Mechanisms of Fatty Acid Effects on Sarcoplasmic Reticulum

III. THE EFFECTS OF PALMITIC AND OLEIC ACIDS ON SARCOPLASMIC RETICULUM FUNCTION—A MODEL FOR FATTY ACID MEMBRANE INTERACTIONS

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The mechanism by which palmitic and oleic acids modify calcium sequestration by sarcoplasmic reticulum vesicles was investigated by examining the effects of these fatty acids on calcium-dependent ATPase activity, on the phosphoenzyme intermediates found during calcium sequestration reactions, and on passive membrane permeability to calcium. The calcium sequestered in the presence of these fatty acids was also characterized by determining the amount exchangeable with the extravesicular pool or released by the ionophore A23187.

In the presence of 50 μM ATP, 18 μM palmitic acid enhanced and 18 μM oleic acid inhibited calcium sequestration, whereas both fatty acids stimulated ATPase activity. Neither fatty acid had significant effects on the amount or distribution of the phosphoenzyme formed during the calcium transport reaction. Palmitic acid stimulated calcium sequestration only when ATP was present. Oleic acid caused the release of a portion of the accumulated calcium during ATP-supported calcium sequestration and also enhanced the release observed in ATP-depleted reactions. A portion of the calcium sequestered in the presence of palmitic acid appears to be incorporated into a nonexchangeable and ionophore-insensitive calcium pool, although the latter was estimated to be considerably larger than the nonexchangeable pool. These data support the hypothesis that oleic acid inhibits calcium sequestration by increasing membrane permeability to calcium, whereas palmitic acid appears to stimulate calcium sequestration by interacting with a portion of the calcium within the vesicles to form a separate, poorly exchangeable calcium pool.

Calcium sequestration by sarcoplasmic reticulum vesicles isolated from skeletal muscle is coupled to the hydrolysis of ATP by a membrane-bound Ca,Mg-dependent ATPase (1, 2). The transport of calcium from the extravesicular to the intravesicular space involves a reaction sequence whereby calcium and MgATP are rapidly bound to the enzyme and the α-phosphate of ATP is transferred to form an acid-stable phos-
the sarcoplasmic reticulum membrane. The effects of palmitic and oleic acids on calcium fluxes and calcium exchange, ATPase activity, and phosphoenzyme levels suggest that palmitic acid enhances calcium sequestration by interacting with a portion of the calcium within the vesicles to form a separate, slowly exchanging, calcium pool. In contrast, oleic acid inhibits calcium sequestration by increasing sarcoplasmic reticulum permeability to calcium.

MATERIALS AND METHODS

Sarcoplasmic reticulum vesicles were prepared from rabbit white skeletal muscle by the method described previously (26). The "light" sarcoplasmic reticulum fraction was used in all of the experiments described in this report and is referred to as sarcoplasmic reticulum.

Unless otherwise stated, calcium sequestration was determined in 120 mM KCl, 1 mM MgCl2, 1 mM MgATP, 8–13 μM 45CaCl2, and 40 mM histidine buffer (pH 6.8). Reactions were initiated by the addition of 6 or 12 μg/ml of sarcoplasmic reticulum protein and terminated at selected times by filtering an aliquot of reaction mixture through type HA (0.45 μm pore size) Millipore filters mounted in Swinney adapters. A 50-μl aliquot of the filtrate was added to 2 ml of Biofluor (New England Nuclear) for counting in a Packard or Searle liquid scintillation spectrophotometer. The effects of palmitic and oleic acids on calcium sequestration were examined by adding the fatty acid in ethanol to reaction mixtures with and without MgATP prior to the initiation of the sequestration reaction, or by transferring at a predetermined time an aliquot of the reaction mixture to separate tubes containing the fatty acid in 1:200 the volume of the transferred aliquot. The final concentration of ethanol, <0.13% (v/v), had no significant effect on calcium sequestration and inhibited ATPase activity less than 10%.

