Phospholipid Synthesis and Exchange in Castor Bean Endosperm Homogenates

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ABSTRACT

Crude organelle preparations from castor bean (Ricinus communis L.) endosperm rapidly incorporate CDP-\(^{14}\)C)choline and CDP-\(^{14}\)C)-ethanolamine into phosphatidylcholine and phosphatidylethanolamine, respectively. Separation of organelles by sucrose density gradient centrifugation following incubation with these substrates demonstrated that most of the \(^{14}\)C phospholipids thus formed were present in the endoplasmic reticulum membranes, although label was also found in mitochondria, proplastids, and glyoxysomes. The phospholipid-synthesizing enzymes, cholinephosphotransferase and ethanolaminephosphotransferase, are exclusively confined to the endoplasmic reticulum membrane fraction, suggesting that the appearance of \(^{14}\)C-phospholipid in other organelles was due to phospholipid exchange. Phospholipid synthesis was inhibited by the cytoplasmic supernatant fraction. The active inhibitor in this fraction was not identified, but the inhibition was not significantly relieved by either dialyzing or boiling the supernatant. Phosphatidylcholine synthesis showed an absolute requirement for Mg\(^{2+}\); the Michaelis constant was 1 mM. Ca\(^{2+}\) was a potent inhibitor of Mg\(^{2+}\)-stimulated phospholipid synthesis and enhanced the decay of \(^{14}\)C-phospholipids from pre-labeled membranes, particularly when the membranes were resuspended in the cytoplasmic supernatant.

The data are consistent with the concept that the endoplasmic reticulum is a major site of membrane proliferation where structural lipids, and possibly proteins, are inserted into, and thus expand, a pre-existing membrane fraction. Other organelle and cellular membranes could therefore originate from the proliferating endoplasmic reticulum by a process of membrane flow and differentiation.

The conversion of stored fats to sucrose which occurs during the early stages of germination in castor bean endosperm tissue is accompanied by a rapid formation of the organelles, glyoxysomes, and mitochondria, which house the enzymes of the gluconeogenic sequence (2). Several of the structural phospholipids present in the membranes of these organelles are synthesized by enzymes located in the endoplasmic reticulum. Phosphatidylcholine (16), phosphatidylethanolamine (4), phosphatidylerine (18), and phosphatidylinositol (18) are all synthesized in this cellular fraction. These findings raise the question of how these phospholipids are transferred from their site of synthesis in the endoplasmic reticulum to the membranes of other organelles. This process is just one aspect of the wider problem of membrane and organelle biogenesis in eukaryotic cells.

Current concepts offer at least two suggested mechanisms for phospholipid transfer from the endoplasmic reticulum. Electron micrographs have frequently shown direct continuity between the endoplasmic reticulum and microbody membrane (9, 10, 22) and the endoplasmic reticulum and outer mitochondrial membrane (5, 8, 19). These observations have been interpreted as visualizations of the final stage in the assembly of the microbody or outer mitochondrial membranes, which are derived from the preformed endoplasmic reticulum membrane by a process of vesiculation and membrane differentiation. This model is strengthened by the classical studies of Palade and coworkers and their interpretations of the developmental relationships between the endoplasmic reticulum membranes, Golgi membranes, and vesicles and the plasmamembrane (6, 21). Clearly this model offers one mechanism which explains how phospholipids synthesized on, and presumably incorporated into, the endoplasmic reticulum membrane could be transferred to the microbody or outer mitochondrial membranes, but it is difficult to extend such a model to include the inner mitochondrial membrane (which also contains a large proportion of endoplasmic reticulum-derived phospholipids such as phosphatidylcholine). Several of the inner mitochondrial membrane proteins and phospholipids (e.g., cardiolipin) are synthesized by the organelles themselves. Components of the inner membrane not synthesized by mitochondria might be individually transferred from their sites of synthesis to mitochondrial assembly sites. The recent demonstration of cytoplasmic proteins in animal cells which specifically catalyze the exchange of phospholipids between cellular membrane fractions provides a second mechanism for the transfer of phospholipids from the endoplasmic reticulum to other organelles (14, 17, 25).

The present paper reports a preliminary investigation of the fate of newly synthesized phosphatidylcholine and phosphatidylethanolamine in castor bean endosperm homogenates. The bulk of the newly formed phospholipid is recovered in the endoplasmic reticulum membranes, but some evidence for exchange into other cellular membranes is presented, although the situation is complicated by the inhibitory effect of the cytoplasmic supernatant fraction on phospholipid synthesis.

