Membrane Lipid Metabolism in Germinating Castor Bean Endosperm[1]

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ROBERT P. DONALDSON
Thimann Laboratories, University of California, Santa Cruz, California 95064

ABSTRACT

Castor bean (*Ricinus communis* L. var. Hale) endosperms, excised after 2 days germination at 30 C, were incubated 5 min to 8 hr with ^{14}C-acetate and ^{3}H-glycerol. Homogenates were fractionated by sucrose gradient centrifugation. Organelles found to be active in lipid synthesis were the lipid bodies and the endoplasmic reticulum. The products of incorporation in the lipid bodies were ^{3}H-diglycerides containing ^{14}C-fatty acids of more than 20 carbons. In contrast, the endoplasmic reticulum produced ^{3}H-phospholipids as well as ^{3}H-diglycerides rich in ^{14}C-linoleate. The phospholipids synthesized and their acyl contents were of the types known to be the major components of organelle membranes in this tissue. Phospholipids and diglycerides containing ^{14}C and ^{3}H were found in the glyoxysomes and mitochondria subsequent to their appearance in the endoplasmic reticulum. The results show that germinating castor bean endosperm synthesizes membrane lipids *de novo* from acetate rather than reutilizing stored lipid components directly. It is also apparent that the endoplasmic reticulum is responsible for several steps in membrane lipid production.

Germination of castor bean involves the breakdown of lipid storage bodies in the endosperm and the biogenesis of membranous organelles such as endoplasmic reticulum, mitochondria, and glyoxysomes. The lipid compositions of these organelles are known (7). Phosphatidylcholine and phosphatidylethanolamine are the predominant phospholipid components of all membranes. Also present are free fatty acids and small amounts of diglycerides. The important acyl contents of each lipid class are linoleate, palmitate, and oleate. Included in the inventory of storage lipid, which consists mainly of triricinolein, are small proportions of these fatty acyls (1). The capacity to synthesize these species has been demonstrated in germinating (9, 11) as well as developing (ripening) (10, 12, 14) castor bean endosperm using cell-free preparations.

The purpose of this investigation is to define further some of the steps in lipid synthesis in the germinating tissue, especially the synthesis of membrane components. It is known that the capacity to install polar groups into phospholipids resides in the endoplasmic reticulum (3, 4, 13). It is not known whether the acyl groups are derived directly from the storage pool or from *de novo* synthesis. In this study lipid synthesis was examined *in vivo* by applying precursors to the intact tissue. Evidence is given herein for *de novo* synthesis of membrane components from acetate. Also, the results point to the sites of synthesis, desaturation, and esterification.

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MATERIALS AND METHODS

The substrates used and the amounts applied to five endosperms were as follows: Na-acetate-1,2-^{14}C, 12.5 μCi (0.25 μmole); glycerol-1-^{3}H, 62.5 μCi (0.025 μmole); and Na-malonate-2-^{14}C, 14.7 μCi (2.3 μmoles).

The substrates were applied to the inner surfaces of castor bean (*Ricinus communis* L. var. Hale) endosperm halves after 1 to 5 days germination (17). Acetate and glycerol were often provided as a mixture. The application volume was 5 μl/endosperm half. For each analysis, 10 halves were incubated at 30 C for 5 min to 8 hr in a closed Petri dish containing 300 μl of H_2O to prevent drying. After incubation 6 ml of cold grinding medium (13% [w/w] sucrose, 0.15 M Tricine, 1 mM EDTA, pH 7.5) were added, and the endosperms were chopped with two razor blades for 10 min. The homogenates were filtered through two layers of nylon curtain material and pipetted directly onto sucrose gradients which were centrifuged in a Beckman SW 27.1 rotor at 21,000 rpm for 2 hr. Each 16-ml centrifuge tube contained a 12-ml gradient of 60% to 15% (w/w) sucrose, 1 ml of 15% (w/w) sucrose, and 4.5 ml of the filtered homogenate. The sucrose solutions contained 1 mM EDTA, pH 7.5.

After centrifugation the fat layer (lipid bodies) was collected from the top of the gradient with a pipette. Fractions, 1.2 ml each, containing the sedimentable organelles were collected from the bottom as previously described (13). Lipid extracts of 1-ml samples of each fraction, 0.1 ml of each fat fraction, and 0.1 ml of each original homogenate were prepared (7). Neutral lipid classes were separated by TLC on Eastman 13179 silica gel on plastic layers using petroleum ether (b.p. 60–72 C), diethyl ether and acetic acid (70:30:2, v/v) as the solvent. Phospholipids were separated as previously described (7) using chloroform-methanol-acetic acid-water (65:50:5:3). Fatty acid methyl esters were prepared from each lipid extract and separated by gas chromatography (Fig. 7). The fatty acid methyl esters collected from a stream splitter in capillary tubes and sections cut from the thin layers were counted in toluene scintillant appropriate for use in the Beckman LS-230 counter. Aqueous samples resulting from washing of the lipid extracts were counted in 10% H_2O, 25% Triton X-100, 65% toluene scintillant. In double label experiments, 15% spillover of ^{14}C cpm into the ^{3}H counting channel was accounted for in the calculations.

