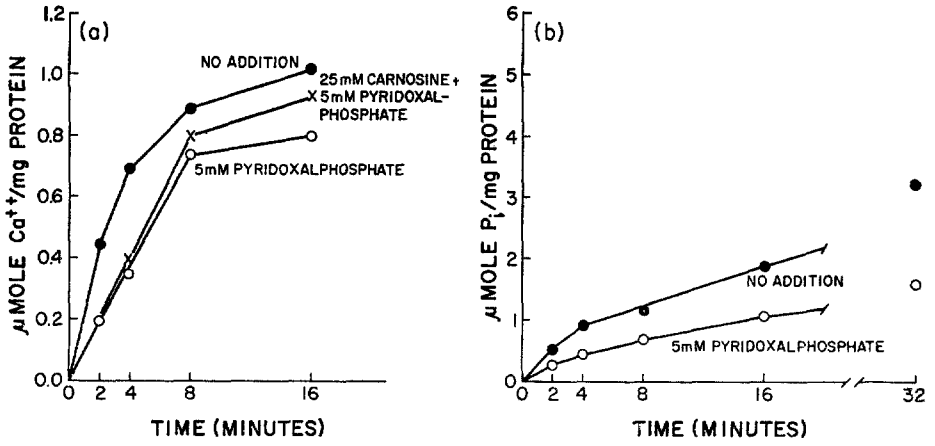

FIGURE 5. Effect of pyridoxalphosphate on (*a*) Ca^{++} uptake and (*b*) ATP splitting of the sarcotubular granules.

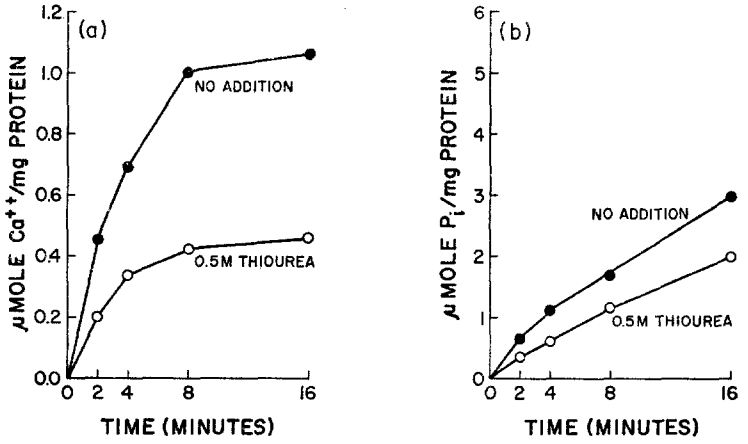

FIGURE 6. Effect of thiourea on (*a*) Ca^{++} uptake and (*b*) ATP splitting of the sarcotubular granules.

tion of myofibrils (microsome-containing?) by the former, and the reversal of this by the latter substance. Pyridoxalphosphate (5 mM, but not 1 mM) inhibited both the Ca^{++} accumulation and the ATPase activity of the microsomes (Fig. 5); thus, this effect is opposite to what would be required to explain its apparent relaxing activity. Carnosine (5 mM) had no effect, either alone or upon the system with pyridoxalphosphate. We also found no effect

of carnosine upon the relaxation of glycerol-extracted muscle fibers (31) brought about by a suspension of microsomes, while this was counteracted by 10^{-4} M Ca^{++}. At high concentration (25 mM), however, carnosine partially

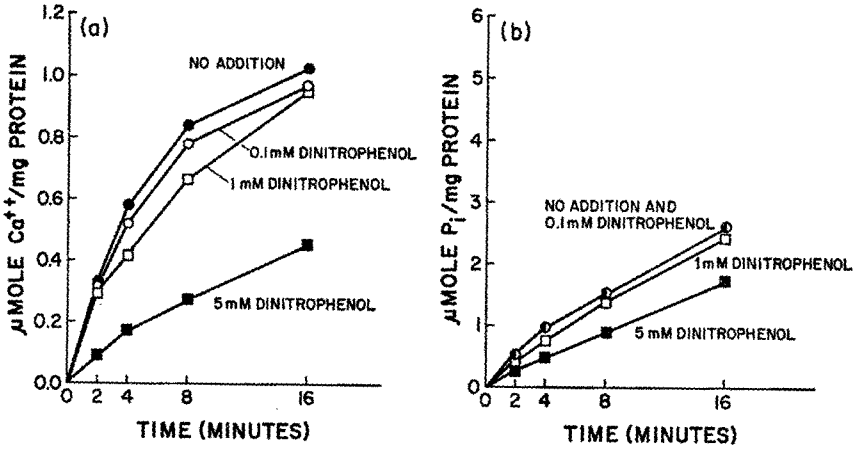

FIGURE 7. Inhibition by 2,4-dinitrophenol of (*a*) Ca^{++} uptake and (*b*) ATPase activity of the sarcotubular granules.