MATERIALS AND METHODS

Seeds of castor bean (Ricinus communis L.) were soaked overnight in running tap water and germinated in moist vermiculite in darkness at 30°C.

Homogenization. Endosperm halves, removed from 3-day-old seedlings, were homogenized by chopping for 10 min with a single razor blade in grinding medium contained in a Petri dish on ice. The grinding medium contained 0.15 M Tricine (pH 7.5), 10 mM KCl, 1 mM MgCl\(_2\), 1 mM EDTA (pH 7.5), and 13% (w/w) sucrose, and was used in the proportion of 1 ml of grinding medium to 2 endosperm halves. The crude homogenate was filtered through four layers of cheesecloth. Mitochondria, proplastids, glyoxysomes, and virtually all the endoplasmic reticulum membranes present in the homogenate were obtained in the pellet after centrifuging for 15 min at 20,000g and 2°C. (This was

1 This work was supported by the Science Research Council through Grant B/RG 66629.
confirmed by assaying various organelle marker enzymes—data not shown.) The crude organelle pellet was gently resuspended in an equivalent volume of fresh grinding medium or, where appropriate, the 20,000g supernatant.

**Phospholipid Synthesis.** Phosphatidylcholine was synthesized by incubating 5 ml of resuspended pellet, 0.25 ml of 0.1 M MgCl₂, and 50 to 200 µl of CDP-(14C)choline (0.1–0.4 µCi). Phosphatidylethanolamine was synthesized in identical reaction mixtures by replacing the CDP-(14C)choline with 50 to 200 µl of CDP-(14C)ethanolamine (0.1–0.4 µCi). In general, reaction mixtures were incubated for 1 hr at 25 C.

Cholinephosphotransferase (EC 2.7.8.2.) activity present in organelles separated by sucrose density gradient centrifugation was determined by incubating collected gradient fractions (1 ml) with 0.1 ml of 0.1 M MgCl₂ and 10 µl of CDP-(14C)choline (0.02 µCi) at 25 C for 30 min.

**Sucrose Density Gradient Centrifugation.** Separation of organelles was achieved by centrifuging samples (approximately 5 ml) into sucrose gradients for 4 hr at 20,000 rpm in an SW 27 rotor on a Beckman L2 65B ultracentrifuge. Gradients consisted of 20 ml of sucrose solution increasing linearly in concentration from 32 to 60% (w/w) over a 2-ml cushion of 60% (w/w) sucrose, and topped with a 5-ml layer of 20% (w/w) sucrose. Gradients were contained in 38.5 ml cellulose nitrate tubes. All sucrose solutions were prepared in 1 M EDTA, pH 7.5. Sucrose concentration was determined refractometrically. After centrifugation, 1-ml fractions were collected from the bottom of the tubes using a Beckman density gradient fractionator.

**Phospholipid Extraction.** Phospholipids were extracted from 1-ml aliquots of reaction mixtures or from collected gradient fractions (1 ml) by adding 2 ml of absolute ethyl alcohol. Precipitated proteins were removed by centrifugation and the pellet was extracted with an additional 1 ml of ethyl alcohol. After centrifugation, the ethyl alcohol phases were combined and mixed with 2 ml of chloroform. Unreacted CDP-14C base was removed by washing the organic phase twice with 5-ml portions of 2 M KCl and twice with 5-ml portions of water. The residual chloroform phase, containing phosphatidyl(14C)choline or phosphatidyl(14C)ethanolamine, was transferred to scintillation vials and evaporated to dryness. Ten milliliters of scintillation fluid were added, and the 14C content was determined in a Packard Tri-Carb liquid scintillation counter.

**Enzyme Assays.** Previously described assays were used for NADH-Cyt c reductase (16), triose phosphate isomerase (3), and isocitrate lyase (7).

**Materials.** CDP-(Me-14C)choline (28 µCi/µmole) and CDP-(2-14C)ethanolamine (60 µCi/µmole) were obtained from the Radiochemical Centre, Amersham, England.

**RESULTS AND DISCUSSION**

The separation of various organelles present in castor bean endosperm homogenates by sucrose density gradient centrifugation is shown in Figure 1. Two peaks of NADH-Cyt c reductase activity were present; the peak at density 1.12 g/cm³ which locates the endoplasmic reticulum membranes; and the peak of antimycin A-sensitive activity at density 1.18 g/cm³ which is due to the mitochondrial enzyme (16). Triosephosphate isomerase (1.22 g/cm³) and isocitrate lyase (1.24 g/cm³) activities locate the positions of proplastids and glyoxysomes, respectively, in the gradient. When the enzymes catalyzing the synthesis of phosphatidylcholine and phosphatidylethanolamine are assayed in fractions collected from similar gradients, activity is exclusively confined to the membranes of the endoplasmic reticulum (4, 16).

