Relation of lipid structure of sarcotubular vesicles to Ca^{++} transport activity

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ABSTRACT The role of lipids of the sarcotubular membranes in their Ca^{++} uptake and Mg–ATPase activities was investigated. Treatment of the membranes with phospholipase C inhibits both processes. Treatment with phospholipase A and phospholipase D, which results in massive hydrolysis of the sarcotubular phospholipids, does not inhibit either the Ca^{++} uptake or the Mg–ATPase activities, nor does treatment with the polyene antibiotics affect these processes. Essential fatty acid deficiency alters sarcotubular membrane lipids; they contain much less stearic, linoleic, and arachidonic acids and much more oleic and eicosatrienoic acids than normally, but do not lose the ability to actively sequester Ca^{++}. It is concluded that neither nonpolar lipids nor the nonpolar regions of polar lipids are involved in Ca^{++} sequestering and Mg–ATPase activities of the sarcotubular membranes.

Of the polar components, the phosphoryl moiety of the phospholipids is required for both activities. However, the phosphoryl group appears to be required for the maintenance of the membranous structure necessary for Ca^{++} sequestration rather than serving specifically in the active transport process. That treatment with phospholipase D, which results in the conversion of much of the sarcotubular phospholipid from a dipolar to an anionic structure, does not affect Ca^{++} uptake activity is a most remarkable finding.

KEY WORDS sarcotubular membranes · active Ca^{++} transport · Mg–ATPase · phospholipase C · phospholipase A · phospholipase D · polyene antibiotics · essential fatty acid deficiency · phospholipids · cholesterol · electron micrographs

IT HAS BEEN SUGGESTED that in excitation–contraction coupling of skeletal muscle the sarcoplasmic reticulum releases Ca^{++}, in response to the action potential, to the myoplasm where it promotes interaction between actin and myosin (1). The sarcoplasmic reticulum is also thought to be involved in muscle relaxation (1) by virtue of the transport of Ca^{++} from the myoplasm into its internal structure, whereby the $[Ca^{++}]$ of the myoplasm is lowered to the point where relaxation of the myofibrils occurs.

When membrane fragments of sarcoplasmic reticulum are isolated from muscle homogenates, they vigorously sequester Ca^{++} in vitro if ATP is available (2). They also have ATPase activity, which is thought to serve in the energy-requiring Ca^{++} sequestration process (2). Such preparations have been used extensively to study the molecular mechanisms involved in Ca^{++} uptake by sarcoplasmic reticulum.

While there is evidence that phospholipids are involved in both the calcium-uptake and the ATPase activities of sarcotubular membranes of skeletal muscle, most of the research on the role of phospholipids was done with crude microsomal fractions that contained not only sarcotubular membranes but other structures, e.g. ribosomes, lysosomes, and considerable amounts of electron-opaque granules of unknown cytologic nature. In 1950 Kielley and Meyerhof (3) inhibited the ATPase activity by treating muscle microsomes with phospholipase C, and they further showed that this inhibition could not be explained by the accumulation of either diglyceride or phosphoryl choline, the products of the phospholipase C reaction with lecithin. Since the calcium-sequestering activity of muscle microsomes is now known to result in relaxing activity, the report of Ebashi (4) in 1958 that phospholipase C treatment inhibits this relaxing activity indicates the involvement of phospholipids in the calcium uptake process. That such is the case has recently been verified by the work of Martonosi (5, 6) in which the hydrolysis of muscle microsomal lecithin by phospholipase C was shown to cause an inhibition of both the ATPase and calcium transport activities. Moreover, Martonosi found that

Abbreviation: FFA, free fatty acid(s).

both the ATPase and calcium transport activities of phospholipase C-treated microsomes could be reactivated by the addition of phospholipids in ultrasonic dispersions.

The role of lipids in the active transport of calcium by sarcotubular membranes has been further investigated in our laboratory. Our studies involved purified sarcotubular membranes rather than crude microsomes and were designed to provide information on the structural components of sarcotubular membrane lipids involved in the calcium uptake process. The results of these studies are presented in this communication.

METHODS

Isolation of Sarcotubular Membranes

Adult male rats (Charles River strain) are decapitated and skeletal muscles from the legs and back are immediately excised, minced with scissors, and blended in 0.3 M sucrose (1:3 w/v) at 2°C for 30 sec in a Waring Blendor at full speed. Microsomes are isolated from the homogenate by usual differential centrifugation procedures. The microsomes are subfractionated by the sequential use of two sucrose density gradient centrifugation systems (7). One of the subfractions contains sarcotubular membranes that appear to be as free from contamination by other cellular components as the sarcotubular membranes obtained from rabbit skeletal muscle by the method of Seraydarian and Mommaerts (8). This subfraction, designated *sarcotubular membranes*, was the only fraction used in the experiments reported below.