RESULTS

Germination Time and Incorporation of ^{14}C-Acetate and ^{3}H-Glycerol into Lipid. The proportion of ^{14}C-acetate incorporated into lipid was greatest in endosperms obtained after 2 to 3 days germination. This agrees with a previous finding (9). After 5 days germination, only 2% of the ^{14}C was found in lipid (2-hr incubation) as reported by Canvin and Beevers (5), whereas as much as 18% ^{14}C-lipid was produced in 2-day endosperms. The remaining ^{14}C was in the aqueous phase, either as unincorpo-

rated ^{14}C-acetate or ^{14}C-metabolites such as sugars and amino acids (5). CO_2 was not recovered or accounted for.

The proportion of ^{14}C-phospholipid relative to total ^{14}C-lipid was also greatest in 2-day endosperm. This correlates with the report that phospholipid-synthesizing enzymes were most active in 2- to 3-day endosperms (4), and the finding that such endosperm was most efficient in the incorporation of ^{32}P into phospholipid (9).

Maximum lipid incorporation of ^{3}H-glycerol occurred in 1-day endosperm. The concentration of glycerol is known to increase 120-fold during the first 4 days of germination (6). This could explain the apparent decrease in lipid synthesis from ^{3}H-glycerol. Similarly, the efficiency of ^{14}C-acetate incorporation could be affected by endogenous acetyl-CoA levels.

Because of the ability to incorporate ^{14}C-acetate and ^{3}H-glycerol into phospholipid, castor beans germinated for 2 days were chosen for detailed studies.

Time Dependence of ^{14}C-Acetate Incorporation into Lipid. The amount of ^{14}C-lipid reached a maximum after a 2-hr incubation with ^{14}C-acetate (Fig. 1), increasing rapidly before 2 hr, and declining slowly thereafter. The decline in ^{14}C-lipid was accompanied by a decrease in the total amount of ^{14}C recovered and thus must reflect the release of ^{14}C as CO_2.

The proportion of ^{14}C-lipid in the lipid bodies (spherosomes or fat storage organelles) relative to that in the sedimentable organelles (the endoplasmic reticulum, mitochondria, and glyoxysomes) also increased with incubation time, reaching a maximum at 2 hr. In this experiment ^{3}H-glycerol was also provided as a substrate. ^{3}H-glycerol lipids were produced maximally at 2 hr.

Organelle Distribution of ^{14}C and ^{3}H-Lipid. Figure 2A shows the sucrose gradient distributions of lipid derived from ^{14}C-acetate and ^{3}H-glycerol in homogenates of 2-day endosperm which had been incubated with the substrates for 15 min. After a

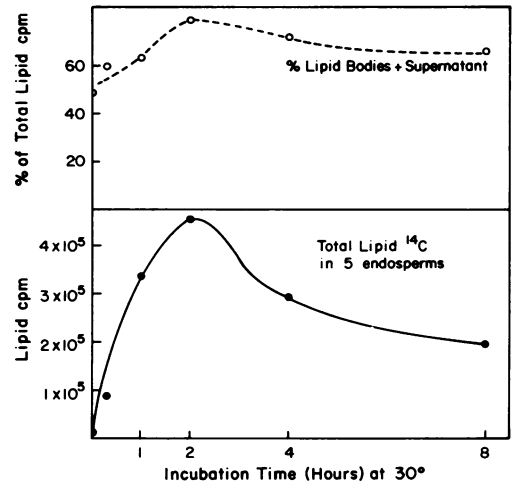

FIG. 1. Time course of acetate-^{14}C incorporation into lipid. After 2 days germination 5 excised castor bean endosperms were incubated with acetate-1,2-^{14}C at 30 C. The percentage of the lipid cpm in the lipid bodies plus supernatant was determined from the distribution of lipid cpm on sucrose gradients such as shown in Fig. 2. The first two time points represent 5 min and 15 min.

