Developmental Changes in the Composition and Function of Sarcoplasmic Reticulum*

(Received for publication, June 13, 1973)

RICARDO BOLAND AND ANTHONY MARTONOSI†‡
From the Department of Biochemistry, St. Louis University School of Medicine, St. Louis, Missouri 63104
THOMAS W. TILLACK
From the Department of Pathology, Washington University School of Medicine, St. Louis, Missouri 63110

SUMMARY

Developmental changes in the protein, phospholipid, cholesterol, and fatty acid composition of skeletal and cardiac microsomes were correlated with Ca\textsuperscript{2+} transport activity in chicken embryos and during the early postnatal period. In skeletal muscle microsomes isolated from leg, superficial pectoralis, or deep pectoralis muscles, the rapid increase in the rate and extent of calcium accumulation around the time of hatching was accompanied by increase in total and Ca\textsuperscript{2+}-sensitive ATPase activity, the concentration of phosphoprotein intermediate and the amount of transport ATPase enzyme determined by gel electrophoresis. The increased enzyme content of the membrane is reflected in its increased density and a marked decrease in phospholipid to protein ratio with development. The concentration of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, sphingomyelin, and phosphatidylinositol changed relatively little during the first 50 days of development but the appearance of calcium transport activity was accompanied by a marked decrease in the palmitate and increase in the linoleate content of membrane phospholipids and a decrease in cholesterol concentration.

These changes were clearly apparent in purified sarcoplasmic reticulum membranes isolated by calcium loading or in less pure membrane fractions obtained by differential or sucrose gradient centrifugation.

Corresponding but much less pronounced changes in the enzymatic activity of cardiac microsomes were observed during development. Although the observations are consistent with nearly all possible mechanisms of membrane assembly they may suggest that the phospholipid-rich membranes of embryonic muscle are gradually converted during development into calcium-transporting structures by stepwise insertion of the ATPase enzyme and other components of the transport system.

Sarcoplasmic reticulum membranes offer a relatively simple system for the analysis of the requirements of active ion transport (2). The transport ATPase constitutes 60 to 70% of the protein content of the membrane (3). Its molecular weight on the basis of polyacrylamide gel electrophoresis is close to 100,000 (3-5). Under appropriate conditions the enzyme protein is separated into several subfragments with molecular weights ranging from 30,000 to 60,000 (3, 6).

The ATPase activity and calcium transport are reversibly inhibited by removal of membrane lipids with phospholipase C (7, 8). The activity can be restored with micellar dispersions of synthetic or natural phospholipids indicating a spontaneous re-formation of the native lipoprotein structures (7-9). Self-assembly of membrane structures from microsomal components solubilized with deoxycholate is also readily demonstrable (10). The morphological and functional characteristics of the reconstituted membranes are reminiscent of native sarcoplasmic reticulum (5, 10).

Developmental changes in the composition (1), function (1, 11-13), and structure of sarcoplasmic reticulum membranes offer a promising insight into the mechanism of intracellular membrane assembly.

During early embryonic development, chicken skeletal muscles are largely inactive and the microsomes isolated from them are devoid of calcium transport activity. The appearance of calcium-sensitive ATPase and Ca\textsuperscript{2+} transport shortly before hatching coincides with increasing muscular activity. Both functions reach a maximum activity in 2-week-old chicks providing readily available material for the study of the assembly of calcium-transporting structures at all stages of completion. As the calcium transport system of sarcoplasmic reticulum is dependent upon membrane phospholipids for activity (8), changes in the lipid composition of the membrane may contribute to the developmental changes in ATPase activity and calcium transport.

The subject of the next two reports is an analysis of the pro-
tein, phospholipid, and fatty acid composition of membrane fractions isolated from skeletal and cardiac muscles of chicks during embryonic and postnatal development. These data are correlated with developmental changes in the calcium transport, ATPase activity, and phosphoprotein concentration of the membranes and with their fine structure as derived from freeze-etch electron microscopy.

**EXPERIMENTAL PROCEDURES**

White Leghorn eggs were incubated in New Brunswick incubator shaker (model G 27) at 38° and a relative humidity of 64 to 70%. After hatching the chicks were maintained on water for 2 days followed by a Ralston Purina Chick StarTena Medicated 20-V 6011 diet ad libitum. Microsomes were prepared from leg, superficial pectoralis, deep pectoralis, and heart muscles obtained during 10 to 130 days of development beginning with the start of incubation. The number of animals used for one microsome preparation was 100 to 142 with 10-day-old embryos, 60 to 70 with 18- to 20-day-old embryos, 20 to 35 with 15- to 18-day-old chicks, and 6 to 7 with 42-day-old chicks. The term embryo is used to denote all stages of development before hatching. Except when otherwise noted, the age of chicks is given in days after hatching.

The muscles were excised, cleaned of fat and connective tissue, minced, and placed in an ice bath made of distilled water until sufficient amount was collected (usually about 1 to 1.5 hours). The muscles were homogenized for 90 s in a Virtis homogenizer or Waring Blender using 4 volumes of 0.1 M KCl, 10 mM imidazole, pH 7.4, and 0.3 M sucrose. The homogenates were centrifuged for 20 min at 8,200 × g in Lourdes 9RA or VRA rotors. The sediment was discarded. The supernatant was filtered through glass wool previously washed with the homogenizing medium. The remaining mitochondria were removed by centrifugation for 30 min at 8,200 × g. The microsomes were sedimented from the supernatant at 50,000 × g for 1 hour in Spinco No. 50 or 30 rotors, depending upon the volume.

The microsomal sediment was dispersed in a solution of 0.6 M KCl, 10 mM imidazole, pH 7.3, and 0.3 M sucrose. After standing for 30 min at 2°, the microsomes were collected by centrifugation at 50,000 × g for 1 hour. The sediment was dispersed in 0.3 M sucrose and centrifuged at 58,000 × g for 30 min. The final sediment was suspended to a protein concentration of 5 to 10 mg per ml in 0.15 or 0.3 M sucrose, and used either for further fractionation or for the various assays.

Two methods were used for further purification of microsomes.

**Sucrose Density Gradient Fractionation—**Centrifugation was performed in Spinco SW 25.1 or 39 rotors at 41,000 × g for 18 to 20 hours using a continuous gradient ranging from 0.3 to 1.50 M sucrose concentration in 10 mM histidine, pH 7.3. The microsome samples were applied in 0.15 or 0.3 M sucrose at the top of the gradient. After centrifugation, the contents of the tubes were collected in five fractions, diluted to 0.3 M sucrose concentration, and centrifuged at 55,000 × g for 1 hour. The sedimented microsomes were suspended in 0.5 M sucrose, 10 mM imidazole, and used for the various assays. Fraction 1 represents the bottom layer, and Fractions 2 to 5, the progressively lighter fractions.