The fate of newly synthesized phosphatidylcholine was investigated by incubating a crude homogenate (5 ml) with CDP-(14C)choline and Mg²⁺ and then separating cellular organelles by centrifuging the mixture on sucrose gradients. Such gradients were fractionated and an aliquot of each fraction was assayed for 14C. The result (Fig. 2A) shows that the bulk of the radioactivity remained in the soluble fraction, although a clear peak of activity was located in the endoplasmic reticulum membranes (fraction 10) with some radioactivity in the mitochondrial, proplastid, and glyoxysomal regions of the gradient. However, when the crude homogenate was centrifuged at 20,000g for 15 min to pellet the bulk of the organelles present which were then carefully resuspended in an equivalent volume of fresh grinding medium prior to incubation with CDP-(14C)choline, a much greater proportion of the radioactivity present in gradient fractions was found in the endoplasmic reticulum peak (Fig. 2A). In order to distinguish between 14C present in phosphatidylcholine and that due to unreacted CDP-(14C)choline, each collected gradient fraction was extracted with ethyl alcohol and chloroform, and the 14C phospholipid content of each fraction was determined. These data (Fig. 2B) confirmed that most of the newly synthesized phospholipid was located in the endoplasmic reticulum fraction with a small but significant amount present in the mitochondrial and proplastid fractions. The enlarged scale in Figure 2C emphasized this incorporation into organelles other than the endoplasmic reticulum membranes. Clear peaks were obtained in the mitochondria (fraction 18) and proplastids (fraction 24), with a shoulder on the proplastid peak corresponding to the position of glyoxysomes (fraction 28). Identical results were obtained when the above experiments were repeated with CDP-(14C)ethanolamine substituted for CDP-(14C)choline in the incubation mixtures. The distribution of phosphatidyl(14C)ethanolamine among gradient fractions is shown in Figure 3; once again considerably more phospholipid was synthesized by organelles removed from crude homogenates by centrifugation and resuspended in fresh grinding medium than by an equivalent volume of crude homogenates. These results (Figs. 2 and 3) indicate the following. (a) Most of the newly synthesized phosphatidylcholine and phosphatidylethanolamine is incorporated into the membranes of the endoplasmic reticulum. (b) Some newly synthesized phospholipid is present in other organelle membranes, suggesting the possibility of phospholipid exchange if the enzymes synthesizing these compounds are exclusively confined to the endoplasmic reticulum. (c) The soluble fraction of the cell inhibits phospholipid synthesis by the endoplasmic reticulum enzymes.
tid/glyoxysome peaks also contained appreciable phosphatidyl(\(^{14}\)C)choline (Fig. 4). However, when fractions from the untreated sample gradient were individually assayed for cholinephosphotransferase activity, activity was exclusively confined to the endoplasmic reticulum peak (Fig. 4). This confirmed that the mitochondrial, proplastid, and glyoxysomal peaks on the gradient were completely free of contaminating endoplasmic reticulum membranes, and indicated that phospholipid exchange between organelles had occurred when mixed organelle preparations were incubated with CDP(\(^{14}\)C)choline prior to gradient centrifugation.

The finding that the exchange process apparently occurs at a greater rate in the absence of the cytoplasmic supernatant fraction differs from the effects observed with animal cells, where crude supernatants stimulate the exchange of phospholipids between organelles (14, 25). It is, however, consistent with recent findings using potato tuber (12) where it was attributed to the presence of lipid-degrading enzymes not found in animal supernatants (24, 25). Although evidence was presented that in the potato tuber system the active fraction which stimulated phospholipid exchange was a protein present in the cytoplasmic supernatant, the rate of exchange was greater for organelles.