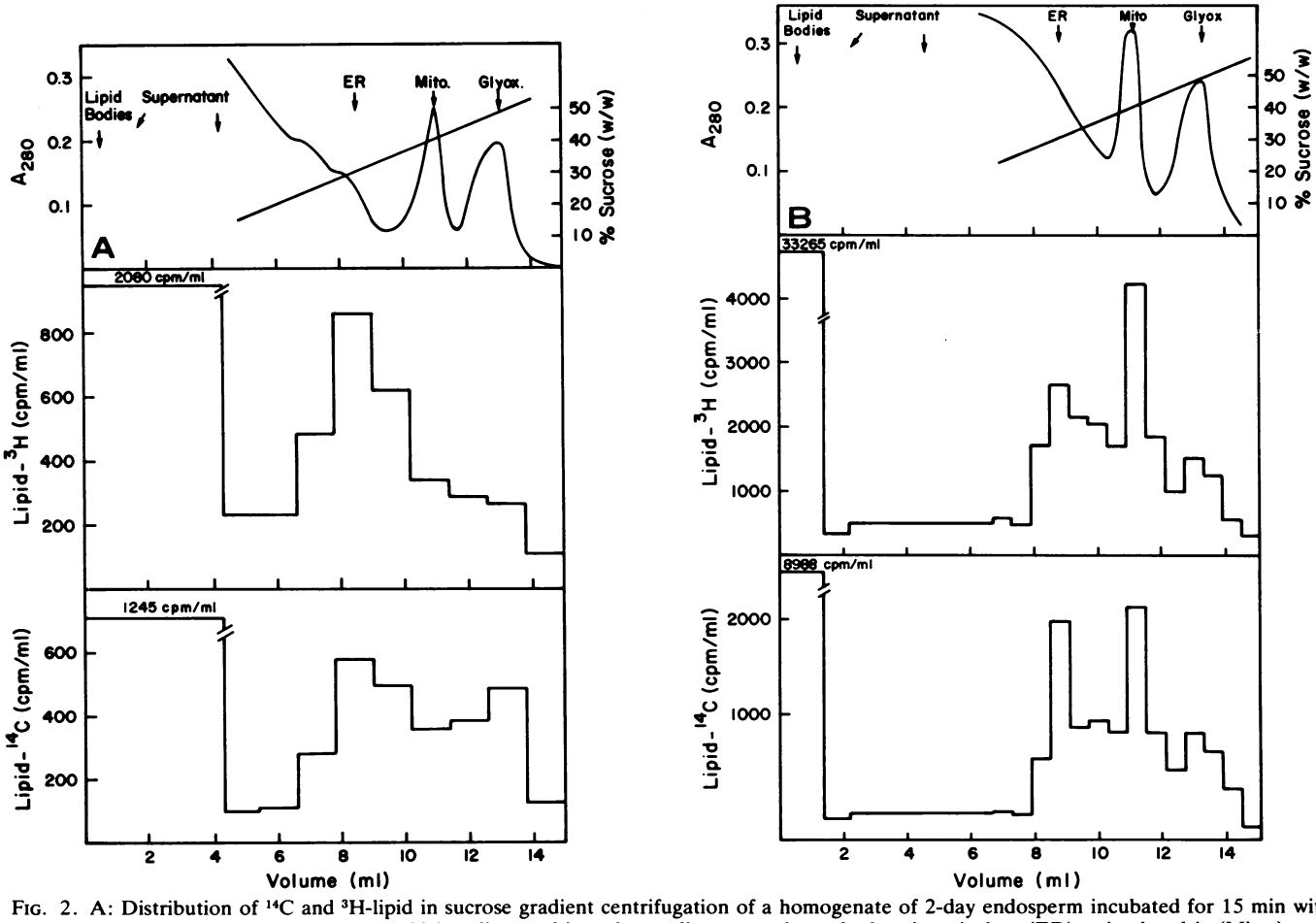

FIG. 2. A: Distribution of ^{14}C and ^{3}H-lipid in sucrose gradient centrifugation of a homogenate of 2-day endosperm incubated for 15 min with glycerol-^{3}H and acetate-^{14}C. The organelles which sedimented into the gradient were the endoplasmic reticulum (ER), mitochondria (Mito.); and glyoxysomes (Glyox.). B: Distribution of ^{14}C- and ^{3}H-lipid after 8 hr incubation.

15-min incubation, 2.9% of the ^{14}C in the homogenate was lipid-extractable. In the sucrose gradient, 60% of the ^{14}C-lipid was associated with the lipid bodies. The sedimentable ^{14}C-lipid appeared in the endoplasmic reticulum and in a high density fraction, perhaps the proplastids.

Most of the ^{3}H-lipid, 70%, was found in the lipid bodies. The remainder was associated with the endoplasmic reticulum. The concurrent appearance of ^{3}H together with ^{14}C-lipid in the lipid bodies and the endoplasmic reticulum indicates that these are sites of esterification.

Similar distributions of ^{3}H- and ^{14}C-lipid among the organelles were observed after a 5-min incubation with the respective substrates. However, after a 1-hr incubation the organelle distribution of the newly synthesized lipid became more general, encompassing the mitochondria and glyoxysomes. After 8 hr (Fig. 2B), the sedimentable organelles, the endoplasmic reticulum, mitochondria, and glyoxysomes all contain lipids derived from the ^{14}C-acetate and ^{3}H-glycerol with a pattern resembling the mass distribution of lipid among these organelles. At this time 26.7% of the ^{14}C in the homogenate was lipid extractable, 7.3% of the ^{3}H. The lipid bodies contained 66% of the ^{14}C-lipid and 76% of the ^{3}H-lipid.