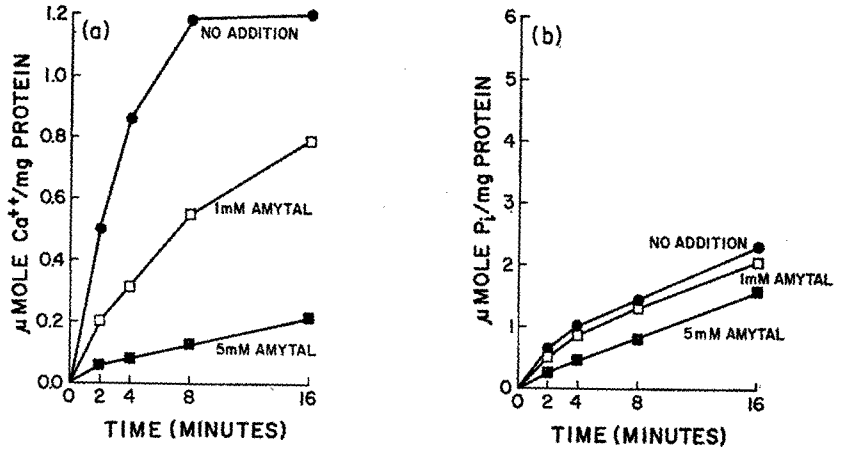

FIGURE 8. Inhibition by amytal of (*a*) Ca^{++} uptake and (*b*) ATPase activity of the sarcotubular granules.

counteracted the effect of pyridoxalphosphate and, when added alone, showed a small initial inhibition.

Effect of Thiourea This substance at 0.5 M concentration inhibited both the Ca^{++} accumulation and the ATP-splitting measurably (Fig. 6). Thiourea had previously been found to inhibit the actin-myosin interaction in skeletal muscle (32) and in the anterior byssus-retractor muscle of *Mytilus edulis* (33);

but not the Mg-activated particulate ATPase of the latter muscle. Our finding may apply to a Ca-activated ATPase only.

Reagents Effecting Oxidative Phosphorylation Dinitrophenol (Fig. 7) inhibited both the calcium pump and ATPase activity; the effects increased

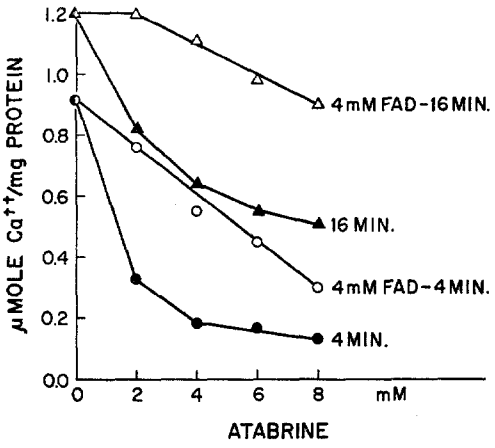

FIGURE 9. Ca^{++} uptake of the sarcotubular granules in the presence of various concentrations of atabrine with and without 4 mM FAD, measured after 4 and after 16 minutes.

