Plant Physiol. (1978) 62, 173–178

Fatty Acid Synthesis in Endosperm of Young Castor Bean Seedlings[1]

Received for publication January 9, 1978 and in revised form March 22, 1978

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ABSTRACT

Enzyme assays on organelles isolated from the endosperm of germinating castor bean (*Ricinus communis*) by sucrose density gradient centrifugation showed that fatty acid synthesis from [^{14}C]malonyl-CoA was localized exclusively in the plastids. The optimum pH was 7.7 and the products was mainly free palmitic and oleic acids. Both NADH and NADPH were required as reductants for maximum activity. Acetyl-CoA, and acyl-carrier protein from *Escherichia coli* increased the rate of fatty acid synthesis, while low O_2 levels suppressed synthesis. In the absence of NADPH or at low O_2 concentration, stearic acid became a major product at the expense of oleic acid. Fatty acid synthesis activity was highest during the first 3 days of germination, preceding the maximum development of mitochondria and glyoxysomes. It is proposed that the plastids are the source of fatty acids incorporated into the membranes of developing organelles.

The most striking metabolic event which occurs in the castor bean endosperm during the first 7 days of growth is the efficient conversion of triglyceride, stored mainly as triricinolein in the spherosomes, into sucrose which is utilized by the developing embryo. This transformation requires the presence of at least two other organelles, the glyoxysomes and the mitochondria. The glyoxysomes convert fatty acids to succinate and generate NADH, while the mitochondria convert succinate to oxaloacetate and generate ATP by coupled oxidation of NADH. The enzymes which synthesize sucrose from oxaloacetate presumably occur in the cytosol.

In the endosperm of ungerminated castor bean seeds, glyoxysomes and mitochondria are barely detectable, but their numbers increase dramatically during the first 5 days of growth (28). The production of these new organelles requires the prior synthesis of membrane constituents, namely protein and phospholipids. Synthesis of the protein most likely occurs by the reassembly of amino acids from degraded protein bodies, while synthesis of most of the phospholipids (2, 13, 17–19, 27) takes place in the ER, beginning with phosphatidic acid synthesis by reaction of glycerol-3-P with fatty acyl-CoA thioesters. Donaldson and Beevers (5) studied the fatty acid composition of phospholipids in the developing organelles of endosperm of germinating castor bean seeds, and showed that the familiar components, palmitic, stearic, oleic, linoleic, and linolenic acids, comprise the membrane fatty acids; ricinoleic, the predominant storage fatty acid, is not present in membranes. Acetate incorporation studies *in vivo* show that the membrane lipids are synthesized *de novo* rather than from storage lipid (4). Thus, the castor bean endosperm, whose major function is to degrade stored fatty acids, must first synthesize another group of fatty acids for inclusion into the membranes of the organelles which degrade the stored lipid.

In this paper, we have investigated the site of synthesis of the fatty acids which are incorporated into developing organelles in germinating castor bean endosperm. Several investigators have previously examined the site of fatty acid synthesis in developing (ripening) castor bean endosperm during the period of rapid storage lipid accumulation, but the literature shows disagreement about the major site of fatty acid synthesis. Harwood and Stumpf (11) showed that the fat fraction had the highest specific activity for fatty acid synthesis, while Zilkey and Canvin (35) and Nakamura and Yamada (20) both reported that the plastids were the most active fraction.

Weaire and Kekwick (30) presented a valuable discussion of the problems encountered in deciding where within the plant cell the process of fatty acid synthesis occurs. They reviewed the experiments showing the role of the chloroplast in fatty acid synthesis in green cells and showed that contradictory reports concerning localization in nongreen cells are probably due to incomplete organelle separation and breakage. They showed that the plastids are the major site of fatty acid synthesis in avocado mesocarp and cauliflower buds and argued that this may be the usual location in plant cells generally.

The data presented here demonstrate that in the endosperm of germinating castor beans, fatty acids are synthesized only in the plastids, and serve as precursors for phospholipid synthesis elsewhere in the cell.

MATERIALS AND METHODS

Materials. [2-^{14}C]Malonyl-CoA (32.1 mCi/mmol) was supplied by New England Nuclear. [1-^{14}C]Acetyl-CoA (54.4 mCi/mmol) and [^{14}C]sodium bicarbonate (55.7 mCi/mmol) were purchased from ICN. Unlabeled malonyl CoA, acetyl-CoA, and CoA were obtained from PL Biochemicals, Inc., Milwaukee, Wisconsin. NADPH, NADH, RuDP[3] (disodium salt), and ATP were from Sigma. ACP was partially purified (about 10% purity) from *Escherichia coli* up to the $(NH_4)_2SO_4$ fractionation and acid precipitation step according to Majerus *et al.* (15). The final protein concentration was about 3.1 mg/ml.