The exchange of phospholipids \textit{in vitro} between endoplasmic reticulum membranes and mitochondria is well documented for animal tissues, and specific phospholipid exchange proteins have been isolated from the cytosol (14, 27). Recently, evidence has been presented for similar exchange reactions in higher plants (1), and a phospholipid exchange protein has been partially purified from potato tuber (12). Before the results obtained in the present study (Figs. 2 and 3) could be interpreted as evidence for phospholipid exchange in castor bean endosperm, it was necessary to eliminate the possibility that some cross contamination of other organelle fractions by endoplasmic reticulum membranes had occurred during gradient centrifugation. Previous studies have established that the type of gradient used here effectively separated endoplasmic reticulum membranes from other organelle fractions (15, 16), and this was confirmed by the following experiment. A crude homogenate (10 ml) was divided into two 5-ml portions which were spun at 20,000g for 15 min. The organelle pellets were each carefully resuspended in 5 ml of grinding medium. CDP(\(^{14}\)C)choline and \(\text{Mg}^{2+}\) were added to one sample which was incubated for 30 min at 25 C, after which time this sample was loaded onto a sucrose gradient and the untreated sample loaded onto an identical gradient. After centrifugation both gradients were fractionated and the \(^{14}\)C-phospholipid content of each fraction from the treated sample gradient was determined. Most of the phosphatidyl(\(^{14}\)C)choline was present in the endoplasmic reticulum membranes, but the mitochondrial and proplas-
resuspended in buffer. The fact that exchange occurs at all in the absence of the supernatant fraction may be due to a significant or accidental association of the phospholipid exchange protein with cellular organelles. The inhibitory effect of the cytoplasmic supernatant fraction on phospholipid synthesis by the endoplasmic reticulum enzymes was confirmed by determining the capacity of organelle pellets resuspended in either fresh grinding medium or in the 20,000g supernatant to synthesize phospholipids. Both phosphatidylethanolamine (Fig. 5A) and phosphatidylethanolamine (Fig. 5B) synthesis were markedly inhibited in the presence of the cellular supernatant in comparison to the rate of synthesis in fresh grinding medium. This effect may have been due to the presence of compounds inhibiting phospholipid synthesis by the endoplasmic reticulum enzymes or to the presence in the supernatant of lipid-degrading enzymes, such as phospholipase D, which could be rapidly degrading newly synthesized phospholipid. The inhibitory effect of the supernatant on phosphatidylethanolamine synthesis was not significantly relieved by either dialysis or boiling prior to incubation.

The addition of 50 mM CaCl₂ completely inhibited phosphatidylethanolamine synthesis by the endoplasmic reticulum membranes resuspended in either the cytoplasmic supernatant or fresh grinding medium (Fig. 6). This suggests that Ca²⁺-stimulated phospholipase D may be degrading the newly formed phospholipid. However, it seems unlikely that such an enzymic degradation would be 100% efficient over the time course studied, and if a soluble phospholipase was acting, the inhibition should have relieved by boiling. It seems more likely that Ca²⁺ simply inhibits phosphatidylethanolamine synthesis by cholinephosphotransferase (23).

Cholinephosphotransferase from animal tissues is known to require divalent cations, particularly Mg²⁺ for maximum activity (23), and this divalent cation has been routinely included in assay mixtures for this enzyme from plants (11, 15, 20). The ability of various divalent cations to activate castor bean endosperm cholinephosphotransferase was determined using organelle pellet resuspended in grinding medium from which Mg²⁺ had been omitted. Only Mg²⁺ of the cations tested, each at a final concentration of 10 mM, significantly activated this enzyme (Table I). Limited activation occurred in the presence of Fe²⁺ and Co²⁺, but other cations did not substitute for Mg²⁺. The failure of Mn²⁺ to stimulate activity is somewhat surprising since this cation activates animal cholinephosphotransferase (23), but only one concentration was tested here. The effect of Mg²⁺ concentration on castor bean endosperm cholinephosphotransferase is shown in Figure 7; the Kₘ for Mg²⁺ was calculated to be 1 mM.