Measurement of Ca^{++} Uptake by Sarcotubular Membranes

The Ca^{++} uptake activity of the sarcotubular membranes is measured in the incubation system developed by Carsten and Mommaerts (9) in which the Ca^{++} is labeled with $^{45}Ca^{++}$. The sarcotubular vesicles (approximately 100 μg of sarcotubular protein) are suspended in 4 ml of incubation medium in 50-ml Erlenmeyer flasks and incubated with shaking at 37°C. All glassware in the incubation and analysis procedures is siliconized. At various time intervals, aliquots are removed; the reaction is terminated and the Ca^{++} sequestered measured by the Millipore filtration method of Martonosi and Feretos (10). In brief, a 0.5 ml suspension of sarcotubular vesicles is rapidly filtered through HA 0.45 μ or GS 0.22 μ Millipore filters 25 mm in diameter (Millipore Corp., Bedford, Mass.), and the filtrate collected. The ^{45}Ca content of the filtrate is assayed by adding 0.1 ml of aqueous sample plus 0.5 ml of 0.11 N NaOH to 15 ml of a liquid scintillation solution and counting in a Packard Tricarb liquid scintillation spectrometer. The liquid scintillation solution is prepared as follows: 7 g of 2,5-diphenyloxazole, 0.3 g of *p*-bis[2-(5-phenyloxazolyl)]benzene, 100 g of naphthalene, and 40 g of Cab-O-Sil are placed in a glass-stoppered 2 liter flask to which 1 liter of dioxane is added. The bottle is vigorously shaken for 30 min; the resultant suspension is stable for weeks.

Measurement of Mg–ATPase Activity of Sarcotubular Membranes

A 2 ml system containing 20 mM Tris–maleate (pH 7.2), 3 mM $MgCl_2$, 3 mM ATP, and sarcotubular membranes containing 50 μg of protein is incubated for 20 min at 37°C; the reaction is stopped by the addition of 0.2 ml of 50% trichloroacetic acid. The P_i (inorganic phosphate) liberated is measured colorimetrically by the method of Fiske and Subbarow (11).

Assay of Sarcotubular Protein

The protein contained in an aliquot of the sarcotubular membrane suspension is solubilized by adding deoxycholate (0.16% final concentration) and heating on a steam bath. The protein concentration is then measured colorimetrically by the method of Lowry, Rosebrough, Farr, and Randall (12).

Treatment of Sarcotubular Membranes with Phospholipase C

The sarcotubular membranes (10.26 mg of protein) are suspended in 10 ml of Tris–maleate buffer (20 mM) in 0.3 M sucrose (pH 7.2) to which 3.42 mg of phospholipase C (from *Clostridium welchii* purchased from Sigma Chemical Co.) is added. A large amount of phospholipase C is necessary because the treatment is carried out in the absence of Ca^{++}. A suspension of sarcotubular membranes without added phospholipase C is processed simultaneously and serves as the control. Both are incubated for 60 min at room temperature; each is mixed periodically. At 0, 20, 40, and 60 min, aliquots are removed for the measurement of Ca^{++} uptake activity, Mg–ATPase activity, and the amount of water-soluble organic phosphate released by the action of phospholipase C. The latter is determined by filtering the aliquots through a Millipore filter and assaying the filtrate by the method of Baginski, Foa, and Zak (13).

Treatment of Sarcotubular Membranes with Phospholipase A

The sarcotubular membranes (6.38 mg) are suspended in 6 ml of Tris–maleate buffer (20 mM, pH 7.2) in 0.3 M sucrose containing 2% albumin (fatty acid-poor) to

which 6.38 mg of phospholipase A (purified grade from *Crotalus adamanteus* venom purchased from Koch–Light Laboratories, Ltd., Colnbrook, Bucks., England) is added. A large amount of phospholipase A is necessary because the treatment is carried out in the absence of Ca^{++}. A suspension of sarcotubular membranes without added phospholipase A is processed simultaneously and serves as the control. Both are incubated for 120 min at room temperature; each is mixed periodically. At 0, 30, 60, 90, and 120 min aliquots are removed for the measurement of Ca^{++} uptake activity, Mg–ATPase activity, and the amount of FFA released by the action of phospholipase A.

The amount of FFA released to the medium is determined by filtering the suspension through a Millipore filter and assaying the filtrate for FFA as follows. FFA are extracted from the filtrate by the method of Trout, Estes, and Friedberg (14). From the heptane phase, 3-ml aliquots are removed and added to 15 ml of isopropanol for assay of the FFA by a modification of the titrimetric method of Meester and Schut (15). The Radiometer TTT-1C titrator, ABO1B autoburette, and TTA4 photometric titration assembly are used. To each FFA sample, 0.05 ml of indicator solution [0.08% 1-(2-hydroxybenzene)-azo-2-naphthol in isopropanol] is added and titration is carried out under a constant flow of pure N_2 bubbled through KOH; the titrant used is 0.02 N NaOH. When the endpoint is reached, the titrator automatically stops delivering titrant and the volume of NaOH used is read directly from the digital read-out. Heptane solutions containing known amounts of palmitic acid are assayed in a similar manner and serve as the basis for all calculations.