Growth Conditions. Castor bean seeds (*Ricinus communis* var. Hale) were soaked for 24 hr in cold running tap water, then planted in moist Vermiculite and germinated in the dark at 30 C in a humidified growth chamber. The time of planting was considered to be time zero in developmental studies.

Homogenization and Fractionation of Cellular Components. The castor beans were harvested after 3 to 4 days when the average weight of an endosperm, with testae and cotyledons removed, was between 0.63 and 0.80 g. Fifteen endosperms were chopped with

[1] This work was supported by Contract EY-76-S-03-0034 from the Energy Research and Development Administration.

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[3] Abbreviations: RuDP: ribulose diphosphate; ACP: acyl-carrier protein.

razor blades for 20 min in 10 ml of a medium containing 150 mM Tricine (pH 7.5), 13% (w/w) sucrose, and 1 mM EDTA. The resulting homogenate was filtered through three layers of nylon cloth and 10 to 12 ml were layered directly onto a sucrose gradient. The sucrose gradient was prepared in centrifuge tubes (2.5 × 7.5 cm) with a 3-ml cushion of 60% (w/w) sucrose, 40 ml of a linear gradient from 60 to 15% sucrose, and 5 ml of 15% sucrose. All sucrose solutions contained 1 mM EDTA (pH 7.5). The gradients were centrifuged for 90 min at 21,000 rpm in a Beckman SW 25.2 rotor. After centrifugation the fat fraction was removed from the top of the gradient and sonicated for 3 to 4 sec in 40 ml of the homogenizing medium. The fat fraction was recentrifuged for 15 min at 21,000 rpm in the SW 25.2 rotor, removed from the top of the centrifuge tube, and resuspended in 1.2 ml of the homogenizing medium. The washed and resuspended fat fraction constituted fraction 1 of the sucrose gradient. The remaining gradient was separated into 1.2-ml portions with a gradient fractionator. The density of each fraction was determined by refractometry.

For the study of changes in enzyme activity with age, the endosperms of 20 seeds from each day of germination were homogenized with a mortar and pestle in 15 to 25 ml of homogenizing medium. Each extract was strained through nylon cloth and centrifuged for 10 min at 12,000*g*. The fat layer was removed and the supernatant decanted into another tube and centrifuged at 140,000*g* for 1 hr. The supernatant was recombined with the 12,000*g* pellet and the resulting suspension was adjusted to 30 ml with homogenizing medium and assayed for enzyme activity.

Enzyme Assays. Fumarase was measured spectrophotometrically by the method of Racker (22). Catalase was assayed according to Lück (14). The assay for acyltransferase has been described previously (27).

Fatty acid synthetase was measured by the incorporation of [2-^{14}C]malonyl-CoA into chloroform-soluble saponified products. The reaction mixture contained 250 mM TES (pH 7.7), 0.05 mM [2-^{14}C]malonyl-CoA (adjusted to 2.25 mCi/mmol with unlabeled malonyl-CoA), 0.3 mM NADPH, 0.3 mM NADH, 0.1 mM acetyl-CoA, 15 μg of ACP, and the appropriate endosperm preparation (0–40 μg of protein) in a final volume of 0.2 ml. The reaction was initiated by the addition of enzyme and allowed to proceed at 30 C. After 20 min the reaction was terminated by the addition of 0.04 ml of 8 N NaOH and heated at 80 C for 30 min. The mixture was cooled and 0.04 ml of concentrated HCl was added, followed by 1 ml of distilled H_2O. Free fatty acids were extracted with 2 ml of $CHCl_3$-CH_3OH solution (2:1, v/v) and centrifuged to separate the phases. The bottom $CHCl_3$ phase was washed once with 1 M malonic acid and recentrifuged. The $CHCl_3$ layer was transferred to a scintillation vial, evaporated to dryness, and assayed for ^{14}C using 0.5% (w/v) PPO in toluene. All analyses of radioactivity were made in a Beckman LS-230 counter and all counts were corrected for instrument efficiency. Reaction rates were expressed as nmol of [2-^{14}C]malonyl-CoA incorporated into fatty acids/min.

RuDP carboxylase was assayed by a modification of the procedure of Benedict (1), which measures the incorporation of $^{14}CO_2$ into acid-stable products in the presence of enzyme and RuDP. The reaction mixture contained 150 mM Tris (pH 7.5), 15 mM $MgCl_2$, 1 mM RuDP, 50 mM $NaH^{14}CO_3$ (0.04 mCi/mmol), and 0.1 ml of enzyme in a total volume of 0.5 ml. The reaction was stopped after 30 min at 30 C by the addition of 0.5 ml of glacial acetic acid. The mixture was boiled for 10 min on a hot plate, cooled, added to 4 ml of 0.5% (w/v) PPO in naphthalene-dioxane (100 g/l), and assayed for ^{14}C.