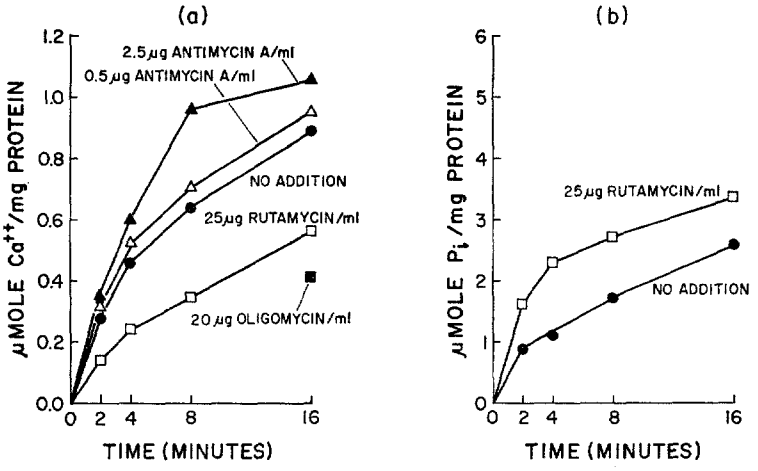

FIGURE 10. Effect of rutamycin, oligomycin, and antimycin A on (*a*) Ca^{++} uptake and (*b*) of rutamycin on ATP splitting of the sarcotubular granules. Since rutamycin, oligomycin, and antimycin A were dissolved in ethanol, the control flask received the same amount of ethanol (0.02 ml).

with increasing concentration of the reagent, no intermediary stimulation of the ATPase being noticed.

Amytal inhibited the calcium uptake strongly, the ATPase weakly (Fig. 8).

Atabrine inhibited the Ca^{++} uptake (Fig. 9); its effect upon ATPase was not established, because of its interference with the phosphate analysis. Its

effect was overcome by flavin-adenine dinucleotide, in keeping with the view that this substance forms a complex with atabrine (34).

Oligomycin in concentrations used to influence mitochondrial metabolism (0.5 to 2.5 μg per ml) had no effect, but in large amount (20 to 25 μg per ml, or 300 μg per mg microsomal protein) clearly inhibited Ca^{++} uptake (Fig. 10 *a*) while slightly stimulating ATPase Rutamycin showed the same behavior (Figs. 10 *a* and *b*), thus following the parallelism in the behavior of these two substances in oxidative phosphorylative systems.

Antimycin A, an inhibitor of oxidative phosphorylation and Ca^{++} uptake in mitochondria, stimulated the Ca^{++} uptake when applied in low concentrations (Fig. 10 *a*) without affecting the ATPase activity.

Ca^{++} uptake as well as ATP splitting proceeded at the same rate under nitrogen or helium as under exposure to air, and the inhibition by atabrine also was the same. FAD overcame this inhibition to the same extent as in air.

DISCUSSION

The uptake of Ca^{++} by sarcotubular vesicles has been looked upon in two ways: as a binding of Ca^{++} by a constituent of the particles (2) or as an active transport process (1). Under the conditions of our experiments, the latter process may be thought to dominate, hence we discuss our findings with emphasis on this interpretation.

The role of the calcium pump in the totality of the relaxation effects observed in muscle models will be considered first. In the latter case, full activity is limited to ATP and CTP (13), clearly different from the nucleotide specificity of calcium accumulation in which CTP, GTP, and ITP are almost as effective as ATP, and UTP not much less. The additional restriction in the complete relaxation factor system might be explained if the actomyosin were to contain NTPase activities preferentially removing GTP, ITP, and UTP (35) or also if the calcium requirement for contraction were to depend on the nucleotide employed. Preferential splitting of GTP, ITP, and UTP in the presence of Ca^{++}, but of ATP and CTP in the absence of Ca^{++}, has so far only been shown for a myosin NTPase. Ca- or Mg-activated actomyosin has been shown to preferentially split ATP > CTP > UTP > ITP > GTP (13). The NTPase of the granules studied here showed slightly different specificity. It is not clear whether the differences in question could be accounted for by a complete relaxation factor system containing myosin or actomyosin NTPases. Furthermore, the apparent relaxing effect of pyridoxalphosphate and its reversal by carnosine (31) are not explained by the action of these substances upon the calcium pump, as they work in the calcium pump in a direction opposite to that in the syneresis of myofibrils. The pharmacological properties of caffeine, considered to depend on whether it is applied to the outer surface of the cell membrane (36) or intracellularly, are not clearly explained by its

small influence upon calcium accumulation. Among the substances grouped together here as modifiers of oxidative phosphorylation, there are some instances of parallelism between their effects upon relaxation and upon calcium uptake; *i.e.*, the inhibition of relaxation by atabrine and amytal (14). It is not yet possible to state whether the combined characteristics of the sarcotubular calcium accumulation and of the calcium requirement of contractility will suffice to explain all phenomena of relaxation in model systems. But the differences in nucleotide specificity of the Ca^{++} accumulation and of the relaxing activity would argue against a complete identification of relaxation with calcium uptake, in line with the demonstration (5, 6) that there is a relaxing substance independent of the transport of calcium.