**Discontinuous Sucrose Gradient Centrifugation of Calcium Oxalate-loaded Microsomes**—Leg microsome suspension (0.5 ml containing 5 to 10 mg of protein) was layered on the surface of 4 ml of 40% sucrose-10 mM imidazole, pH 7.4, solution in an SW 39 tube and centrifuged in SW 39 rotor at 64,000 × g for 2 hours. The supernatant was decanted and mixed with 60-ml solution containing 0.1 M KCl, 10 mM imidazole, 5 mM calcium oxalate, 5 mM MgCl₂, 5 mM ATP, and 2.5 × 10⁻⁴ M CaCl₂ equilibrated at room temperature. In several experiments, Fraction 3 obtained after centrifugation in continuous sucrose density gradient was used directly for the calcium oxalate-loading experiment. After incubation for 8 min at 25° the solution was rapidly cooled, and loaded in 18 ml portions onto the surface of three gradients containing 6 ml of 40% sucrose-10 mM imidazole, and 6 ml of 28% sucrose-10 mM imidazole in SW 25 tubes. After centrifugation at 51,505 × g × g for 1 hour the calcium oxalate-loaded microsomes sedimented to the bottom of the tube containing most of the 45Ca radioactivity added to the medium. The supernatants were diluted with water and the microsomes were collected by centrifugation.

Both fractions were dispersed in water to the desired protein concentration and subjected to the various assays described below.

Samples were taken from crude microsomes, sucrose density gradient-fractionated microsomes, and calcium oxalate-loaded preparations for electron microscopy in thin sections and by the freeze-etch method.

**Measurement of Calcium Uptake**—Calcium uptake was measured on the day of microsome preparation at 23° in a medium of 0.1 M KCl, 10 mM imidazole, 5 mM MgCl₂, 5 mM ATP, 5 mM potassium oxalate, and 0.1 mM CaCl₂ at several protein concentrations ranging from 0.03 to 0.3 mg per ml. Samples were taken for Millipore filtration (14) after 1, 2, 5, and 10 min of incubation. The rate of calcium uptake and the calcium capacity of the microsomes were evaluated on the basis of the values obtained after 1 and 10 min incubation, respectively.

**ATPase Activity**—ATPase activity was measured in three different media. Medium A contained 0.1 M KCl, 10 mM imidazole, 5 mM ATP, 5 mM MgCl₂, 0.5 mM EGTA, and 0.45 mM CaCl₂. Medium B was identical with A but with CaCl₂ omitted. Medium C was in addition to KCl, imidazole, and ATP, 5 mM CaCl₂ as activator. The reaction was started with the addition of microsomes (0.015 to 0.075 mg per ml) and after 5 to 20 min incubation at 25° was stopped with trichloroacetic acid.

Each sample was tested at least two different protein concentrations. The liberated inorganic phosphate was assayed according to the method of Fiske and SubbaRow (15). The "extra" or "calcium-sensitive" ATPase activity is obtained as the difference between the rates measured in Mediums A and B. Only this calcium-sensitive ATPase activity is related to the calcium pump.

**Measurement of Phosphoprotein Intermediate**—The reaction was performed under three different conditions as described earlier (16). The assay system contained 0.05 M KCl, 5 mM imidazole, 0.5 mM [32P]ATP, 0.3 to 0.6 mg of microsomal protein per ml, and either 5 mM MgCl₂, 0.5 mM EGTA, and 0.45 mM CaCl₂ (Medium I) or 5 mM MgCl₂ and 0.5 mM EGTA (Medium II) or 5 mM CaCl₂ (Medium III) as activators. Incubation was carried out in ice for 20 s. The reaction was stopped as described earlier (16). In experiments with crude microsomes, the processing of samples involved repeated centrifugation as reported earlier (16). In the experiments with microsomes purified by sucrose density gradient centrifugation or calcium oxalate sedimentation, after two initial centrifugations, a sample was taken for protein determination and the rest of the microsomes were filtered through Millipore filter. After three to four additional washings with 2% trichloroacetic acid, the filter was dried and counted in toluene-based scintillation fluid using 2,5-di-
phenyloxazole (PPO) and 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) as scintillators.

Protein was determined by the Lowry (17) method. In most experiments, the method of Loftfield and Eigner (18) was used for measurement of radioactivity.

The protein composition of crude microsomes, and the membranes purified by sucrose density gradient centrifugation or calcium oxalate loading was analyzed by polyacrylamide gel electrophoresis essentially as described earlier (3). The electrophoretic gels were stained with Amido black and an approximate estimate of the relative amounts of the various proteins was obtained by scanning with Joyce-Loebl Chromoscan densitometer.

For determination of phospholipids, microsomes were extracted with chloroform-methanol according to the method of Folch et al. (19) and separated by thin layer chromatography on Silica Gel G using petroleum ether-ethyl ether-acetic acid (90:10:1, v/v) as solvent. For two-dimensional separation the solvent systems were: chloroform-methanol-concentrated NH$_4$OH (65:25:4, v/v) and chloroform-acetone-methanol-acetic acid H$_2$O (50:20:10:10:5, v/v). Normally the phospholipids were detected by exposure to iodine vapors. In some cases, Zinsser's reagent (20) or ninhydrin was also used.

For determination of fatty acid composition the total lipid extract of microsomes was evaporated to dryness and subjected to transesterification in methanol-H$_2$SO$_4$ (95:5, v/v) under nitrogen for 90 min at 100°C (21, 22). The resulting methyl esters were extracted and concentrated according to the method of Keenan and Morre (23). Gas chromatography was performed on a Barber-Colman series 5000 instrument equipped with an electron capture detector using a glass column (6 feet × 3 mm diameter) packed with 10% EGSS-X on Chromosorb Q (100 to 120 mesh) obtained from Applied Science Laboratory, State College, Pa. The flow rate of argon (Chemotron Corp., Chicago) was 40 ml per min. The carrier gas was dehydrated by passage through a molecular sieve column (Applied Science Laboratories). Column temperature: 180°C. Detector temperature: 270°C.