Calcium exchange and calcium influx were determined by a modification of the method of Katz et al. (26) described by Takenaka et al. (5). An aliquot of an ongoing calcium sequestration reaction initiated with 45CaCl2 was transferred to high specific activity, carrier-free 45CaCl2. Sequestration of the labeled calcium was then determined at selected times by the methods described. An identical reaction was initiated with 45CaCl2 to determine the calcium concentration outside the vesicles and the amount of calcium sequestered by the vesicles at the times when calcium exchange and influx were measured.

Sarcoplasmic reticulum ATPase activity was measured under the same conditions as calcium sequestration except that reactions were initiated with either 1 mM MgATP or 50 μM [γ-32P]ATP. Liberation was determined after reactions were terminated at appropriate times by addition of an aliquot of the reaction mixture (0.5 ml) to 10% (w/v) Na2CO3, and 0.1 mM KH2PO4 at 95 °C for 1 h. Two aliquots were collected, and one was assayed for protein by the method of Lowry et al. (28); approximately 60% of the total protein was consistently recovered. The second aliquot was added to 10 ml of Biofluor and counted for 32P activity. ADP-sensitive EP (E,P) was determined by measuring the phosphoprotein present in the reaction mixture quenched by trichloroacetic acid 4 s after the addition of 1 mM ADP. ADP-insensitive EP (E′P) was calculated as the difference between the total EP and the ADP-sensitive EP.

All reagents used were reagent grade. ATP, purchased as the disodium salt from Sigma, was changed to the potassium salt by passage over Dowex AG 50W-X8 (K+ form), neutralized with Tris, and stored at –20 °C with equimolar MgCl2. [γ-32P]ATP was prepared by the method of Johnson and Walseth (29) and stored at –20 °C in

FIG. 1. Effect of fatty acids on calcium sequestration and phosphate liberation of sarcoplasmic reticulum vesicles. Calcium sequestration (closed symbols) was assayed as described under “Materials and Methods” and P, liberation (open and partially open symbols) was measured spectrophotometrically. Reactions were carried out in the presence of 12 μg/ml of protein, 1 mM MgATP, and 10 μM added Ca in control (A), and 12 μM palmitic acid and 12 μM oleic acid were present from the onset (B and C, respectively). Each data point is the mean ± S.D. of three experiments.

FIG. 2. Concentration dependence of the effects of palmitic and oleic acids on sarcoplasmic reticulum vesicles. Calcium sequestration (A) was determined 2 min after the onset of the reaction in the presence of palmitic acid (●) or oleic acid (□). ATPase activity (B) was calculated from P, liberation over the first 2.5 min of the reaction. The lower abscissa expresses the fatty acids in nanomoles per mg of protein. Conditions were as in the legend to Fig. 1. The data points are mean ± S.D. of five experiments.

1 The abbreviations used are: EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid.
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2 μM ATP. Contaminating calcium in a standard reaction mixture was 3 μM, determined by atomic absorption spectroscopy, and was taken into account in all calculations. The fatty acids were purchased from Sigma and stored at -20 °C as stock solutions in ethanol. Each fatty acid produced a single band when examined by thin layer chromatography. A25187 was a gift from Lilly. Deionized water was glass distilled prior to use.