^{14}C-Diglyceride and ^{14}C-Phospholipid in Organelle Fractions. The lipid classes were separated by TLC, and the amount of ^{14}C in each was expressed as a per cent of the total ^{14}C-lipid within the organelle fraction (Fig. 3). In the lipid bodies, ^{14}C-diglyceride was the major component, 65% at 15 min, and 72% at 8 hr, while in the sedimentable organelles there was considerable ^{14}C-phospholipid. After a 15-min incorporation, phospholipid accounted for 45% of the ^{14}C-lipid in the endoplasmic reticulum while there was less in the mitochondria and glyoxysomes. The endoplasmic reticulum is known to be the location of the phospholipid-synthesizing enzymes (3, 4, 13). After 8 hr, the proportion of ^{14}C-phospholipid increased in all sedimentable organelles. For example, the amount increased from 20% in the glyoxysomes at 15 min to 50% at 8 hr.

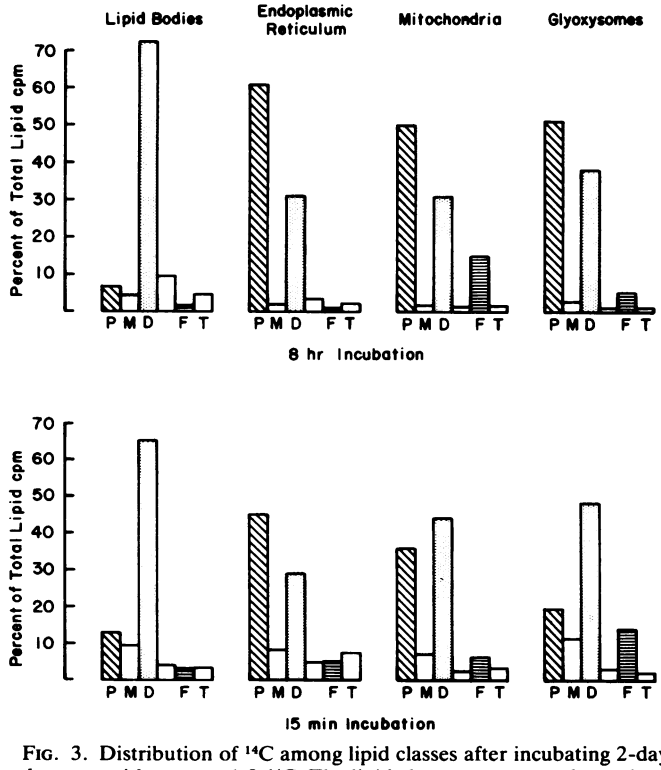

FIG. 3. Distribution of ^{14}C among lipid classes after incubating 2-day endosperm with acetate-1,2-^{14}C. The lipid classes represented are phospholipid (P) (origin); monoglyceride (M); diglyceride (D); free fatty acid (F); triglyceride (T). The lipids are given in order of the separation on TLC.

Some free ^{14}C-fatty acid was detected in the mitochondria and glyoxysomes. Significant quantities of free fatty acid (10–13% of the total acyl lipid) have been observed in mass analysis of the membrane lipids of endoplasmic reticulum, glyoxysomes, and mitochondria obtained from 4.5-day germinated endosperms (unpublished). Figure 3 shows 14% ^{14}C-free fatty acid in the glyoxysome fraction at 15 min. At 5 min there was 23% free fatty acid, probably palmitate, in this fraction. The maximum amount of ^{14}C-fatty acid at this time was actually associated with a fraction slightly lighter than the glyoxysomes, suggesting that the proplastids were responsible for this fatty acid synthesis (14, 22). Although the mass amount of diglyceride is small (2–4% of the acyl lipid) in these membranes, considerable ^{14}C-diglyceride was found in each, even after 8 hr.

The same lipid classes which incorporated ^{14}C-acetate also received ^{3}H-glycerol, except for the free fatty acids. The proportion of ^{3}H in each lipid class within the lipid bodies at any given time was almost identical to the proportion of ^{14}C. However, the percentage of ^{3}H-phospholipid in the endoplasmic reticulum, mitochondria, and glyoxysomes was greater than the percentage of ^{14}C-phospholipid. For example, the glyoxysomes contained 26% ^{3}H-phospholipid compared to 20% ^{14}C-phospholipid at 15 min. At 8 hr the comparison was 60% to 50%, respectively.

Rate of ^{3}H-Glycerol Incorporation Compared with ^{14}C-Acetate. The amount of ^{3}H-lipid relative to ^{14}C-lipid was high after a short incubation with the respective substrates, especially in the endoplasmic reticulum (Fig. 4). The ratio dropped to a minimum at 1 hr and gradually increased thereafter. This indicates that glycerol was rapidly incorporated into lipid while the incorporation of acetate was delayed by the processes of fatty acid synthesis. Intermediates in fatty acid synthesis such as acyl-CoA and acyl-carrier protein would not be lipid-extractable. The increase in the ratio after 1 hr may reflect preferential catabolism of acyl moieties.