The methyl esters of fatty acids were identified by two methods. (a) Comparison of their retention time with those of known fatty acid methyl esters. (b) Analysis of their mass spectra using an LKB model 9000 gas chromatograph-mass spectrometer equipped with a Becker-Rylieage molecule separator.

For all fatty acid methyl esters except one earlier identified (1) as myristoleate (14:1), the mass spectra were identical with those of authentic fatty acid methyl esters. The compound with the retention time of myristoleate yielded a mass spectrum which is tentatively assigned to the 14:1 fatty aldehyde; it represents a minor component of membrane phospholipids.

Quantitative evaluation of fatty acid composition was performed by half-height analysis of the peaks of gas chromatograms.

The carboxylic ester bond content was determined according to the method of Stern and Shapiro (24).

The fatty acid composition of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositides and sphingomyelin was determined after separation by thin layer chromatography as described above for total phospholipids.

For determination of sterols, Folch extracts of muscle homogenates and microsomes were fractionated by thin layer chromatography on Silica Gel G into phospholipids and neutral lipids using petroleum ether-ethyl ether-acetic acid (90:10:1, v/v) as solvent (25). The sterols were extracted from the plate with chloroform-methanol (4:1, v/v) and evaporated to dryness (25). The residue was dissolved in chloroform and was analyzed on a model 402 Hewlett-Packard, F & M gas chromatograph using 3% QF, on Chromosorb Q (100 to 120 mesh) (26). Column temperature was 225°C. The instrument was equipped with a flame ionization detector equilibrated to 245°C. Helium was used as carrier gas at a flow rate of 60 ml per min. A Disc integrator was used for quantitative evaluation of the area under the peaks. Cholesterol represents the bulk of the sterols in muscle microsomes but the presence of smaller amounts of other sterols cannot be ruled out. Cholesterol and cholestanol had identical retention times in several systems tested.

RESULTS

Developmental Changes in Calcium Transport, ATPase Activity, and Phosphoprotein Concentration in Crude Microsome Preparations Prepared by Differential Centrifugation—During 10 to 14 days of embryonic development the microsomes isolated from leg (Fig. 1), superficial pectoralis (Fig. 2), heart (Fig. 3), and deep pectoralis (not shown) muscles of chicken embryos show little calcium transport or ATPase activity and the concentration of phosphoprotein is barely measurable. In skeletal muscle microsomes (Figs. 1B and 2B), the ATPase activity measured with Mg$^{2+}$, Mg$^{2+}$ + Ca$^{2+}$, or Ca$^{2+}$ as activators rapidly increases during the 3rd week of incubation reaching maximum values of 3 to 4 μmoles per mg of protein per min at hatching, followed by a gradual decline. The "extra" ATPase activity (Figs. 1A and 2A) calculated as the difference between the Mg$^{2+}$ + Ca$^{2+}$-activated and the magnesium-activated rate of ATP hydrolysis from the data of Figs. 1B and 2B increases with development at a slower rate than the total ATPase activity, maximum values being reached only 2 to 3 weeks after hatching.

The rise in "extra" ATPase activity in leg and superficial pectoral muscle microsomes during development was closely accompanied by an increase in the rate and extent of calcium accumulation (Figs. 1A and 2A) and in the steady state concentration of phosphoprotein intermediate measured with 5 mM Mg$^{2+}$ and 10$^{-5}$ M Ca$^{2+}$ or with 5 mM Ca$^{2+}$ as activators (Figs. 1C and 2C). Only marginal phosphoprotein concentrations were obtained with 5 mM MgCl$_2$ and 0.5 mM EGTA as activators (Figs. 1C and 2C). The excellent correlation between extra ATPase activity, the rate of calcium transport and the steady state concentration of phosphoprotein suggest that the concentration of calcium transport sites in the membrane increases with development.

The data of Figs. 1 and 2 reveal a close similarity between the calcium uptake capacity of the microsomes isolated from the white superficial pectoralis and the predominantly red leg muscles of chicken. This is unexpected in view of the marked differences in Ca$^{2+}$ transport between red and white mammalian muscle microsomes (2).

In agreement with the observations of Holland and Perry (12), the rise in magnesium + EGTA activated (magnesium-insensitive) ATPase activity of leg and superficial pectoral muscle microsomes during the last week of incubation (Figs. 1B and 2B) occurs much earlier than the increase in the rate of calcium transport or in the level of phosphoprotein. Furthermore the calcium-insensitive ATPase (or ATPases) is associated with membrane fractions which are readily separated from the calcium-transporting elements of sarcoplasmic reticulum.

The maximum rates of extra ATPase activity and calcium transport of heart muscle microsomes (Fig. 3A) are much lower than those of their skeletal counterparts and show only minor changes with development. In keeping with the slow rate of...
FIG. 1 (left). Calcium uptake, ATPase activity, and phosphoprotein concentration of leg muscle microsomes during development. The calcium uptake (A), ATPase activity (B), and phosphoprotein concentration (C) were measured as described under "Experimental Procedures." The rate of extra ATPase was calculated as the difference between measurements made in Mediums A and B (Fig. 1B) and is presented in Fig. 1A for comparison with the rate of calcium transport. A, O-O, calcium uptake after 1 min incubation; O-O, calcium uptake after 10 min incubation; Δ-Δ, extra ATPase; B, △-△, ATPase activity in Medium A; Δ-Δ, ATPase activity in Medium B; O-O, ATPase activity in Medium C; C, •-•, phosphoprotein concentration in Medium 1; O-O, phosphoprotein concentration in Medium II; ×-×, phosphoprotein concentration in Medium III.

FIG. 2 (center). The enzymatic activity of microsomes isolated from superficial pectoralis muscles at various stages of development. For details, see "Experimental Procedures" and the legend to Fig. 1.

FIG. 3 (right). The calcium transport, ATPase activity, and phosphoprotein concentration of heart microsomes isolated at various stages of development. For details, see "Experimental Procedures" and the legend to Fig. 1.

FIG. 4. Sucrose density gradient patterns of leg muscle microsomes isolated at various stages of development. 1, 14-day-old embryo; 2, 19-day-old embryo; 3, 12-day-old chick; 4, 16-day-old chick; 5, 120-day-old chick; 6, adult rabbit skeletal muscle microsomes.