The effect of various divalent cations on cholinephosphotransferase activity in the presence of optimum Mg²⁺ concentration is shown in Table II. All cations tested inhibited phosphatidylcholine synthesis in comparison to the Mg²⁺ control, but in some cases (Fe²⁺, Ba²⁺, and Co²⁺) this may simply represent a competition with the stimulatory Mg²⁺ ions for the enzyme sites. Other cations, particularly Ca²⁺ and Hg²⁺, completely inhibited the Mg²⁺-stimulated activity, possibly by irreversibly binding to the enzyme-active site. The effect of Ca²⁺ concentration on the inhibition of cholinephosphotransferase activity in the presence of 20 mM Mg²⁺ is shown in Figure 8; 50% inhibition occurred with 1 mM Ca²⁺. With respect to Mg²⁺, Ca²⁺ was a mixed inhibitor of castor bean endosperm cholinephosphotransferase (Fig. 9). The possibility that divalent cations present in the cytoplasmic supernatant might inhibit cholinephosphotransferase activity is shown in Table II. All cations tested inhibited phosphatidylcholine synthesis in comparison to the Mg²⁺ control, but in some cases (Fe²⁺, Ba²⁺, and Co²⁺) this may simply represent a competition with the stimulatory Mg²⁺ ions for the enzyme sites. Other cations, particularly Ca²⁺ and Hg²⁺, completely inhibited the Mg²⁺-stimulated activity, possibly by irreversibly binding to the enzyme-active site. The effect of Ca²⁺ concentration on the inhibition of cholinephosphotransferase activity in the presence of 20 mM Mg²⁺ is shown in Figure 8; 50% inhibition occurred with 1 mM Ca²⁺. With respect to Mg²⁺, Ca²⁺ was a mixed inhibitor of castor bean endosperm cholinephosphotransferase (Fig. 9). The possibility that divalent cations present in the cytoplasmic supernatant might inhibit cholinephosphotransferase activity is shown in Table II. All cations tested inhibited phosphatidylcholine synthesis in comparison to the Mg²⁺ control, but in some cases (Fe²⁺, Ba²⁺, and Co²⁺) this may simply represent a competition with the stimulatory Mg²⁺ ions for the enzyme sites. Other cations, particularly Ca²⁺ and Hg²⁺, completely inhibited the Mg²⁺-stimulated activity, possibly by irreversibly binding to the enzyme-active site. The effect of Ca²⁺ concentration on the inhibition of cholinephosphotransferase activity in the presence of 20 mM Mg²⁺ is shown in Figure 8; 50% inhibition occurred with 1 mM Ca²⁺. With respect to Mg²⁺, Ca²⁺ was a mixed inhibitor of castor bean endosperm cholinephosphotransferase (Fig. 9). The possibility that divalent cations present in the cytoplasmic supernatant might inhibit cholinephosphotransferase activity is shown in Table II. All cations tested inhibited phosphatidylcholine synthesis in comparison to the Mg²⁺ control, but in some cases (Fe²⁺, Ba²⁺, and Co²⁺) this may simply represent a competition with the stimulatory Mg²⁺ ions for the enzyme sites. Other cations, particularly Ca²⁺ and Hg²⁺, completely inhibited the Mg²⁺-stimulated activity, possibly by irreversibly binding to the enzyme-active site. The effect of Ca²⁺ concentration on the inhibition of cholinephosphotransferase activity in the presence of 20 mM Mg²⁺ is shown in Figure 8; 50% inhibition occurred with 1 mM Ca²⁺. With respect to Mg²⁺, Ca²⁺ was a mixed inhibitor of castor bean endosperm cholinephosphotransferase (Fig. 9).
ferase was explored by adding varying concentrations of EDTA to chelate such cations prior to incubation with 10 mM Mg^{2+} and CDP-(^{14}C)choline. Increasing concentrations of EDTA up to 5 mM resulted in enhanced cholinephosphotransferase activity (Table III). Higher concentrations were inhibitory, probably due to lowering the Mg^{2+} concentration effectively available to the enzyme. The inhibitory effect of the cytoplasmic supernatant on phospholipid synthesis is therefore apparently not solely due to the presence of lipid-degrading enzymes but may involve other inhibitors, such as Ca^{2+} ions, which bind to the enzyme sufficiently tightly as to resist removal by dialysis.

The turnover of labeled phospholipids in castor bean endo-

sperm membrane fractions was also investigated. Phosphatidy-

choline and phosphatidylethanolamine were labeled by incubating organelle pellets with either CDP (^{14}C)choline or CDP-

(^{14}C)ethanolamine. The organelles were repelleted and washed, and then resuspended in either fresh grinding medium or the 20,000g supernatant fraction in the presence or absence of 50 mM CaCl_2. At various time intervals aliquots were removed and the ^{14}C-phospholipid was extracted and determined (Figs. 10 and 11). Phosphatidylcholine and phosphatidylethanolamine were relatively stable in membranes resuspended in grinding medium, but decayed more rapidly when the membranes were resuspended in the 20,000g supernatant fraction. The addition of 50 mM CaCl_2 to the medium in which the membranes were resuspended greatly enhanced the rate of (^{14}C)phospholipid turnover, particularly in the case of membranes resuspended in the 20,000g supernatant (Figs. 10 and 11). The mechanism of this Ca^{2+}-stimulated membrane ^{14}C phospholipid decay is not clear. Possible explanations include Ca^{2+}-stimulated lipolytic enzyme activity, for example, phospholipase D, or Ca^{2+}-stimu-

lated exchange reactions such as the exchange of ^{14}C choline or ^{14}C ethanolamine present in the ^{14}C phospholipids with unla-

beled bases.