Time-dependent Changes in ^{14}C-Lipid Classes in Glyoxysomes. The amount of ^{14}C in each lipid class is expressed in Figure 5 as a percentage of the total ^{14}C-lipid in the glyoxysome fraction. The percentages of ^{14}C-free fatty acid and monoglyceride were greatest at 5 to 15 min and subsequently decreased. Diglyceride contained ^{14}C from the beginning. The proportion of ^{14}C-phospholipid increased with time rapidly during the 1st hr of incubation and more slowly after that. Since the phospholipids (and probably the other lipids) are not synthesized in the glyoxysomes, this curve is a function of both the rate of synthesis in the endoplasmic reticulum and the rate of delivery.

Similar time-dependent changes in the ^{14}C-lipid classes were observed in the mitochondria and the endoplasmic reticulum, although the proportion of ^{14}C-phospholipid in the endoplasmic reticulum was high (40–50%) even at 5 min. Changes in the ^{3}H-lipids were comparable to the ^{14}C-lipids.

Incorporation of ^{14}C-Acetate into Phosphatidylcholine and Phosphatidylethanolamine. The percentages of ^{14}C-phospholipid classes in the endoplasmic reticulum after 1, 4, and 8 hr incubation are shown in Figure 6. The predominant product of acetate incorporation after 1 hr was phosphatidylcholine (66%) along with small amounts of phosphatidylethanolamine and neutral lipid. After 4 hr the amounts of phosphatidylethanolamine and neutral lipid had increased and after 8 hr there was even more phosphatidylethanolamine. Although phosphatidylinositol accounts for 8% of the phospholipid in the endoplasmic reticulum (Table I), this lipid did not receive comparable amounts of ^{14}C (or ^{3}H) at any time. The distribution of ^{3}H among the phospholipids was almost identical to the ^{14}C pattern, showing the same time-dependent changes. Similar trends of phospholipid incorporation also occurred in the glyoxysomes and mitochondria. These results indicate that the rates of synthesis for the

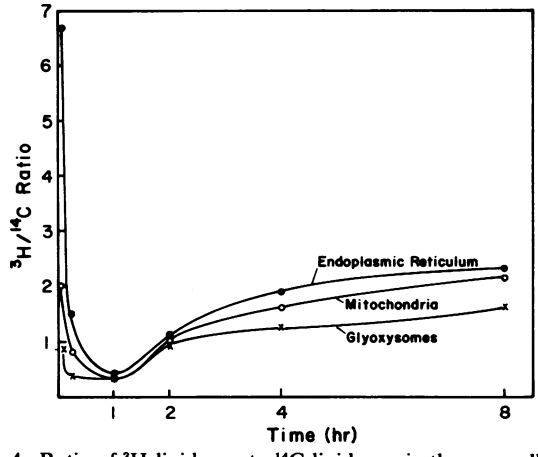

FIG. 4. Ratio of ^3H-lipid cpm to ^{14}C-lipid cpm in the organelles of 2-day endosperm after incubation with ^3H-glycerol and ^{14}C-acetate.

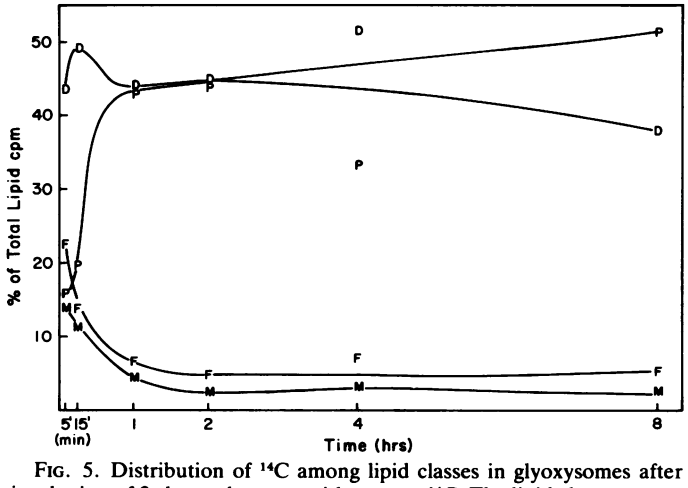

FIG. 5. Distribution of ^{14}C among lipid classes in glyoxysomes after incubation of 2-day endosperm with acetate-^{14}C. The lipid classes represented are diglyceride (D); free fatty acid (F); monoglyceride (M); phospholipid (P).

individual phospholipids vary. Phosphatidylcholine is synthesized the most rapidly, phosphatidylethanolamine more slowly, and phosphatidylinositol very slowly or not at all.