The specific activity of magnesium + EGTA-activated (Ca²⁺-insensitive) ATPase increases with decreasing density reaching a maximum in Fraction 5. This is a consistent observation throughout the whole range of development although the maximum specific activity is found in microsomes isolated from 19-day-old embryos and 16-day-old chicks. On the other hand the Ca²⁺-sensitive "extra" ATPase which represents an increasing fraction of the total ATPase activity with development, appears to be concentrated in the heavier gradient layers, and its contribution to the total ATPase activity decreases with decreasing density. For example in microsomes from 120-day-old chicks 82.05% of the ATPase is Ca²⁺-sensitive in Fraction 1, 63.12% in Fraction 3, but only 2.43% in Fraction 5. Unfortunately the extra ATPase activity of Fraction 1 represents only...
Microsomes obtained by differential centrifugation were separated on sucrose gradients ranging from 0.3 to 1.5 M sucrose concentration. In this gradient the calcium oxalate-loaded vesicles represent calcium-containing vesicles formed a pellet while the lighter calcium-free vesicles remain in the supernatant. It is reasonable to assume that the calcium oxalate-loaded vesicles represent calcium-transporting sarcoplasmic reticulum membranes in essentially pure form. Using this criterion, the calcium-transporting sarcoplasmic reticulum membrane mass expressed as percentage of the total mass of microsomal membranes increases with development from 2.6% in 14-day-old embryos to 17.2% just before hatching, and stays close to 20% up to 4 months of age (Table I). The amount of calcium oxalate accumulated in the pellet fraction is 20 to 30 times greater than that found in the microsomal vesicles of the supernatant and increases from 1.70 μmoles per mg of protein in 19-day-old embryos to 6.92 μmoles per mg of protein in microsomes isolated from the leg muscles of a 4-month-old chicken. Repeated tests of the calcium uptake activity of the supernatant fraction after separation yielded data similar to those presented in Table II. These data together with those obtained on crude leg muscle microsomes (Fig. 1A) suggest that even in muscles of adult animals less than one-third of the membrane mass of microsome preparations isolated by simple differential centrifugation represent calcium-accumulating structures. However this may be a minimum estimate. The difference between crude microsomes and the calcium oxalate pellet fraction is less pronounced with respect to the extra ATPase activity (Table III). The supernatant fraction obtained after the removal of calcium oxalate-loaded vesicles still retains sizable calcium-sensitive "extra" ATPase activity (Table III). This observation may imply the presence of microsomes in the supernatant which contain calcium pump but are unable to accumulate Ca++, perhaps due to the leakiness of the membrane.

The customary increase in calcium uptake and extra ATPase activity with development is accompanied by a comparable increase in the concentration of phosphoprotein (Table II) which reaches considerably higher steady state concentration in 4-month-old chicken leg microsomes purified by calcium oxalate sedimentation than in either crude or sucrose gradient-fractionated preparations.

<table>
<thead>
<tr>
<th>Stage of Development and Fraction No.</th>
<th>Protein recovered %</th>
<th>Ca++ Uptake</th>
<th>Phosphoprotein</th>
<th>ATPase</th>
<th>Mg++-Ca++ activated</th>
<th>Mg++-Ca++-EGTA activated</th>
<th>Extra ATPase</th>
<th>Mg++ sensitive ATPase</th>
<th>Ca++ sensitive ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 day 1</td>
<td>3.38</td>
<td>0.003</td>
<td>0.019</td>
<td>0.090</td>
<td>0.020</td>
<td>0.020</td>
<td>0.010</td>
<td>0.30</td>
<td>0.04</td>
</tr>
<tr>
<td>20 day 2</td>
<td>1.97</td>
<td>0.003</td>
<td>0.017</td>
<td>0.114</td>
<td>0.020</td>
<td>0.020</td>
<td>0.010</td>
<td>0.30</td>
<td>0.04</td>
</tr>
<tr>
<td>21 day 3</td>
<td>11.17</td>
<td>0.018</td>
<td>0.015</td>
<td>0.191</td>
<td>0.057</td>
<td>0.057</td>
<td>0.010</td>
<td>0.30</td>
<td>0.04</td>
</tr>
<tr>
<td>22 day 4</td>
<td>3.87</td>
<td>0.014</td>
<td>0.015</td>
<td>0.141</td>
<td>0.050</td>
<td>0.050</td>
<td>0.010</td>
<td>0.30</td>
<td>0.04</td>
</tr>
<tr>
<td>23 day 5</td>
<td>2.57</td>
<td>0.017</td>
<td>0.017</td>
<td>0.145</td>
<td>0.010</td>
<td>0.010</td>
<td>0.010</td>
<td>0.30</td>
<td>0.04</td>
</tr>
<tr>
<td>24 day 6</td>
<td>10.72</td>
<td>0.017</td>
<td>0.017</td>
<td>0.145</td>
<td>0.010</td>
<td>0.010</td>
<td>0.010</td>
<td>0.30</td>
<td>0.04</td>
</tr>
<tr>
<td>25 day 7</td>
<td>3.65</td>
<td>0.017</td>
<td>0.017</td>
<td>0.145</td>
<td>0.010</td>
<td>0.010</td>
<td>0.010</td>
<td>0.30</td>
<td>0.04</td>
</tr>
<tr>
<td>26 day 8</td>
<td>1.83</td>
<td>0.015</td>
<td>0.018</td>
<td>0.060</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.30</td>
<td>0.04</td>
</tr>
<tr>
<td>27 day 9</td>
<td>1.99</td>
<td>0.014</td>
<td>0.014</td>
<td>0.060</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.30</td>
<td>0.04</td>
</tr>
<tr>
<td>28 day 10</td>
<td>2.70</td>
<td>0.018</td>
<td>0.017</td>
<td>0.145</td>
<td>0.010</td>
<td>0.010</td>
<td>0.010</td>
<td>0.30</td>
<td>0.04</td>
</tr>
<tr>
<td>29 day 11</td>
<td>3.52</td>
<td>0.017</td>
<td>0.017</td>
<td>0.145</td>
<td>0.010</td>
<td>0.010</td>
<td>0.010</td>
<td>0.30</td>
<td>0.04</td>
</tr>
<tr>
<td>30 day 12</td>
<td>1.61</td>
<td>0.015</td>
<td>0.018</td>
<td>0.060</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.30</td>
<td>0.04</td>
</tr>
<tr>
<td>31 day 13</td>
<td>2.90</td>
<td>0.018</td>
<td>0.017</td>
<td>0.145</td>
<td>0.010</td>
<td>0.010</td>
<td>0.010</td>
<td>0.30</td>
<td>0.04</td>
</tr>
<tr>
<td>32 day 14</td>
<td>3.86</td>
<td>0.018</td>
<td>0.018</td>
<td>0.145</td>
<td>0.010</td>
<td>0.010</td>
<td>0.010</td>
<td>0.30</td>
<td>0.04</td>
</tr>
<tr>
<td>33 day 15</td>
<td>2.86</td>
<td>0.018</td>
<td>0.018</td>
<td>0.145</td>
<td>0.010</td>
<td>0.010</td>
<td>0.010</td>
<td>0.30</td>
<td>0.04</td>
</tr>
</tbody>
</table>
FIG. 5. Electron micrographs of microsomes prepared by differential centrifugation from leg muscles of A, 10-day embryos; B, 15-day embryos; C, 6-week chicks. The majority of the vesicles have diameters of 1,000 to 2,000 Å. The microsomes show less variability in size and density with development. Particularly noticeable is the preponderance of sharply defined, elongated membrane profiles and the absence of dense osmiophilic bodies after hatching. Magnification is approximately X 32,000.