RESULTS

Effects of Palmitic and Oleic Acids on Calcium Sequestration—As previously reported (17) palmitic acid caused a concentration-dependent enhancement of calcium sequestration (Figs. 1 and 2). The stimulatory effect of palmitic acid was observed at 10-15 s, the initial time examined after the onset of the reaction, and the effect persisted for at least 10 min in reactions carried out at high concentrations of ATP (Fig. 1). In the absence of ATP, where 10 nmol of Ca/mg of sarcoplasmic reticulum protein became associated with the sarcoplasmic reticulum vesicles under the standard conditions of these experiments, palmitic acid had no effect on calcium association with the sarcoplasmic reticulum (17). Addition of palmitic acid to sarcoplasmic reticulum vesicles that had sequestered a maximal amount of calcium caused a rapid increase in calcium sequestration (Fig. 3). In contrast, oleic acid (6-24 μM) inhibited calcium sequestration when present from the onset of the reaction (Figs. 1 and 2) and when added to calcium-filled vesicles caused the release of a portion of the sequestered calcium (Fig. 3). At higher concentrations (approximately 36 μM) oleic acid induced the release of all but approximately 10 nmol/mg of the sequestered calcium, which represents the amount of calcium associated with sarcoplasmic reticulum in the absence of ATP.

Effects of Palmitic and Oleic Acids on ATPase Activity—At concentrations (6-24 μM) that had opposite effects on calcium sequestration, both palmitic and oleic acids stimulated Ca2+-activated ATPase activity (Figs. 1 and 2). In the presence of 1 mM ATP, the control ATPase activity was 0.04 ± 0.04 μmol of P2~ per mg per min, whereas in the presence of 18 μM palmitic and oleic acids, ATPase activity increased to 2.12 ± 0.23 and 1.81 ± 0.14 μmol/mg·min, respectively. Neither fatty acid affected the "basal" ATPase activity (approximately 0.04-0.07 μmol/mg·min) determined in the presence of 1 mM EGTA. Furthermore, at concentrations of 6 μM, where palmitic acid was without effect and oleic acid slightly inhibited calcium sequestration, both fatty acids stimulated ATPase activity by more than 75% (Fig. 2). Thus, the ability of these fatty acids to stimulate ATPase activity could be dissociated from their effects on calcium sequestration.

Effects of Palmitic and Oleic Acids on the Phosphoprotein Intermediates of the Sarcoplasmic Reticulum ATPase—To determine if the stimulatory effects of the two fatty acids on Ca2+-activated ATPase activity were associated with different alterations in the amount or type of phosphorylated enzyme intermediates, the influence of these compounds on total and ADP-sensitive E2P was examined. Thirty seconds after starting the reaction in the presence of 50 μM [γ-32P]ATP, total E2P level reached a maximum that was maintained for 2 min. The rate of P2~ liberation was also virtually constant during

![Fig. 3](http://www.jbc.org/)

**Fig. 3.** The effect of the addition of palmitic and oleic acids to calcium-filled sarcoplasmic reticulum vesicles. Palmitic acid, 12 μM (A; O→C) and oleic acid, 12 μM (B; C→O), were added to control reactions (●) 5 min after onset and calcium sequestration was determined at the indicated times after addition. Conditions were as in the legend to Fig. 1. Each point is the mean ± S.D. of three experiments.

![Fig. 4](http://www.jbc.org/)

**Fig. 4.** The influence of fatty acids on the time course of P2~ liberation and the total amount of phosphorylated intermediate during a sarcoplasmic reticulum ATPase reaction. P2~ liberation (open symbols) and E2P levels (closed symbols) in control (upper panel), in the presence of 18 μM palmitic acid (middle panel), and in the presence of 12 μM oleic acid (lower panel) were determined in reactions containing 5 μM added Ca2+, 12 μg/ml of protein and 50 μM Mg[y-32P]ATP. The data points are mean ± S.E. of three experiments for the fatty acids and the results of a single experiment for the control.