Phospholipid Content of Lipid Bodies. The lipid bodies from 2-day endosperm contained considerable amounts of phospholipids, more than any other organelle fraction (Table I). Also, a greater variety of phospholipid species was observed in the lipid body fraction. The predominant components were phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol, the same as the other fractions. In contrast to the other organelles, the lipid bodies contained lysophospholipid, relatively less phosphatidylethanolamine, and more phosphatidylinositol. Ungerminated castor beans contain these phospholipids (16), suggesting that those found after 2 days germination were not synthesized *de novo* although some incorporation of ^{14}C-acetate into lipid body phospholipid was observed (Fig. 3).

Fatty Acid Composition of ^{14}C-Lipid. The ^{14}C-acyl components of the lipid extracts were analyzed by gas chromatography (Fig. 7). After endosperms were incubated 15 min with ^{14}C-acetate, the ^{14}C-acyl products found in the lipid bodies were palmitate, oleate, linoleate, and long chain fatty acids, greater than C20. After 8 hr the proportion of long chain species was greater. C22 and C24 account for most of this; very little ^{14}C-ricinoleate, which has an equivalent chain length of C29, was detected. As shown above (Fig. 3), most of the ^{14}C-fatty acid in the lipid bodies was in diglyceride.

Acetate-^{14}C was rapidly incorporated into linoleate in the endoplasmic reticulum, which, therefore, may be the site of oleate desaturation. After 8 hr the ^{14}C-fatty acid pattern in the endoplasmic reticulum resembled the mass distributions of fatty acid which are known for the component phospholipids (unpublished). This was also true for the mitochondria.

The glyoxysomes contained much ^{14}C-palmitate after a short incubation. The levels of ^{14}C-oleate and linoleate were increased after 8 hr. However, there was still proportionally more palmitate than would be expected in the phospholipid. Much of this was probably in the diglyceride which represented 40% of the ^{14}C-lipid in the glyoxysomes at 8 hr (Fig. 3).

Little or no ^3H was found in the fatty acids. The $^3H/^{14}C$ ratio was 0.007 at 15 min and 0.02 at 8 hr, whereas the ratios for phospholipid and diglyceride at 15 min were 6.3 and 4.9, respectively. There was little metabolic spillover of ^3H-glycerol into acetate pools.

^{14}C-Malonate Incorporation. When ^{14}C-malonate was pro-

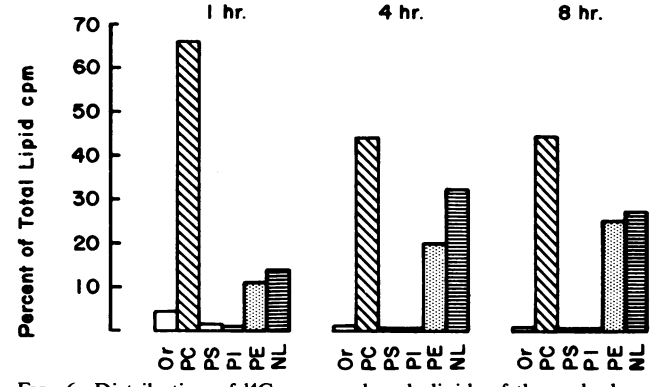

FIG. 6. Distribution of ^{14}C among phospholipids of the endoplasmic reticulum. The phospholipids are given in order of their separation on TLC: origin (Or); phosphatidylcholine (PC); phosphatidylserine (PS); phosphatidylinositol (PI); phosphatidylethanolamine (PE); neutral lipid (NL) (mostly diglyceride, Fig. 3).

Table I. *Phospholipid Content of Lipid Bodies and Other Organelles from 2-day Endosperm*

Thirty endosperms (14 g) were homogenized and fractionated by sucrose gradient centrifugation. The phospholipids were separated by TLC, and the phosphorus content was analyzed. The phospholipids are given in order of their separation on TLC. The region of the chromatogram corresponding to cardiolipin and the solvent front contained three visible phosphorus containing spots in the case of the lipid bodies. The details of the analytical methods have been described elsewhere (7).

	Lipid Bodies	Endoplasmic Reticulum	Mitochondria	Glyoxysomes
	nmoles/5 endosperm			
Total Phospholipid	723	84	95	16
	percent of lipid phosphorus			
Origin	8.6	3.1	3.5	5.4
Lysophospholipid	9.0			
Phosphatidyl Choline	30.4	46.4	35.7	40.6
Phosphatidyl Serine	7.0	4.0	4.0	5.3
Phosphatidyl Inositol	13.0	7.5	3.5	6.7
Phosphatidyl Ethanolamine	17.1	30.0	33.7	29.0
Phosphatidyl Glycerol or Phosphatidic Acid	5.9	4.0	4.2	5.7
Cardiolipin and Solvent Front	8.0	5.0	15.4	7.2