Treatment of Sarcotubular Membranes with Phospholipase D

The sarcotubular membranes (12.65 mg) are suspended in 10 ml of Tris–maleate buffer (20 mM, pH 7.2) in 0.3 M sucrose to which 12.65 mg of phospholipase D (purified grade from cabbage from Koch–Light Laboratories) is added. A large amount of phospholipase D is necessary because the treatment is carried out in the absence of Ca^{++}. A suspension of sarcotubular membranes without added phospholipase D is processed simultaneously. Both are incubated for 120 min at room temperature; each is mixed periodically. At 0, 30, 60, 90, and 120 min, aliquots are removed for the measurement of Ca^{++} uptake activity, Mg-ATPase activity, and the amount of choline released by the action of phospholipase D. The amount of choline released to the medium is determined by filtering the aliquots through a Millipore filter and assaying the filtrate for choline by the method of Hack (16).

Treatment of Sarcotubular Membranes with Polyene Antibiotics

Nystatin was supplied by the Squibb Institute for Medical Research, New Brunswick, N. J., filipin by The Upjohn Co., Kalamazoo, Mich., and pimaricin by Lederle Laboratories Div., American Cyanamid Co., Princeton, N. J. To a 10 ml system containing Tris–maleate buffer (20 mM, pH 7.2) in 0.3 M sucrose is added: (a) 10 μmoles of nystatin and sarcotubular membranes (11.04 mg of protein); or (*b*) 5 μmoles of filipin and sarcotubular membranes (10.5 mg of protein); or (*c*) 10 μmoles of pimaricin and sarcotubular membranes (10.5 mg of protein). A suspension of sarcotubular membranes without antibiotics is processed simultaneously. These systems are incubated for 90 min at room temperature; each is mixed periodically. At 0, 30, 60, and 90 min, aliquots are removed for the measurement of Ca^{++} uptake activity, Mg–ATPase activity, and when possible the amount of antibiotic bound to the sarcotubular membranes. The amount of filipin bound is determined by filtering the aliquots through a Millipore filter and assaying the filtrate for absorbancy at 338 mμ.[1] Spectrophotometric methods for estimating the extent of binding to the other two antibiotics are not available.

Phospholipid and Phosphatidyl Chlorine Content of Sarcotubular Membranes

An aliquot of sarcotubular membrane suspension is lyophilized and the dry pellet extracted with $CHCl_3$–CH_3OH 2:1; approximately 10 ml of solvent is used per g (dry weight) of pellet. The pellet and solvent are ground together in a Potter–Elvehjem homogenizer, transferred to a centrifuge tube, and centrifuged. The supernatant fraction is collected and the pellet is extracted twice more (including grinding) with $CHCl_3$–CH_3OH 2:1. The lipid extract is washed to free it of nonlipid phosphorus (17). The lipid phosphorus content of an aliquot of the washed-lipid extract is determined by previously described techniques (17). With another aliquot, choline-containing phospholipids are separated from other lipids by silicic acid column chromatography (17), and the amount of phosphorus in these phospholipids is measured. The sarcotubular membranes of four preparations contained per mg of protein 0.608–0.616 μeq of lipid P (average 0.611 μeq) and 0.425–0.472 μeq of choline-containing lipid P (average 0.432 μeq).

Fatty Acid Composition of Lipid Esters of Sarcotubular Membranes

The lipids are extracted from the sarcotubular mem-

[1] Personal communication for method of quantitative analysis of filipin from G. B. Whitfield, Jr., of the Upjohn Company.

branes as described above. The fatty acid moieties of the lipid esters are converted to methyl esters of fatty acids by the method of Kuchmak and Dugan (18). The composition of the methyl esters is determined by gas–liquid chromatography in a Packard gas–liquid chromatograph model 7839 equipped with an argon ionization detector. The column is 20% diethylene glycol adipate polyester plus 3% H_3PO_4 on 60–80 mesh Chromosorb W and the system is run isothermally at 185°C. Fatty acid methyl esters used as standards were purchased from Applied Science Laboratories Inc., State College, Pa. The amount of a fatty acid methyl ester present was determined by planimetry of the peaks traced.

Preparation of Essential Fatty Acid-Deficient Rats

Weanling male rats (Charles River strain) were divided into two groups. Group 1 was fed the following synthetic diet: 70% glucose, 18% casein, 8% corn oil, 4% salt, and vitamin mix (including the fat-soluble vitamins). Group II was fed the same synthetic diet except that the corn oil was omitted and glucose made up 78% of the diet. The diet was fed for 3–6 months, the rats in Group II being killed when their general appearance was that of an essential fatty acid-deficient rat. At the time of sacrifice the 15 rats in Group I weighed 350 g on the average and the 35 rats in Group II weighed 170 g.

RESULTS

The time course of Ca^{++} uptake by the sarcotubular membranes is presented in Fig. 1. By 4 min the uptake reaches a maximum and after 8 min or so there is some loss of the sequestered Ca^{++} to the medium.

The effect of phospholipase C treatment on Ca^{++} uptake by sarcotubular membranes is shown in Fig. 2. The loss in ability to take up Ca^{++} correlates with the amount of sarcotubular phospholipid hydrolyzed. There is decreased uptake when 55% of the phospholipid has been hydrolyzed and almost no uptake at all when about 75% of the phospholipid has been hydrolyzed.