TABLE II
Phosphoprotein concentration and Ca\textsuperscript{2+} transport in microsomal subfractions isolated after calcium oxalate loading

<table>
<thead>
<tr>
<th>Stage of development</th>
<th>Protein in calcium oxalate sediment as percent of total protein</th>
<th>Ca\textsuperscript{2+} uptake (mole/mg protein)</th>
<th>Phosphoprotein intermediate (mole ATP/10\textsuperscript{5} g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-day embryo</td>
<td>2.60</td>
<td>0.075</td>
<td>0.88</td>
</tr>
<tr>
<td>19-day embryo</td>
<td>7.03</td>
<td>0.075</td>
<td>1.70</td>
</tr>
<tr>
<td>20-day embryo</td>
<td>17.20</td>
<td>0.195</td>
<td>4.01</td>
</tr>
<tr>
<td>16-day chick</td>
<td>16.43</td>
<td>0.215</td>
<td>6.92</td>
</tr>
<tr>
<td>120-day chick</td>
<td>19.58</td>
<td></td>
<td>3.03</td>
</tr>
</tbody>
</table>

The fractionation and the various assays were performed as described under "Experimental Procedures."

The electron microscope appearance of the calcium-accumulating membrane material in thin sections (not shown) is not readily distinguishable from sucrose gradient-fractionated microsomes (Fig. 6), except that early embryonic preparations obtained by sucrose gradient fractionation are slightly more heterogeneous.

**Protein Composition of Muscle Microsomes during Development**—Polyacrylamide gel electrophoresis of microsomes isolated from leg muscles by differential centrifugation, showed a marked increase in the concentration of the M protein with development (Fig. 7). The M protein was previously identified in rabbit skeletal muscle microsomes with the ATPase enzyme involved in the calcium transport (27). The change in the concentration of M protein closely accompanied the increase in extra ATPase activity, calcium transport, and the concentration of phosphoprotein. During the same period there was some decrease in the relative amount of small molecular weight proteins which do not have clearly assigned functional significance. Essentially
TABLE III
ATPase activity of microsomal subfractions isolated after calcium oxalate loading

For details see "Experimental Procedures."

<table>
<thead>
<tr>
<th>Stage of development</th>
<th>Mg$^{2+}$ + Ca$^{2+}$-activated ATPase</th>
<th>Mg$^{2+}$ + EGTA-activated ATPase</th>
<th>Extra ATPase</th>
<th>Extra ATPase as per cent of Mg$^{2+}$ + Ca$^{2+}$-activated ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calcium oxalate supernatant</td>
<td>Calcium oxalate sediment</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>μmoles Pi/mg protein/min</td>
<td>μmoles Pi/mg protein/min</td>
<td>μmoles Pi/mg protein/min</td>
<td></td>
</tr>
<tr>
<td>14-day embryo</td>
<td>0.265</td>
<td>0.200</td>
<td>0.045</td>
<td>16.98</td>
</tr>
<tr>
<td>19-day embryo</td>
<td>1.30</td>
<td>0.73</td>
<td>0.27</td>
<td>0</td>
</tr>
<tr>
<td>16-day chick</td>
<td>1.64</td>
<td>0.71</td>
<td>0.45</td>
<td>20.73</td>
</tr>
<tr>
<td>120-day chick</td>
<td>0.85</td>
<td>0.89</td>
<td>0.80</td>
<td>55.78</td>
</tr>
</tbody>
</table>

Fig. 7. Protein composition of muscle microsomes during development. Polyacrylamide gel electrophoresis was carried out as described under "Experimental Procedures." A, leg muscle microsomes; B, heart muscle microsomes. M indicates the position of transport ATPase.

Identical developmental changes were observed in the protein composition of microsomes isolated from superficial pectoralis and deep pectoralis muscles.

In heart muscle microsomes the developmental changes in protein composition were much less pronounced in accord with the nearly constant ATPase activity, calcium transport, and phosphoprotein concentration (Fig. 7).

As purification of microsomes by sucrose gradient centrifugation or calcium oxalate sedimentation permits the separation of fractions with varying calcium transport and ATPase activity, the protein composition of the different membrane fractions isolated from leg muscles during development was investigated. Surprisingly only minor differences are apparent between the protein composition of crude microsomes subjected to differential centrifugation (Fig. 7), microsomes purified by sucrose gradient centrifugation (Fig. 8A), the calcium oxalate pellet which represents pure sarcoplasmic reticulum membranes (Fig. 8B), and the calcium oxalate supernatant (not shown) which contains particles that are essentially devoid of calcium transport activity.

The developmental changes in the protein composition of crude microsomes (Fig. 7) are also reflected in the protein composition of sucrose gradient-purified (Fig. 8A) or calcium oxalate-sedimented preparations (Fig. 8B). Even sucrose gradient fractions of widely differing densities which showed large differences in the specific activity of calcium-sensitive ATPase and in phosphatidylethanolamine and sphingomyelin content had similar protein compositions (Fig. 8C).

Phospholipid Content of Microsomes at Various Stages of Development—During the early embryonic period the microsomal membranes isolated from leg muscles contain relatively large amounts of phospholipids (Fig. 9). The phospholipid to protein ratio declines during development to 0.6 to 0.7 μmole of lipid-P per mg of protein and remains at that level from 2 weeks to 4 months after hatching. There is only slight difference in the phospholipid to protein ratio of crude microsomes obtained by differential centrifugation and subfractions isolated by sucrose gradient or by calcium oxalate loading. The major decrease in phospholipid to protein ratio coincides with the sharp increase in ATPase activity and calcium transport around the time of hatching. Part of this change is attributable to the increased concentration of the M protein in the membrane. The decrease in phospholipid to protein ratio during development is reflected in the increased density of microsomes as determined by sucrose density gradient centrifugation.