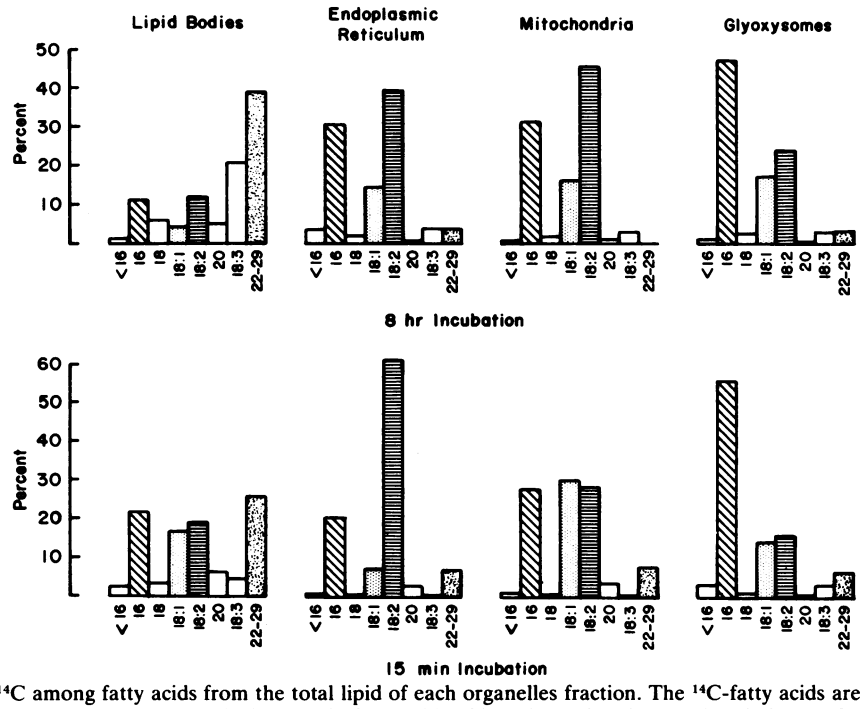

FIG. 7. Distribution of ^{14}C among fatty acids from the total lipid of each organelles fraction. The ^{14}C-fatty acids are given in order of elution of their methyl esters from gas chromatography: <16, short chain fatty acids of less than 16 carbons; 16, palmitate; 18, stearate; 18:1, oleate; 18:2, linoleate; 20, arachidate; 18:3, linolenate; 22-29, long chain fatty acids, mostly behenate and lignocerate. Fatty acid methyl esters were prepared from dried samples of each lipid extract by adding 5 ml of 5% H_2SO_4 in methanol and heating at 70 C for 2 hr. The methyl esters were taken up in 1 to 2 ml of petroleum ether after adding 5 ml of H_2O and separated by gas chromatography on a glass column (2 m × 2 mm) containing 15% diethyleneglycolsuccinate on Chromasorb W-HP (100/200 mesh) at 200 C, 35 cm^3/min He flow, in a Beckman GC 65 and flame ionization detector (250 C).

vided as the substrate, saturated fatty acids, especially long chain species, were the products which appeared in all organelles (Table II). Most of the malonate was incorporated by the lipid body fraction, which, for example, contained 67% of the ^{14}C-lipid on a sucrose gradient following a 1-hr incubation. Much of the ^{14}C-fatty acid was in the free form or in an unidentified lipid running between phospholipid and monoglyceride. This lipid was especially prominent in the endoplasmic reticulum.

DISCUSSION

These results demonstrate that germinating castor bean endosperm has the capacity to synthesize membrane lipids from pools of acetate and glycerol *in vivo*. Since membrane lipid synthesis is the cooperative effort of several subcellular sites, complete synthesis can only be observed in an intact system. The kinetics of incorporation of acetate and glycerol call attention to two subcellular organelles, the lipid bodies and the endoplasmic reticulum, which both contain ^{14}C-acyl-^{3}H-glycerol lipids after short incubations (5-15 min).

The rapid incorporation of acetate into lipids of several organelle fractions suggests that there is more than one site of fatty acid synthesis. The synthesis of long chain fatty acids (C22 and C24) occurs in the lipid bodies. The kinetics of synthesis in the lipid bodies seem to be slower than in the other organelles (Fig. 1). Linoleate first appears in the endoplasmic reticulum. Palmitate is the predominant product in a high density fraction, perhaps the proplastids. This is the only fraction containing significant quantities of ^{14}C-free fatty acid. There is no evidence here for fatty acid synthesis in the cytoplasm. The possibility remains that acyl-carrier protein or acyl-CoA is produced in the cytoplasm since such products were not recovered in this study.

The use of fractions isolated from developing (ripening) castor beans to study fatty acid synthesis *in vitro* has led to conflicting results. Some reports indicate that the proplastids are responsible for the synthesis, the main product being oleate (14, 22).