Phospholipase C treatment also inhibits the Mg–ATPase activity of sarcotubular membranes (Fig. 2). The extent of depression correlates with the amount of phospholipid that has been hydrolyzed, with almost no activity remaining in sarcotubular membranes in which 75% of the phospholipid has been hydrolyzed.

Fig. 3 shows electron micrographs of untreated sarcotubular membranes (A) and of phospholipase C-treated membranes (B) that lost 75% of their phospholipids. The untreated preparation primarily contains irregular, large membranous vesicles. The phospholipase C-treated vesicles exhibit the following alterations: they

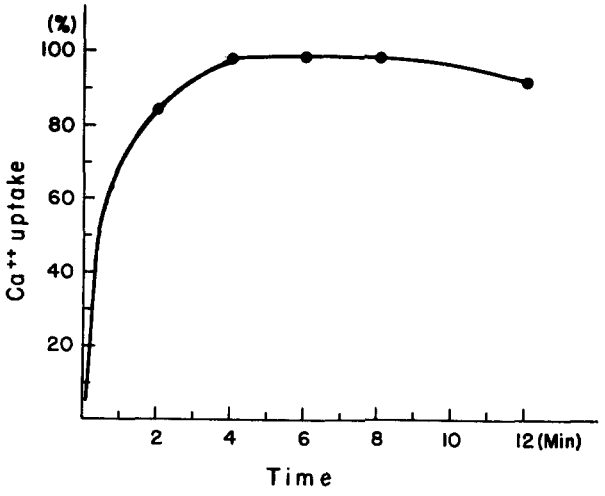

FIG. 1. Time course of Ca^{++} uptake by sarcotubular membranes. The system contains 150 μg of sarcotubular proteins and 4 μmoles of Ca^{++}. The Y axis refers to percentage of the 4 μmoles of Ca^{++} taken up by the sarcotubular membranes. The results are from a typical experiment taken from more than 50 such experiments.

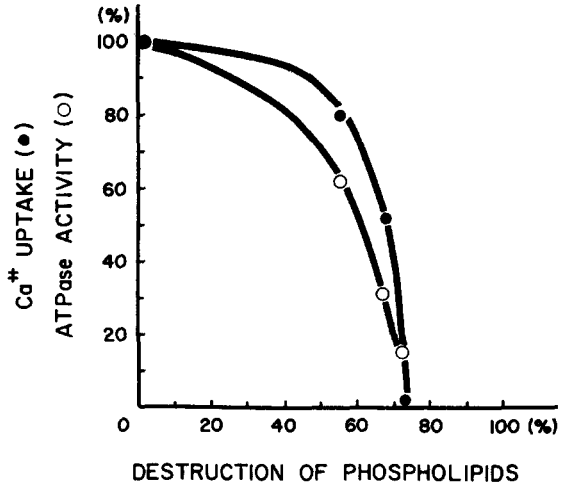

FIG. 2. Effect of phospholipase C on Ca^{++} uptake and Mg–ATPase of sarcotubular membranes. The Y axis refers either to the maximum uptake of Ca^{++} (closed circles) by the phospholipase-treated sarcotubular membranes as a percentage of the maximal uptake observed with untreated sarcotubular membranes processed identically, or to the Mg–ATPase (open circles) expressed in the same way. Each point for Ca^{++} uptake was obtained by time-course experiments like that described in Fig. 1. The X axis refers to the percentage hydrolysis of phospholipid based on a phospholipid/protein ratio of 0.611 μeq of lipid P/mg of protein. The experiment reported is typical of the six experiments carried out. A seventh experiment was done with commercial phospholipase C that was purified by the method of Ikezawa, Yamamoto, and Murata (19), which increased the specific activity 3-fold; the results with this enzyme preparation were similar to those in the figure.

are smaller, are more regularly ovoid, have thinner walls, and tend to aggregate more than untreated vesicles.

Treatment with phospholipase A causes little loss in the amount of Ca^{++} uptake by sarcotubular membranes (Fig. 4). The time course of uptake (not shown in