The carboxylic ester bond content of the membrane determined on crude microsomes (Fig. 9) is about that expected from the phospholipid content at all stages of development. Consequently the di- and triglyceride content of the preparations is probably negligible. Esterified cholesterol was not detected.

The principal phospholipid fractions were identified by two-dimensional thin layer chromatography as phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, sphingomyelin,
FIG. 8. Protein composition of microsomal subfractions at various stages of development. A, sucrose gradient Fraction 3. 1, 14-day-old embryo; 2, 19-day-old embryo; 3, 15-day-old chick; 4, 120-day-old chick. 5, calf calcium oxalate sediment fraction. B, sucrose gradient fraction of leg muscle microsomes from 4-month-old chicken. 9, Fraction 1; 10, Fraction 2; 11, Fraction 3; 12, Fraction 4.

FIG. 9. The phospholipid to protein ratio of microsomal membranes during development. The phospholipid content of microsomes was measured after Folch extraction as described under "Experimental Procedures." For the measurement of carboxylic ester bonds, the method of Stern and Shapiro was used (21). ○, crude microsomes; ■, peak fraction after sucrose density gradient centrifugation; △, sediment after calcium oxalate loading; Δ, supernatant after calcium oxalate loading; +++, carboxylic ester bonds.

FIG. 10. Thin layer chromatogram of microsomal lipids isolated from sucrose gradient-fractionated leg muscle microsomes of 14-day-old embryos (A) and 5-week-old chicks (B). PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; SM, sphingomyelin; PI, phosphatidylinositol; NF, neutral fat.

and phosphatidylinosides (Fig. 10). The relative amounts of these phospholipids show minor changes with development.

A quantitative comparison of the phospholipid content of crude microsomes and their subfractions obtained by sucrose density gradient centrifugation or calcium loading at various stages of development is shown in Fig. 11. In general, there is less phosphatidylcholine and more phosphatidylethanolamine and phosphatidylserine in chicken than in adult rat or rabbit skeletal muscle microsomes. Phosphatidylcholine comprises 40 to 50% of microsomal phospholipids and remains essentially constant during development. Further work is required to evaluate the significance of the slight developmental changes observed in the concentration of phosphatidylethanolamine (25 to 30%). The combined phosphatidylinositol and sphingomyelin fraction and phosphatidylserine which constituents 2 to 10% of the total phospholipids showed slight decrease during development.

Although there were no striking differences in phospholipid composition between crude microsomes and the purified fractions represented in Fig. 11, a systematic analysis of all sucrose gradient fractions revealed considerable heterogeneity. Sucrose gradient fractions obtained from leg muscle microsomes of 19-day-old embryos and 4-month-old chickens show increasing phospholipid to protein ratios with decreasing density (Table IV). Fraction 3 contains usually most of the calcium transport and extra ATPase activity. The contribution of calcium-
TABLE IV

Phospholipid composition of membrane subfractions obtained by sucrose gradient centrifugation

Sucrose density gradient centrifugation was performed as described under "Experimental Procedures." Fractions 1 to 5 represent progressively lighter portions of the gradient.

<table>
<thead>
<tr>
<th>Age</th>
<th>Fraction number</th>
<th>Phospholipid to protein ratio</th>
<th>Phospholipid content, per cent of total lipid-P</th>
<th>Unidentified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Phosphatidylcholine</td>
<td>Phosphatidylethanolamine</td>
<td>Phosphatidylserine</td>
</tr>
<tr>
<td>19-day embryo</td>
<td>1</td>
<td>0.58</td>
<td>0.50</td>
<td>24.28</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.83</td>
<td>45.07</td>
<td>22.18</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.20</td>
<td>33.73</td>
<td>24.77</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.30</td>
<td>37.33</td>
<td>24.77</td>
</tr>
<tr>
<td>120-day chicken</td>
<td>1</td>
<td>0.60</td>
<td>55.60</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.60</td>
<td>60.00</td>
<td>24.30</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.63</td>
<td>55.50</td>
<td>20.70</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.86</td>
<td>47.60</td>
<td>25.50</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.30</td>
<td>37.33</td>
<td>24.77</td>
</tr>
</tbody>
</table>

sensitive ATPase to the total ATPase activity decreases with decreasing density (Table I) reaching a minimum value in Fraction 5 which also has the highest sphingomyelin and lowest phosphatidylcholine content. Since more than 75% of the total microsomal mass is represented by Fraction 3 together with most of the calcium transport and ATPase activity, the fractions with the most unusual phospholipid to protein ratios do not have great influence on the average values reported in Fig. 11.

Fig. 11. Phospholipid composition of microsome preparations at various stages of development. For technical details, see "Experimental Procedures." O—O, crude microsomes; ——, peak fraction after sucrose density gradient centrifugation; □—□, sediment after calcium oxalate loading; ■—■, supernatant after calcium oxalate loading. Unidentified phospholipids accounted for less than 12% of the total lipid-P.

Fig. 12. Gas chromatogram of methylated fatty acids isolated from total lipids of sucrose gradient-fractionated leg muscle microsomes of 14-day-old embryos (A) and 16-day-old chicks (B). The peak marked ? is unidentified but on the basis of its mass spectrum probably represents the dimethyl acetal derived from the 14:1 fatty aldehyde. All other components were identified by their retention time and mass spectra.

Fatty Acid Composition of Microsomal Phospholipids during Development—In view of the well known influence of fatty acid chain length and unsaturation upon the permeability of artificial lipid membranes for Ca" (28) developmental changes in the fatty acid composition of sarcoplasmic reticulum membrane phospholipids were investigated by gas-liquid chromatography. Typical examples of the separation of fatty acid methyl esters are shown in Fig. 12 representing sucrose gradient-purified microsomes from leg muscles of 14-day-old embryos and 4-month-old chickens, respectively. There is a striking decrease in palmitate (16:0) and increase in linoleate (18:2) content with development. The data are summarized in Fig. 13A for crude and in Fig. 13B for sucrose gradient-fractionated microsomal lipids over a period of development ranging from 10-day-old embryos to 4-month-old chickens. In microsomes isolated from leg muscles of 10- to 11-day-old embryos the saturated fatty acids (palmitate and stearate) constitute about 54% of the total esterified fatty acids. During the next 2 to 3 weeks of development the concentration of palmitate decreases while that of linoleate increases so that in the microsomal lipids of 12-day-old chicks the saturated fatty acids represent only 37% of the fatty acid content. The short chain unsaturated fatty acids are minor components representing less than 5% of the total fatty acids. The major unsaturated fatty acids in addition to linoleate are oleate (18:1) and arachidonate (20:4) which show some decrease with development. In chicken microsomes fatty aldehydes are not found in amounts reported for rabbit sarcoplasmic reticulum (29) and on the basis of mass spectra only the 14:1 aldehyde appears to be present in significant quantity.