Table II. *Incorporation of Malonate-2-^{14}C into Lipids*

Incubation time was 3 hr. The unknown lipid was found between the origin and monoglyceride in the TLC system described under "Materials and Methods". Fatty acids were analyzed as described in Fig. 7.

Lipid	Glyoxysomes	Mitochondria	Endoplasmic Reticulum
	% of total cpm		
Phospholipids			
Origin	0.9	0.8	0.6
Phosphatidylcholine	0.8	3.1	4.2
Phosphatidylethanolamine	4.1	10.5	7.7
Neutral lipids			
Unknown	28.2	22.5	54.0
Monoglyceride	10.0	16.8	11.0
Fatty acid	43.1	23.8	13.6
Diglyceride	5.5	7.6	3.3
Triglyceride	7.1	14.5	5.5
Fatty Acid			
<16:0	3.6	1.5	2.6
16:0	15.7	12.3	9.6
16:1	3.2	0.8	1.6
18:0	0.7	4.7	4.9
18:1	4.7	4.8	3.8
18:2	2.1	4.4	3.2
18:3	1.5	4.6	1.0
20:0	14.0	12.8	12.0
20:2	1.8	1.7	4.3
22:0	21.0	21.0	23.0
>24:0	31.8	31.3	33.9

Others suggest that most of the fatty acid synthesis takes place in the lipid bodies (10, 12).

The results presented here demonstrate that the pool of malonyl-CoA involved in the synthesis of the unsaturated compo-

nents of membrane phospholipids is not accessible to externally provided malonate. The products of malonate incorporation were, rather, neutral lipids containing long chain saturated fatty acids. This agrees with observations obtained using preparations from avocado fruit (18, 20).

Several important lipid synthetic functions evidently occur in the endoplasmic reticulum. Linoleate, the major component of all membrane phospholipids (unpublished), appears very rapidly (5 min) in this fraction. The desaturation of oleate to lineoleate may occur in this membrane. This activity has been characterized as microsomal in developing (ripening) safflower seed (19). Also, esterification apparently takes place in the endoplasmic reticulum since ^{14}C-acetate and ^{3}H-glycerol are rapidly incorporated into lipids, especially diglyceride and phosphatidylcholine, in this fraction. The high ratio of ^{3}H/^{14}C which was initially observed in the endoplasmic reticulum also supports this idea. Avocado microsomes are capable of esterification *in vitro* (2).

It has been previously demonstrated that the endoplasmic reticulum is the site of phospholipid synthesis (3). The data presented here show that the individual phospholipids are not synthesized in a concerted manner. Phosphatidylcholine is the most rapidly produced, followed by phosphatidylethanolamine. Phosphatidylinositol is apparently not being synthesized at all in 2-day endosperm. It may be synthesized very slowly as it is in certain cultured animal cells (8) or it may be recycled. There is a potential supply of phosphatidylinositol in the lipid bodies. These observations suggest that membrane assembly is not concerted.

The kinetics of synthesis indicate flow of phospholipid from the endoplasmic reticulum into other membranes. Phospholipids are rapidly synthesized in the endoplasmic reticulum and subsequently appear in the glyoxysomes and mitochondria. Similar kinetics are observed when ^{14}C-choline is provided as the substrate (13). A possible explanation for this flow is that portions of the endoplasmic reticulum become the membranes of other organelles. Another possibility is that individual phospholipid molecules are transported between membranes by specific cytoplasmic carrier proteins (21).

The presence of diacyl-^{14}C-glycerol-^{3}H in the glyoxysomes is indicative of lipid traffic among organelles. Diglyceride is unlikely to be synthesized in the glyoxysomes. Although there is a large proportion of diacyl-^{14}C-glycerol-^{3}H in the organelle, diglyceride is a very minor component on a mass basis (unpublished). Further metabolism of the diacylglycerol would be delayed by the lack of synthetic machinery such as that present in the endoplasmic reticulum. Diglyceride in the glyoxysome membrane may be considered an ephemeral by-product of metabolism. An analogous argument could explain the presence of quantities of free fatty acids in the membranes (unpublished).

The metabolic origin of diglyceride is not defined by these results, although the rapid appearance of diacyl-^{14}C-glycerol-^{3}H gives the impression that direct esterification of glycerol occurs. Animal cells are capable of direct acylation of monoglyceride to form diglyceride (15). Diglyceride is usually thought to be derived from glycerol phosphate and phosphatidic acid (2). Phosphatidic (^{3}H or ^{14}C) acid was not detected in the castor bean studies. Nevertheless, a small, rapidly turning over pool of phosphatidic acid could be responsible for diglyceride production.

These results emphasize the need for enzymic studies to determine the cellular site(s) of fatty acid desaturation and esterification. Also required is a further assessment of the role of the lipid bodies (which have lipid synthetic capacities and contain phospholipids) in membrane synthesis during germination in castor bean.

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