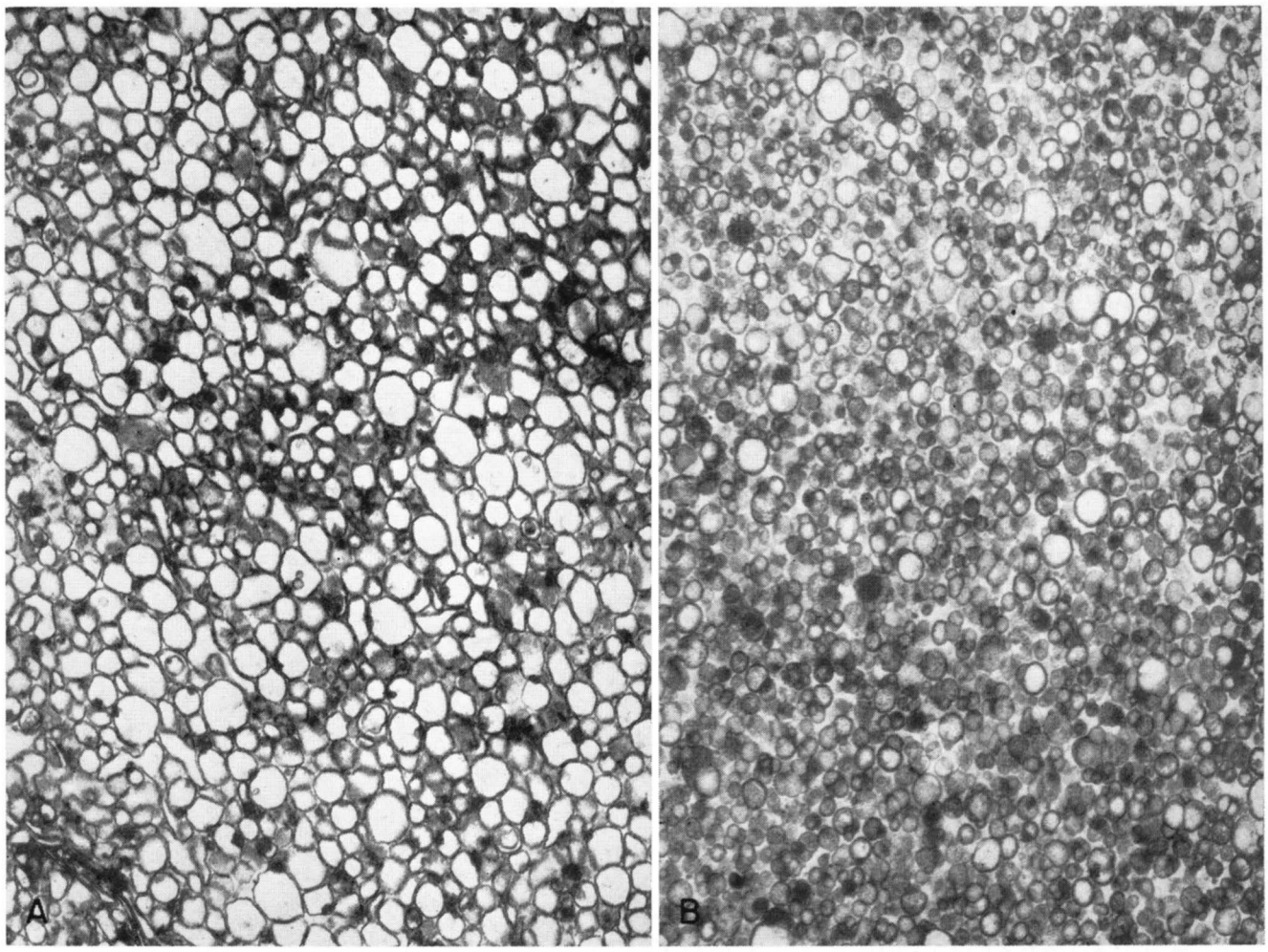

FIG. 3. Electron micrographs of untreated (A) and phospholipase C-treated (B) sarcotubular membranes. The pellet containing the sarcotubular preparation was fixed with 1% OsO_4 in 0.067 M collidine buffer, pH 7.0, embedded in Epon 812, and stained with lead citrate. ×32,000.

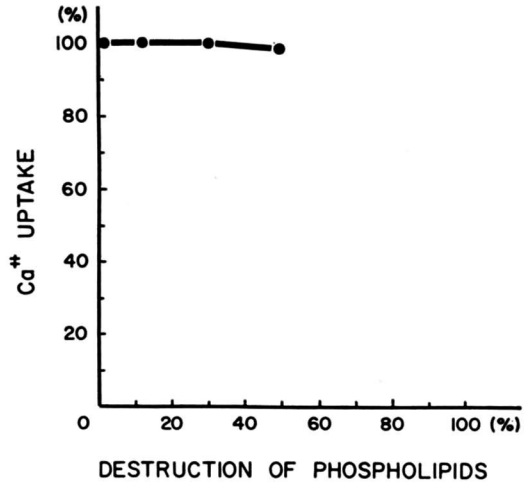

FIG. 4. Effect of phospholipase A on Ca^{++} uptake by sarcotubular membranes. For explanation of Y and X axes see Fig. 2. The experiment reported is typical of six experiments carried out.

Fig. 4) is not changed by phospholipase A treatment, which indicates that the rate of uptake is not affected. The small decrease in Ca^{++} uptake observed with sarcotubular membranes in which half of the phospholipid has undergone hydrolysis is probably due to the FFA liberated during the phospholipase A treatment. It has been shown that FFA are potent inhibitors of sarcotubular Ca^{++} pump activity (20). The data reported in Fig. 4 are from experiments in which the medium contained 2% albumin (fatty acid-poor); when sarcotubular membranes are treated with phospholipase A in the absence of albumin, there is a marked loss in Ca^{++} uptake activity.

In the presence of albumin, phospholipase A does not affect sarcotubular Mg–ATPase activity; in its absence, there is a small but significant increase in the Mg–ATPase activity.