Comparing the NTP specificities for both the calcium accumulation and the NTPase activity of the granules, it is found that these activities vary in a similar fashion in the sequence AtetraP < UTP < CTP, GTP, ITP < ATP, only ITP being split somewhat more rapidly relative to this order. ADP takes an abnormal position as described. A detailed discussion of this relation would require complete kinetic investigations under a variety of nucleotide and ion concentrations. The available data (Fig. 2) certainly support the assumed connection between calcium movement and NTPases (1, 4). If interpreted in terms of the formation of a high energy intermediate utilized for calcium transport (29) (see below), the curvilinear relation in Fig. 2 would suggest that the availability of this intermediate is relatively more favorable at a lower over-all rate of ATP splitting.

Further considerations regarding the coupling between the two processes will result from comparisons with transport-ATPases and with the calcium uptake by mitochondria. It must be stressed that our observations do not depend on admixed mitochondrial fragments; these can be removed as shown by the absence of cytochrome oxidase (17). Conversely, the differences also indicate that the Ca^{++} uptake by mitochondria is not due to admixed reticular fragments. With respect to the action of modifiers, our system behaved rather differently from mitochondrial systems. Dinitrophenol (37, 38) inhibited both biochemical activities in our case, in contrast to its stimulation of the ATPase of myosin (35) and of mitochondrial ATPase (39–41), but in agreement with its inhibition of mitochondrial calcium uptake (42, 43). Amytal interfered with calcium uptake in our case as it does with mitochondria (42) and inhibited the sarcotubular ATPase. Atabrine, known to inhibit mitochondrial (41, 44), myofibrillar (45), and sarcotubular (14) ATPases, clearly inhibited the Ca^{++} uptake of the granules. Oligomycin (0.5 to 3.0 μg per ml) inhibits mitochondrial ATPase (46) and calcium transport in the absence of substrate (47); in our case, calcium uptake was inhibited and ATPase slightly stimulated, but this required tenfold higher concentrations of oligomycin and rutamycin, similar to those causing inhibition of a particulate ATPase of brain (24, 48).

And, in notable contrast, antimycin A, which is an inhibitor of mitochondrial oxidative phosphorylation (46) and of calcium accumulation (42, 43), markedly stimulated calcium uptake in our case, without effect upon ATPase.

Thus the differences outweight the similarities. Yet one is tempted to discuss mitochondrial oxidative phosphorylation and sarcotubular calcium transport in the same connection, because there may be common mechanisms. In the case of atabrine, its effect in connection with relaxation phenomena (14) was actually ascribed to its action upon a flavoprotein-catalyzed oxidative step. This proposal is questionable as it has been shown (34) that flavin nucleotide directly reacts with atabrine, and thus counteracts the inhibition. Although the calcium pump seems to be able to function without oxygen, the various substances studied here may well exert their effects preferentially upon phosphorylations associated in mitochondria with specific sites of the oxidative chain. We refer to current discussions about the several partial reactions associated with each of these oxidation sites (37, 38, 49–53), and propose that in the sarcotubular system, too, ATP stands in relation to high energy intermediates A $\sim$ C or C $\sim$ P (54, 55) the utilization of which is directly connected with the calcium accumulation or other transport phenomena (47, 51, 56). Since these partial reactions may differ quantitatively in the several sites, a wide variety of effects exerted by various modifiers becomes possible.

Despite the intense efforts by leading investigators, there is no unanimity in formulating a reaction equation for oxidative phosphorylation, much less for mitochondrial calcium transport. We will be justified, therefore, in keeping this first investigation along such lines on the sarcotubular system free of detailed theoretical proposals. However, the occurrence of common mechanisms in mitochondrial and sarcotubular reactions may have a deeper reason. We consider that mitochondria and the sarcotubular system, and transport-ATPases for that matter, may be cytogenetically related. They may originate at some stage from a primitive surface membrane of the cell (57); ordinarily, they will specialize towards different structures and physiological functions, but may retain some common biochemical mechanisms.

Note After preparation of this manuscript a paper appeared (58) which touches upon some of the same topics as our investigation, but with some different results. In several instances the authors determined end-states rather than velocities of calcium uptake. Thus they may not have seen the different rates with different triphosphates. Other results such as the activity of deoxy-ATP do not seem to be explainable by this. Perhaps their main sarcotubular fraction, obtained by centrifugation between 8000 and 28,000 $\times$ *g*, contained a larger amount of mitochondrial material than ours, obtained between 12,000 and 40,000 $\times$ *g*.

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