The net result of the developmental changes in fatty acid composition is that the early embryonic membranes containing large amounts of saturated fatty acids are converted by the 30
were fractionated by thin layer chromatography. The separated
sters of fatty acids were separated by gas-liquid chromatography
phospholipids were subjected to methanolysis and the methyl esters
method of Keenan and Morre (23) in which about 60% of the fatty acids are unsaturated. This
sucrose density gradient-fractionated (B) leg muscle microsomes
between 14 and 20 days of development, i.e. before hatching.
Development—The fatty acid composition of the two major
microsomal phospholipids shows characteristic differences (Table
Phosphatidylcholine contains more palmitate but less stearate and arachidonate than phosphatidylethanolamine. At
14 days of embryonic development, saturated fatty acids (palmitate
and stearate) constitute about 54 and 35% of the total fatty acids in phosphatidylcholine and phosphatidylethanolamine,
respectively. The corresponding values at 33 days of development are 46 and 33%, respectively. Phosphatidylethanolamine
contains more unsaturated fatty acids, primarily arachidonate, at an early embryonic stage than lecithin and changes relatively little during subsequent development. The concentration of linoleate and the increase in linoleate content
during development are of similar magnitude in the two classes of phospholipids. The fatty acid composition of phospholipids
isolated from crude microsomes or from purified fractions obtained by sucrose density gradient centrifugation or calcium
oxalate loading are practically identical. Insufficient amounts of material permitted only semi-quantitative analysis of the
fatty acid composition of phosphatidylserine, which resembles that of phosphatidylethanolamine. Phosphatidylinositol and sphingomyelin analyzed as combined fractions had a fatty acid composition similar to lecithin.
Sterol Content of Leg Muscle Microsomes during Development—The sterols present in chicken skeletal muscle microsomes and the various microsome subfractions were analyzed at four stages of development after thin layer chromatographic separation by gas liquid chromatography. No sterified sterols were detected and a major part of the sterol content corresponds to cholesterol. In whole muscle homogenates the sterol content decreases with development from 0.261 mg per g of muscle in 14-day-old embryos to 0.172 mg per g of muscle in 35-day-old chicks (Table V1). It is generally assumed that a major part of the cholesterol is associated with the cell surface membrane and therefore the observed decrease in cholesterol content may result from the increase in the size of muscle cells during development.

Fig. 13. Fatty acid composition of total lipids in crude (A) and
succrose density gradient-fractionated (B) leg muscle microsomes
as function of development. The fatty acid composition of ex-
tracts prepared according to the method of Folch was analyzed by
gas-liquid chromatography after methanolysis according to the
method of Keenan and Morre (23). •, palmitic; □, stearic; □, oleic; O, linoleic; △, arachidonate; +, unidentified; ●, palmitoleic.
The unidentified fraction has a retention time similar to that of
methyl myristate but on the basis of its mass spectrum it is
tentatively identified as the dimethyl acetal derived from the
14:1 fatty aldehyde.

Table V

Fatty acid composition of phosphatidylcholine and phosphatidylethanolamine in chicken skeletal
muscle microsomes isolated at various stages of development.

<table>
<thead>
<tr>
<th>Stage of Development</th>
<th>Microsome Fraction</th>
<th>Phosphatidylcholine</th>
<th>Phosphatidylethanolamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>14:0</td>
<td>14:1</td>
</tr>
<tr>
<td>14 day chick embryo</td>
<td>Crude</td>
<td>0.82</td>
<td>0.59</td>
</tr>
<tr>
<td>18-19 day chick</td>
<td>Crude</td>
<td>1.72</td>
<td>2.84</td>
</tr>
<tr>
<td>embryo</td>
<td>Su-collagen loaded</td>
<td>1.06</td>
<td>1.77</td>
</tr>
<tr>
<td>6 day chick</td>
<td>Crude</td>
<td>0.92</td>
<td>0.88</td>
</tr>
<tr>
<td>12 day chick</td>
<td>Crude</td>
<td>0.98</td>
<td>0.74</td>
</tr>
<tr>
<td>16 day chick</td>
<td>Crude</td>
<td>0.70</td>
<td>0.88</td>
</tr>
<tr>
<td>18 day chick</td>
<td>Crude</td>
<td>0.74</td>
<td>2.15</td>
</tr>
<tr>
<td>35 day chick</td>
<td>Crude</td>
<td>0.47</td>
<td>0.68</td>
</tr>
<tr>
<td>42 day chick</td>
<td>Crude</td>
<td>0.56</td>
<td>1.29</td>
</tr>
<tr>
<td>120 day chick</td>
<td>Crude</td>
<td>0.30</td>
<td>3.01</td>
</tr>
</tbody>
</table>
The data suggest that sarcoplasmic reticulum membranes contain a small but significant amount of cholesterol. There is a sharp decrease in the cholesterol content of all microsome preparations during development which coincides with the increase in calcium transport and extra ATPase activity and with the changes in the phospholipid to protein ratio of the membrane. No significant further change in cholesterol was observed between 33 and 56 days of development.

**DISCUSSION**

Developmental changes in the protein composition, ATPase, and calcium transport activity of muscle microsomes provide a slow motion picture of the assembly of functionally competent sarcoplasmic reticulum membranes endowed with calcium transport activity.

At 10 to 14 days of embryonic development the ATPase and calcium transport activity of microsomes isolated from leg, superficial pectoralis, and deep pectoralis muscles are low, comparable to that of the heart. The membranes contain primarily low molecular weight proteins and on gel electrophoresis the M band which is known to contain the calcium transport ATPase is only a minor protein component. These embryonic membranes are rather rich in phospholipids and cholesterol and the fatty acid content of phospholipids is characterized by relatively high concentration of palmitic and stearic acid.