Fig. 5 shows that phospholipase D has no effect on

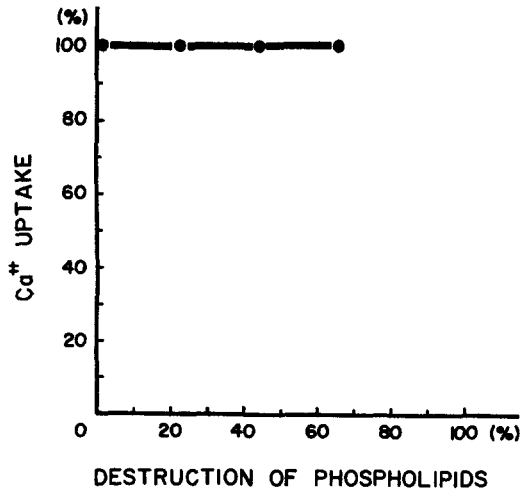

FIG. 5. Effect of phospholipase D on Ca^{++} uptake by sarcotubular membranes. For explanation of Y axis see Fig. 2. The X axis refers to percentage hydrolysis of choline-containing phospholipids based on a choline-containing phospholipid/protein ratio of 0.43 μmole of choline-containing phospholipid/mg of protein. The experiments reported is typical of the six experiments carried out.

sarcotubular Ca^{++} uptake even if almost 70% of the choline-containing phospholipids have undergone hydrolysis. Phospholipase D treatment causes a small but significant increase in Mg–ATPase activity.[2]

Treatment of vesicles with phospholipase A resulting in a 50% removal of the fatty acid at the β-position of the sarcotubular phospholipids causes changes in the ultrastructure similar to those noted with phospholipase C but not as marked (Fig. 6A). Treatment with phospholipase D resulting in 70% loss of choline from the choline-containing phospholipids of the sarcotubular vesicles causes less change in the ultrastructure of the vesicles than phospholipase A (Fig. 6B).

Since the polyene antibiotics are known to interact with sterols of biological membranes, causing reorientation of membrane lipids (22), their effect on Ca^{++} uptake by sarcotubular membranes was studied. Although the sarcotubular membranes removed more than 50% of the filipin added to the incubation medium (~0.25 μmole of filipin per mg of sarcotubular protein), neither the ability to take up Ca^{++} nor the Mg–ATPase activity was affected (Fig. 7). Treatment with nystatin and pimaricin also failed to have any affect.

Data from analysis of the fatty acid composition of the lipids of sarcotubular membranes of normal and essential fatty acid-deficient rats are presented in Table 1. The

[2] Since this manuscript was submitted for publication a paper by Martonosi, Donley, and Halpin (21) has appeared in which the treatment of muscle microsomes with phospholipase D was studied. They also found that Ca^{++} uptake was not inhibited by phospholipase D treatment and that ATPase activity was somewhat increased.

TABLE 1 EFFECT OF ESSENTIAL FATTY ACID DEFICIENCY ON FATTY ACID COMPOSITION OF SARCOPLASMIC RETICULUM

Fatty Acid	Control	EFA-Def.
	moles % ± SD	
16:0	31 ± 1.2	28 ± 2.0
16:1	9 ± 1.6	13 ± 1.9
18:0	19 ± 0.8	5 ± 0.5
18:1	4 ± 1.1	39 ± 2.3
18:2	18 ± 1.7	1 ± 0.9
20:3	1 ± 0.6	10 ± 1.8
20:4	10 ± 2.1	2 ± 0.8

Lipids were extracted from sarcotubular vesicles pooled from two to five rats; five such pools were analyzed both for control rats and for essential fatty acid-deficient (EFA-Def.) rats. Fatty acids are designated by *number of carbon atoms:number of* carbon bonds.

sarcotubular lipids of essential fatty acid-deficient rats contain much less stearic, linoleic, and arachidonic acids and much more oleic and eicosatrienoic acids than do sarcotubular lipids from normal rats. The amount of Ca^{++} taken up by sarcotubular membranes is not affected by essential fatty acid deficiency; the rate of uptake is certainly not decreased and may be increased (Fig. 8).

DISCUSSION

In the present investigation, the findings of Kielley and Meyerhof (3) and of Martonosi (5) that phospholipase C treatment inhibits both calcium uptake and ATPase activities of crude muscle microsomes were confirmed with highly purified sarcotubular membranes. Kielly and Meyerhof and Martonosi presented evidence that the hydrolysis products are not responsible for these inhibitory effects. Moreover, Martonosi found that if the phospholipase C-treated microsomes were incubated with micellar phospholipid, calcium uptake and ATPase activities could be fully restored. Such data strongly suggest that the loss of phospholipid structure, rather than the action of an inhibitory product, is responsible for the loss of these activities. The way in which phospholipids function biochemically in the calcium sequestration process, however, was not delineated. It is interesting that in the case of the purified sarcotubular membranes, hydrolysis of 55% of the phospholipid by the catalytic action of phospholipase C causes only a modest fall in capacity to take up Ca^{++}. In contrast, when phospholipase C causes a chemically undetectable destruction of phospholipid in the renal tubules, there is a pronounced reduction in Rb^{+} uptake (23). That a 55% loss of phospholipid is required to cause a significant decrease in Ca^{++} uptake activity indicates either (*a*) that a large amount of other phospholipid must first be hydrolyzed before phospholipid structures specifically linked to the uptake process become