![Fig. 7. Effect of MgCl_2 concentration on phosphatidylcholine synthesis.](image)

**Table II. Effect of Divalent Cations on Mg^{2+}-stimulated Phosphatidylcholine Synthesis**

<table>
<thead>
<tr>
<th>Cation</th>
<th>Phosphatidylcholine Formed</th>
<th>Relative Rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6,200</td>
<td>100</td>
</tr>
<tr>
<td>Mn</td>
<td>700</td>
<td>11</td>
</tr>
<tr>
<td>Ca</td>
<td>30</td>
<td>0.5</td>
</tr>
<tr>
<td>Ni</td>
<td>230</td>
<td>4</td>
</tr>
<tr>
<td>Fe</td>
<td>2,650</td>
<td>43</td>
</tr>
<tr>
<td>Ba</td>
<td>2,640</td>
<td>43</td>
</tr>
<tr>
<td>Hg</td>
<td>10</td>
<td>0.2</td>
</tr>
<tr>
<td>Co</td>
<td>2,480</td>
<td>40</td>
</tr>
<tr>
<td>Cu</td>
<td>170</td>
<td>3</td>
</tr>
<tr>
<td>Zn</td>
<td>240</td>
<td>4</td>
</tr>
</tbody>
</table>

![Fig. 8. Effect of Ca^{2+} concentration on Mg^{2+}-stimulated cholinephosphotransferase activity.](image)

![Fig. 9. Double reciprocal plot showing the inhibition of cholinephosphotransferase activity at various MgCl_2 concentrations by Ca^{2+}.](image)

**Table III. Effect of EDTA on Mg^{2+}-stimulated Phosphatidylcholine Synthesis by Crude Homogenates**

<table>
<thead>
<tr>
<th>EDTA mM</th>
<th>MgCl_2 mM</th>
<th>Phosphatidylcholine Formed cpm</th>
<th>Relative Rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>10</td>
<td>3750</td>
<td>100</td>
</tr>
<tr>
<td>0.15</td>
<td>10</td>
<td>4800</td>
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<td>0.31</td>
<td>10</td>
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<td>133</td>
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<tr>
<td>0.62</td>
<td>10</td>
<td>5140</td>
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<tr>
<td>1.25</td>
<td>10</td>
<td>5510</td>
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</tr>
<tr>
<td>2.5</td>
<td>10</td>
<td>5560</td>
<td>149</td>
</tr>
<tr>
<td>5.0</td>
<td>10</td>
<td>5710</td>
<td>152</td>
</tr>
</tbody>
</table>
The present results indicate that, in the in vitro systems studied, virtually all the phospholipids synthesized by enzymes exclusively confined to the endoplasmic reticulum membranes are themselves initially incorporated into these membranes. Previous studies have shown that \(^{14}\)C choline supplied to castor bean endosperm is rapidly incorporated into radioactive phosphatidylcholine in the endoplasmic reticulum membranes and subsequently appears in the membranes of the mitochondria and glyoxysomes (13).

The results presented here further indicate that a small proportion of the phospholipid synthesized by the endoplasmic reticulum in crude homogenates is transferred to other organelles. It seems likely that this would be achieved by the transfer of individual phospholipids between the endoplasmic reticulum and already existing organelles rather than by a residual generation of new organelles in crude homogenates involving membrane flow from the endoplasmic reticulum. Clearly, further work is required here to clarify this. To provide evidence for the presence of specific phospholipid transfer proteins in this tissue, but the characterization of such proteins from certain animal and plant tissue, together with the present data, make it reasonable to speculate that phospholipid exchange between organelles might occur in vivo in castor bean endosperm cells. Such a mechanism could explain how structural phospholipids are transferred from their site of synthesis in the endoplasmic reticulum to the inner mitochondrial membrane, a problem not easily explained by a membrane flow model since many of the structural components of the inner membrane are synthesized by the mitochondria. Phospholipid metabolism in vivo could be regulated by cellular components such as Ca\(^{2+}\) ions which influence both the rate of synthesis and turnover-transfer of these compounds.

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LITERATURE CITED