**TABLE I**

Effects of palmitic and oleic acids on the ATPase activity, E2P amount, and the E2P decay rate constant

<table>
<thead>
<tr>
<th>Reaction</th>
<th>ATPase</th>
<th>E2P</th>
<th>V/E2P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/mg·min</td>
<td>nmol/mg</td>
<td>nmol P/nmol E2P·min</td>
</tr>
<tr>
<td>Control</td>
<td>250 ± 23</td>
<td>0.98 ± 0.11</td>
<td>255</td>
</tr>
<tr>
<td>18 μM palmitic acid</td>
<td>711 ± 24</td>
<td>0.94 ± 0.09</td>
<td>760</td>
</tr>
<tr>
<td>18 μM oleic acid</td>
<td>591 ± 60</td>
<td>1.12 ± 0.12</td>
<td>528</td>
</tr>
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</table>
the initial 2 min of the reaction in the presence or absence of either fatty acid (Fig. 4). Although Ca**+-activated ATPase activity in the presence of 50 μM ATP is 34% of that determined in 1 mM ATP (Fig. 2), the per cent stimulation of this activity by the fatty acids was the same at both ATP concentrations (Fig. 2 and Table I). There was no significant difference in the total EP 1.5 min after initiation of the reaction in the control (3.51 ± 0.18 nmol/mg), 18 μM palmitic acid (3.28 ± 0.22), or 18 μM oleic acid (3.20 ± 0.22) reactions (Fig. 5). Furthermore, neither fatty acid altered the relative amounts of E1P or E2P under the steady state conditions examined (Fig. 5). These results demonstrate that these fatty acids neither increased the amount of phosphoprotein intermediate nor altered the distribution of E1P and E2P during the steady state of the ATPase reaction. Both fatty acids increased the rate constant of E2P decay calculated from the ratio of the rate of P liberation: E2P amount from 255 to 760 and 528 for palmitic and oleic acids, respectively (Table I).

Effects of Fatty Acids on Spontaneous Calcium Release—When calcium sequestration and P liberation were examined under conditions where a low (20 μM) ATP concentration initiated the reaction, the initial calcium sequestration was followed by a spontaneous calcium release that occurs when more than 90% of the added ATP was hydrolyzed (Fig. 6). Palmitic acid at a concentration of 18 μM had no effect on this spontaneous calcium release (Fig. 6), suggesting that palmitic acid does not increase calcium sequestration by decreasing calcium efflux from the sarcoplasmic reticulum vesicles. However, under similar conditions, 18 μM oleic acid accelerated this calcium release (Fig. 6).

Effect of Palmitic Acid on Calcium Influx—As the calcium sequestered by sarcoplasmic reticulum vesicles rapidly exchanges with the extravesicular calcium (5, 9, 18), we determined whether the ability of palmitic acid to enhance sequestration resulted from a stimulation of calcium influx. The unidirectional calcium influx rate was examined by adding a small amount of carrier-free 45CaCl2 during the plateau of calcium content during calcium sequestration reactions started with 45CaCl2. No significant effects of palmitic acid on this calcium influx were observed (Fig. 7). Calculation of the apparent influx rates (5, 18) from experiments such as those shown in Fig. 7 demonstrated that despite enhanced steady state calcium sequestration, 18 μM palmitic acid did not stimulate calcium influx (657 ± 89 nmol/mg-min) above control values (675 ± 66 nmol/mg-min).2 There was no difference in the apparent amount of 45Ca sequestered 2 s after its addition to the extravesicular calcium pool in the absence (46.6 ± 3.1 nmol/mg, mean ± SE, n = 17) or presence of 18 μM palmitic acid (48.2 ± 3.7 nmol/mg, mean ± SE, n = 17) providing further evidence that palmitic acid had no appreciable effect on steady state calcium influx. The ability of

![Fig. 7. The effect of palmitic acid on calcium influx into calcium-filled sarcoplasmic reticulum vesicles. Control influx (■) was determined by adding 45Ca to an ongoing reaction after the sarcoplasmic reticulum vesicles had sequestered a maximal amount of unlabeled calcium (arrow); influx (O—O) was determined when calcium sequestration had been maximally enhanced by the presence of 18 μM palmitic acid. The closed symbols represent identical reactions initiated in the presence of 45CaCl2. The conditions were as in the legend to Fig. 1 except the added calcium was 5 μM. Data points are mean ± S.E. for five experiments.](http://www.jbc.org/)