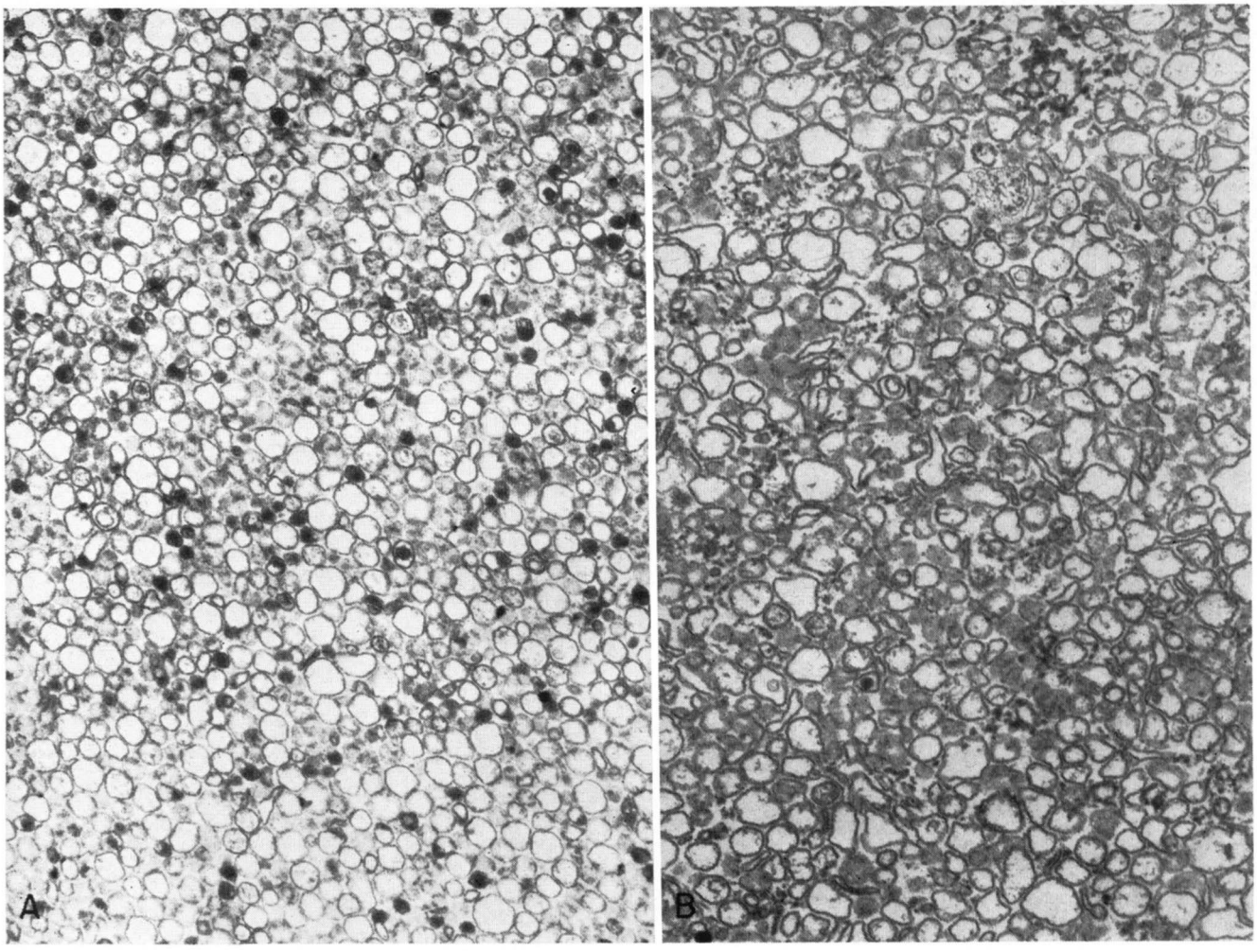

FIG. 6. Electron micrographs of phospholipase A- (A) and phospholipase D-treated (B) sarcotubular membranes. The preparation procedure was the same as for Fig. 3. ×32,000.

accessible to the enzyme; or (*b*) that phospholipids do not function specifically in the Ca^{++} sequestration process but rather are important in maintaining the vesicular structure of sarcotubular membranes; or (*c*) that after much membrane phospholipid is destroyed a Ca^{++} binding protein is released from the membrane. However, the loss of the Mg–ATPase activity must relate to inactivation of the enzyme since the assay used measures both membrane-bound and soluble Mg–ATPase.