In skeletal muscle microsomes between 14 and 30 days of development there is a spectacular increase in the extra ATPase activity, phosphoprotein concentration, and Ca\(^{2+}\) transport together with an increase in the concentration of M protein, which in 1- to 2-week-old chicks constitutes already 60 to 70% of the protein content of microsomes. Parallel with these changes the phospholipid and cholesterol content of the membranes decreases and there is a large increase in the linoleate content of membrane phospholipids with corresponding decrease in palmitate. The concentration of arachidonic acid decreases to a lesser extent. These changes in the unsaturation and average chain length of the fatty acids of microsomal phospholipids may be of importance in maintaining the proper fluidity of the lipid phase of microsomal membranes at physiological temperatures.

In heart muscle microsomes the developmental changes in protein composition and enzymatic functions are much less pronounced.

The average concentration of the various phospholipid classes remains relatively constant during the rather striking changes in the fatty acid composition of the membrane. Phosphatidylcholine is characterized by higher palmitate and lower stearate and arachidonic acid content than phosphatidylethanolamine. The developmental changes in the fatty acid composition of phosphatidylcholine and phosphatidylethanolamine are qualitatively similar. It is unlikely that the observed alteration in fatty acid composition during development could be explained by dietary factors alone since significant changes are apparent before hatching. It is possible however that the unusual aspects of the fatty acid composition of 120-day-old chick microsomes could have resulted from dietary factors.

Phosphatidylethanolamine had fatty acid composition similar to phosphatidylethanolamine. This is not surprising in view of the common biosynthetic pathway shared by these two phospholipids. The phospholipid composition of the combined sphingomyelin-phosphatidylcholine fraction was qualitatively similar to that of phosphatidylcholine.

The developmental changes in fatty acid composition are of similar magnitude in crude, or sucrose density gradient- and calcium oxalate-fractionated microsomes indicating that all membrane systems which are represented in crude microsome preparations participate in the changes to a similar extent.

Sarcoplasmic reticulum membranes defined as the rapidly sedimenting material obtained after calcium oxalate loading account for an increasing fraction of the total microsomal membrane mass with development. This may contribute to the striking increase in the homogeneity of vesicle populations observed by electron microscopy in microsome preparations isolated from older animals.

The increase in calcium transport and calcium sensitive ATPase activity is preceded during development by the appearance of a magnesium-activated ATPase enzyme which does not require Ca\(^{2+}\) for activity and reaches its maximum rate shortly before hatching. The subsequent decline in the activity of this enzyme during development correlates well with the enhancement of the Ca\(^{2+}\) transport-related functions and with the increase in the average density of microsomal membranes. The calcium-insensitive ATPase is obtained with the highest specific activity in the lighter sucrose gradient fractions, while the calcium-sensitive transport ATPase is localized primarily in the heavier layers.

Several interpretations of these observations are possible.

1. The calcium-insensitive ATPase may be entirely independ-
ent from the calcium pump and performs some metabolic function associated with development.

2. The calcium-insensitive ATPase may be a precursor of the transport ATPase which acquires calcium sensitivity at a relatively late stage of development. The hypothetical "coupling factor" which links ATP hydrolysis to Ca\(^{2+}\) transport and imparts Ca\(^{2+}\) sensitivity upon the system may be a protein subunit which links the various elements of the Ca\(^{2+}\) pump into a functioning complex. The accumulation of M protein of 100,000 molecular weight with the appearance of Ca\(^{2+}\)-sensitive ATPase and calcium transport activity during later development may represent the assembly of fully developed Ca\(^{2+}\)-sensitive ATPase enzyme from preformed subunits. Alternatively the assembly of the transport ATPase into a functional enzyme complex may proceed only when the phospholipid and fatty acid composition of the membrane becomes optimal.

The apparent correlation between the appearance of ATPase enzyme molecule and the change in the fatty acid composition of phospholipids with development is consistent with most conceivable mechanisms of membrane assembly. The following possibilities may be considered.

1. The simplest assumption is that the synthesis of lipid and protein constituents of the membrane occurs independently but is so coordinated that the fatty acid composition of the membrane becomes optimal when the transport ATPase is fully developed.

2. Alternatively, although the synthesis of ATPase enzyme protein or its subunits is independent of the fatty acid composition of membrane phospholipids the coupling of ATPase activity to Ca\(^{2+}\) transport, the assembly of the enzyme from its subunits or the insertion of the enzyme protein into the membrane may require some unsaturated fatty acids. The dependence of calcium-sensitive ATPase activity upon phospholipids is well known (30). The evidence concerning specific requirement for unsaturated fatty acids in the in vitro reconstitution of calcium transport and ATPase activity from lipid-depleted membranes is conflicting (8, 9, 16, 31).

It seems unlikely that the phospholipid requirement for the appearance of calcium transport during development would be related to a simple permeability function of phospholipids since phospholipids containing saturated fatty acids are excellent permeability barriers for Ca\(^{2+}\) (28). The calcium permeability of microsomal membranes is four to six orders of magnitude greater than that of pure phospholipid bilayers. Therefore other membrane components, presumably proteins, are the principal determinants of ion permeability under physiological conditions.

Considering the evidence so far available the developmental changes in Ca\(^{2+}\) transport and ATPase activity are consistent with a stepwise assembly of the calcium transport system from preformed subunits which are present in the membrane before significant calcium transport becomes demonstrable. Although the requirement for phospholipids in ATPase activity and calcium transport is well established, their possible effects upon the interaction between the subunits of the Ca\(^{2+}\) pump requires further investigation.

The presence of cholesterol in purified sarcoplasmic reticulum preparations suggests but does not fully establish that cholesterol is a genuine membrane constituent of sarcoplasmic reticulum. Contamination of the calcium oxalate sediment fraction with less than 20% surface membrane could account for the observed cholesterol content. Since cholesterol is not required for ATP hydrolysis or calcium transport (32), the significance of the developmental changes in cholesterol content remains to be established.

Acknowledgments—Our thanks are due to Dr. W. H. Elliott for his help with the analysis of sterols and the use of mass spectrometer, to Mr. C. E. Bussmann for the evaluation of mass spectrometographs, and to Shirley Carroll for assistance with the electron microscopy.

REFERENCES

Developmental Changes in the Composition and Function of Sarcoplasmic Reticulum
Ricardo Boland, Anthony Martonosi and Thomas W. Tillack


Access the most updated version of this article at http://www.jbc.org/content/249/2/612

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/249/2/612.full.html#ref-list-1