1 Calcium influx rates are calculated assuming that the specific activity of the calcium outside the vesicles was constant. Because the calcium sequestered remains constant, the calcium influx and efflux rates are equal, so that a portion of the unlabelled intravesicular calcium will enter the labeled extravesicular pool and decrease the specific activity causing an underestimate of the influx rate. When efflux-dependent changes in specific activity were taken into account (see Ref. 5 for details), calculated influx rates remained unaffected by palmitic acid.
palmitic acid to enhance steady state calcium sequestration thus cannot be explained as resulting from stimulation of steady state calcium influx.

Effect on the Exchangeable and A23187-sensitive Calcium Pool within the Sarcoplasmic Reticulum Vesicles—By labeling the extravesicular calcium during a calcium sequestration reaction started with nonradioactive CaCl₂ and assuming that all the calcium present in the reaction was available to distribute with the added label, the amount of sequestered calcium that was exchangeable with the extravesicular calcium could be estimated. As the amount of labeled ⁴⁵CaCl₂ sequestered reached a plateau 10 s after addition of the labeled calcium, the exchange was complete within this time. For both control reactions and reactions containing 18 μM oleic acid, all of the calcium sequestered by the sarcoplasmic reticulum vesicles at 1 min was found to be exchangeable (Fig. 8). In contrast, after the sarcoplasmic reticulum vesicles sequestered calcium in the presence of 18 μM palmitic acid, a portion (35–40 nmol/mg) of the sequestered calcium remained nonexchangeable up to 4 min after addition of the ⁴⁵Ca label (Fig. 8).

To characterize further the state of the calcium sequestered by the sarcoplasmic reticulum in the presence of palmitic acid, A23187 was added to sarcoplasmic reticulum vesicles that had accumulated calcium in the presence and absence of fatty acid. Addition of 1 μM A23187 to calcium-filled sarcoplasmic reticulum vesicles 5 min after the start of a control reaction resulted in a rapid release (within 30 s) of all but 5–10 nmol/mg of the sequestered calcium (Fig. 9). The calcium sequestered in the presence of oleic acid was similarly sensitive to the ionophore (Fig. 9), whereas in the presence of 18 μM palmitic acid (which enhanced calcium sequestration by 100%) approximately 160 nmol of calcium/mg of protein remained associated with the sarcoplasmic reticulum after A23187 addition (Fig. 9). When the effects of 12, 18, and 24 μM palmitic acid on 12 μg/ml of sarcoplasmic reticulum were examined in a separate series of experiments, the size of the A23187-insensitive calcium pool was dependent on the amount of palmitic acid present and was 52 ± 2 (S.D., n = 3), 114 ± 8 (n = 3), and 161 ± 4 (n = 5) in the presence of 12, 18, and 24 μM fatty acid, respectively. Increasing the amount of added ionophore to 5 μM under similar conditions had no effect on the amount of calcium remaining associated with the sarcoplasmic reticulum, suggesting that the inability of the ionophores to cause calcium release could not be explained by an artefact arising from an effect of palmitic acid to decrease the amount of ionophore available to interact with the sarcoplasmic reticulum.

When a calcium sequestration reaction was started in the presence of 5 μM A23187, only approximately 10 nmol of calcium/mg of protein were associated with the membrane. Palmitic acid (18 μM), added at 2 min after the onset of an ionophore-inhibited reaction, did not stimulate calcium sequestration (data not shown). These results can be explained if the ability of palmitic acid to enhance calcium sequestration requires the presence of a high calcium concentration within the sarcoplasmic reticulum vesicles.

**DISCUSSION**

The experiments described above confirm earlier reports (17, 18, 20) that micromolar concentrations of palmitic and oleic acids have different effects on calcium sequestration by isolated sarcoplasmic reticulum vesicles. Palmitic acid enhanced calcium sequestration by sarcoplasmic reticulum vesicles while oleic acid inhibited this reaction (Figs. 1 and 2). The effects caused by both fatty acids were rapid, being fully developed within 10 s after addition of the fatty acids to calcium-filled vesicles (Fig. 3).