More striking than the loss of calcium-sequestering activity caused by the action of phospholipase C on the sarcotubular fragments is the total lack of effect noted with phospholipase A, phospholipase D, essential fatty acid deficiency, and the polyene antibiotics. Essential fatty acid deficiency totally changes the spectrum of fatty acids (Table 1) in the sarcotubular membrane without significantly influencing Ca^{++} uptake activity. When phospholipase A treatment is carried to the point at which approximately one-half the fatty acid at the β-position has been removed from the phospholipid structure, it still does not affect the Ca^{++} uptake process. From these results it would seem that the nonpolar structures of the phospholipids have no role in calcium uptake by sarcotubular membranes. Apparently these marked alterations in the nonpolar structure of the phospholipids do not even influence the conformation of the molecular structures of the membrane sufficiently to alter either the ATPase activity or Ca^{++} sequestration. Moreover the amount of filipin taken up by the sarcotubular membranes (Fig. 7) is sufficient to interact with every cholesterol molecule in the membrane. Such a marked interaction of the cholesterol in the membrane with the polyene antibiotics should lead to a reorientation of the relationship of cholesterol with other membrane structures. Yet surprisingly, the polyene antibiotics have no effect on the calcium sequestration and Mg–ATPase activities. While phospholipase D has no influence on the nonpolar structure of lipids it markedly alters the polar regions of the phospholipids. However, treatment with phospholipase D sufficient to cause a 70% loss of the

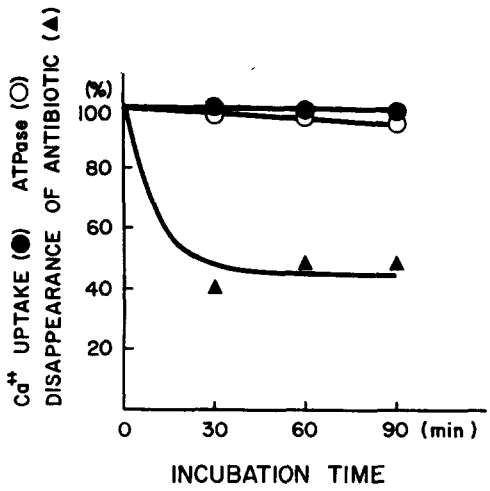

FIG. 7. Effect of filipin on Ca^{++} uptake by sarcotubular membranes. When the Y axis refers either to maximum uptake of Ca^{++} by filipin-treated sarcotubular membranes in terms of percentage of the maximal uptake observed with untreated sarcotubular membranes processed identically (closed circles); to Mg–ATPase activity expressed in the same way (open circles); or to the percentage of the added filipin taken up by the sarcotubular membranes (solid triangles). The X axis refers to the time of incubation with filipin. The experiment reported is typical of the four experiments carried out.

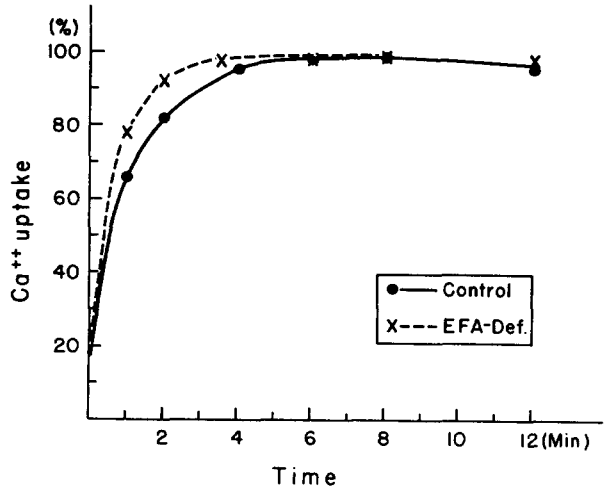

FIG. 8. Comparison of the uptake of Ca^{++} by sarcotubular membranes from normal and essential fatty acid-deficient (EFA-Def.) rats. The system is the same as described in Fig. 1. Closed circles designate "normal" sarcotubular membranes, and X's designate "essential fatty acid-deficient" sarcotubular membranes. The sarcotubular membranes assayed were obtained from pools of sarcotubular vesicles isolated from 2–5 rats. Five such pools were prepared from the essential fatty acid-deficient rats and the normal rats. The results reported are from one of these five pools and represent typical data.

choline from the choline-containing phospholipids (the major type of phospholipid present in the membrane) resulted in no loss of Ca^{++} sequestration activity, which indicates that changing the polar structure of the phospholipids of the sarcotubular membranes from a dipolar to an anionic form does not involve a change in the Ca^{++} sequestration process, a most surprising finding.

The conclusion drawn, therefore, is that most of the lipid structure is not significantly involved in the Ca^{++} sequestration and Mg–ATPase activities of sarcotubular membranes. Indeed, the only lipid structure that seems to be related in any way to these processes is the phosphoryl moiety of the phospholipids. Whether this moiety is involved in the Ca^{++} sequestration process per se, or merely contributes to the maintenance of sufficient structural integrity of the sarcotubular membranes for Ca^{++} sequestration to occur, cannot be definitely answered by the data presented. That such a large amount of the phosphoryl structure of the sarcotubular phospholipids must be eliminated before there is appreciable loss of Ca^{++} uptake activity favors the concept that the phosphoryl unit of phospholipids functions primarily in the maintenance of membrane structure. In contrast to the difficulty in precisely delineating the function of the phosphoryl moiety of the phospholipid in Ca^{++} sequestration, there is strong evidence for a direct, specific role of the sulfhydryl and the histidyl groups (24, 25) of the sarcotubular proteins in the Ca^{++} sequestration system of the sarcoplasmic reticulum.

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