The present studies demonstrate that the contrasting ef-

![Fig. 8](https://example.com/fig8.png)

**Fig. 8.** The effect of fatty acids on the amount of sequestered calcium that is rapidly exchangeable with the extravesicular calcium pool. Exchangeable calcium was determined by adding ⁴⁵Ca to vesicles filled with unlabeled calcium in the absence (squares) or presence of 18 μM palmitic acid (circles) or 18 μM oleic acid (triangles), and measuring the steady state amount of ⁴⁵Ca sequestered by the vesicles (open symbols). The closed symbols represent identical reactions initiated in the presence of ⁴⁵CaCl₂. Conditions were as in the legend to Fig. 7.

![Fig. 9](https://example.com/fig9.png)

**Fig. 9.** Fatty acid-induced alteration in the A23187-sensitive portion of the calcium sequestered by sarcoplasmic reticulum vesicles. The ionophore (1 μM) was added 6 min after initiation of the reaction in the absence (closed symbols, left) or presence of 18 μM palmitic acid (closed symbols, middle) or 18 μM oleic acid (closed symbols, right), and subsequent calcium sequestration was determined (open symbols). Conditions were as in the legend to Fig. 7.
fects of palmitic and oleic acids on calcium sequestration cannot be explained by different effects on Ca\(^{2+}\)-activated ATPase activity as both fatty acids stimulated steady state ATPase activity to a similar degree at concentrations that have opposite effects on calcium sequestration (Fig. 2). Furthermore, at concentrations that had no effect on calcium sequestration, both fatty acids stimulated ATPase activity (Fig. 2). Examination of the phosphoenzyme intermediates of the ATPase reactions demonstrated that both fatty acids increased the rate of ATP hydrolysis by stimulating the rate of E,P decay without altering significantly either the total amount of EP or the relative proportions of E,P and E,P (Fig. 4 and Table 1). These findings demonstrate that the stimulation of the membrane enzyme activity is not due to uncovering of latent calcium pump sites, a mechanism suggested previously to explain amphiphile-induced membrane sequestration, both fatty acids stimulated ATPase activity in a manner that decreased the amount of ionophore incorporated into, or the effectiveness of the ionophore to move calcium across, the sarcoplasmic reticulum (35, 36). However, the observation that a 5-fold increase in ionophore concentration did not increase the amount of calcium release argues against this explanation. Alternatively, the ionophore could increase the amount of exchangeable calcium through an effect on a calcium-palmitate complex (25).

A number of studies have demonstrated that fatty acids associate with biological membranes (23), including the sarcoplasmic reticulum (8, 18, 24). Freeze-fracture electron micrographic studies of sarcoplasmic reticulum membrane vesicles exposed to high concentrations of palmitic acid have demonstrated protein-free “lakes” in the membrane fracture face that may represent intramembranous accumulation of palmitic acid. However, such lakes were not seen at the low palmitic acid concentrations used in this study. Instead, incubation of sarcoplasmic reticulum vesicles with palmitic acid...
under the conditions of these studies resulted in the replacement of membrane phospholipid with fatty acid (24). These findings are consistent with the view that the added palmitic acid gains access to the environment of high calcium concentration at the intravesicular membrane surface (24). The potential ability of the anionic carboxylic group of palmitic acid to enter the vesicles is of significance in light of studies of the interaction of calcium with a series of fatty acids in aqueous solution. These studies have demonstrated that micromolar concentrations of palmitic acid readily form a calcium-fatty acid complex (25). The calcium sequestered in this calcium-palmitate complex, like the additional calcium sequestered by the sarcoplasmic reticulum vesicles (Fig. 8), is poorly exchangeable with the ionized calcium in the surrounding solution and not rapidly released by A23187.

It would be of interest also to examine calcium influx and phosphoprotein levels at palmitic acid concentrations where enhancement of calcium sequestration was near maximal (Fig. 8 and Ref. 25). However, the potential for calcium-palmitate interaction outside the sarcoplasmic reticulum vesicle at high

Fig. 10. Schematic representation of possible mechanisms of palmitic acid-induced enhancement of sarcoplasmic reticulum calcium sequestration. a, exposure of sarcoplasmic reticulum membrane to palmitic acid (●) could result in the insertion of the aliphatic chain of the fatty acid into the membrane bilayer with the carboxylic acid anionic group (▲) residing near the phospholipid head group (○) and exposed to the ionic environment of either the intravesicular or the extravesicular water space. At the extravesicular site the ionized calcium concentration is below that necessary for interaction with the palmitic acid, however, the high ionized calcium concentration within the vesicular space generated by the activity of the calcium pump ATPase results in the complexing of a portion of the sequestered calcium with the carboxylic acid portion of the fatty acid (25). (Although a 2:1 stoichiometry of fatty acid to calcium is suggested by previous studies (25), the possible role of anionic phospholipid headgroups serving as one of the complexing sites cannot be excluded in this membrane scheme). The aliphatic chains of the palmitic acid within and near the membrane surface may act to impart a hydrophobic barrier between the complexed calcium and the intravesicular water space. This isolated calcium would not exchange readily with the noncomplexed intravesicular Ca2+ and could be insensitive to ionophore. Complexing of calcium with only intramembranous palmitic acid (b) would also create a second calcium pool, but this calcium would be expected to reach rapid equilibrium with the remaining intravesicular calcium upon addition of ionophore. c, the fatty acid could form a lamellar or micellar structure within the vesicular space (a structure in equilibrium with intramembranous and intravesicular monomeric fatty acid) whose carboxylic acid portions would interact with and lower the intravesicular ionized calcium concentration (25). The inability to identify such fatty acid structures within the vesicular space and the evidence suggesting that the majority of the fatty acid is in the membrane bilayer argues that if present these structures are small and not likely to account for the total enhancement of calcium sequestration (24).
palmitic acid concentrations would make these determinations and their interpretations unreliable (25). Experiments were therefore carried out at palmitic acid concentrations where enhanced calcium sequestration was clearly developed and the extravesicular calcium-palmitate interaction was minimal (Fig. 8 and Ref. 25).

The phenomena described here are consistent with the hypothesis that, shortly after the initiation of a calcium transport reaction, intravesicular Ca2+ concentration reaches a level that can form a calcium-palmitate complex. The appearance of this complex would lead to the incorporation of a portion of this calcium, present at high concentrations within the vesicles, into a “pool” that, by lowering intravesicular Ca2+ concentration, reduces the inhibitory effect of intravesicular calcium concentration on calcium transport into the vesicles. Such an effect of palmitic acid is similar to the well known effect of oxalate and phosphate to promote calcium uptake by the sarcoplasmic reticulum (37). Therefore, palmitic acid may promote calcium sequestration by sarcoplasmic reticulum vesicles by acting as a calcium-complexing anion (Fig. 10). If palmitic acid could exert such an effect in vivo, where sarcoplasmic reticulum calcium content may be submaximal (38), a decrease in the intraluminal free calcium concentration would be expected. The decreased driving force across the sarcoplasm reticulum could reduce the amount and rate of calcium release from the sarcoplasmic reticulum lumen. These phenomena have been observed in ischemic heart, a condition where cytosolic fatty acid levels rise (39, 40), it might contribute to the well known decline in myocardial contractility (41).

Acknowledgments—We are grateful for the excellent technical assistance of P. Pinto and for the valuable discussions and suggestions of Dr. A. M. Katz and Dr. L. Herbette in the preparation of this manuscript.

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