Acetyl-CoA carboxylase was assayed by the same method as for RuDP carboxylase, except that the reaction mixture contained 120 mM Tris (pH 7.5), 150 mM $MgCl_2$, 1 mM acetyl-CoA prepared according to Ochoa (21), 50 mM $NaH^{14}CO_3$ (0.04 mCi/mmol), 2 mM ATP, and 0.1 ml of enzyme in a final volume of 0.5 ml.

Protein was measured by a modified Lowry method (7).

Analysis of Products. When the products of fatty acid synthesis were to be analyzed, the reaction mixture contained 250 mM TES (pH 7.7), 1.6 μM [2-^{14}C]malonyl-CoA (32.1 mCi/mmol), 0.3 mM NADPH, 0.3 mM NADH, 0.1 mM acetyl-CoA, 75 μg of ACP, 150 μg of enzyme in a final volume of 1 ml. The reaction was allowed to proceed for 2 hr. The method of Mancha *et al.* (16) was used to determine the distribution of ^{14}C in acyl-ACP, acyl-CoA, and neutral and polar lipid. For the analysis of fatty acids, the reaction mixture was saponified and extracted by the method described under "Enzyme Assays," esterified with diazomethane in ethyl ether, and injected onto a gas chromatography column (2 m × 2.5 mm i.d.) containing 10% Silar 10C on 100/120 mesh Gas-chrom Q. The column was operated isothermally for 6 min at 170 C, then programmed from 170 to 250 C at 5 C/min. The products which separated on the column were trapped in glass capillary tubes at 15-sec intervals, transferred to scintillation vials, and assayed for ^{14}C using 0.5% (w/v) PPO in toluene.

RESULTS

Intracellular Location of Fatty Acid Synthesis. Figure 1A shows the distribution of soluble and particulate protein after centrifuging an extract of endosperm tissues from 4-day-old castor beans on a sucrose gradient. The ER was identified by acyltransferase at density 1.13 g/cm^3 and the mitochondria by fumarase at 1.19 g/cm^3 (Fig. 1D). Catalase showed the location of the glyoxysomes at 1.24 g/cm^3 and RuDP carboxylase was used as a marker for the plastids, 1.22 g/cm^3 (Fig. 1C). The high activity of RuDP carboxylase, a stromal enzyme, in the soluble region of the gradient (fractions 2–13) reflects the fragility of the plastids, and indicates that only about 22% of the plastids remained intact during the organelle isolation procedure.

The synthesis of fatty acids from malonyl-CoA occurred in a single peak at 1.22 g/cm^3 coinciding with the RuDP carboxylase activity of the plastids (Fig. 1). There was no synthesis of fatty acids in the fractions from the soluble region of the gradient that showed RuDP carboxylase activity. If the fatty acid synthetase is a large soluble complex as it is in mammalian systems, then this experiment suggests that the complex was disrupted or otherwise inactivated upon breakage of the plastids. The possibility that some or all of the enzymes of fatty acid synthesis are associated with the membrane of the plastid cannot be ruled out. It should be noted that the washed and resuspended fat fraction (Fig. 1B, fraction 1) was unable to incorporate [2-^{14}C]malonyl-CoA into fatty acids. A fat fraction which had not been washed or sonicated was also unable to synthesize fatty acids.

Another enzyme involved in fatty acid biosynthesis, acetyl-CoA carboxylase, was also measured (Fig. 1B). Its activity paralleled that of the stromal enzyme, RuDP carboxylase, suggesting that acetyl-CoA carboxylase from germinating castor bean endosperm was also localized in the stroma of plastids as it is in developing seeds (24).

Fatty Acid Synthesis in the Plastids. Plastids isolated from endosperm of 4-day castor beans were used to determine the characteristics of the fatty acid synthetase. Under the conditions described under "Materials and Methods," the rate of incorporation of [2-^{14}C]malonyl-CoA into fatty acids was constant for about 30 min and declined to nearly zero by 60 min. Nearly maximum rates of fatty acid synthesis were obtained when the malonyl-CoA concentration was 20 μM. No incorporation into fatty acids occurred in the absence of plastid protein and the rate was proportional to amount of protein up to 0.3 mg/ml of reaction mixture. In this range the rate of malonyl-CoA incorporation was 18 nmol/hr · mg of protein. The pH optimum for the reaction was 7.7 (Fig. 2).

Both NADH and NADPH were required for maximum fatty acid synthetase activity. Figure 3A demonstrates the effect of increasing NADPH concentration in the presence or absence of NADH. The upper curve shows that in the presence of 0.3 mM

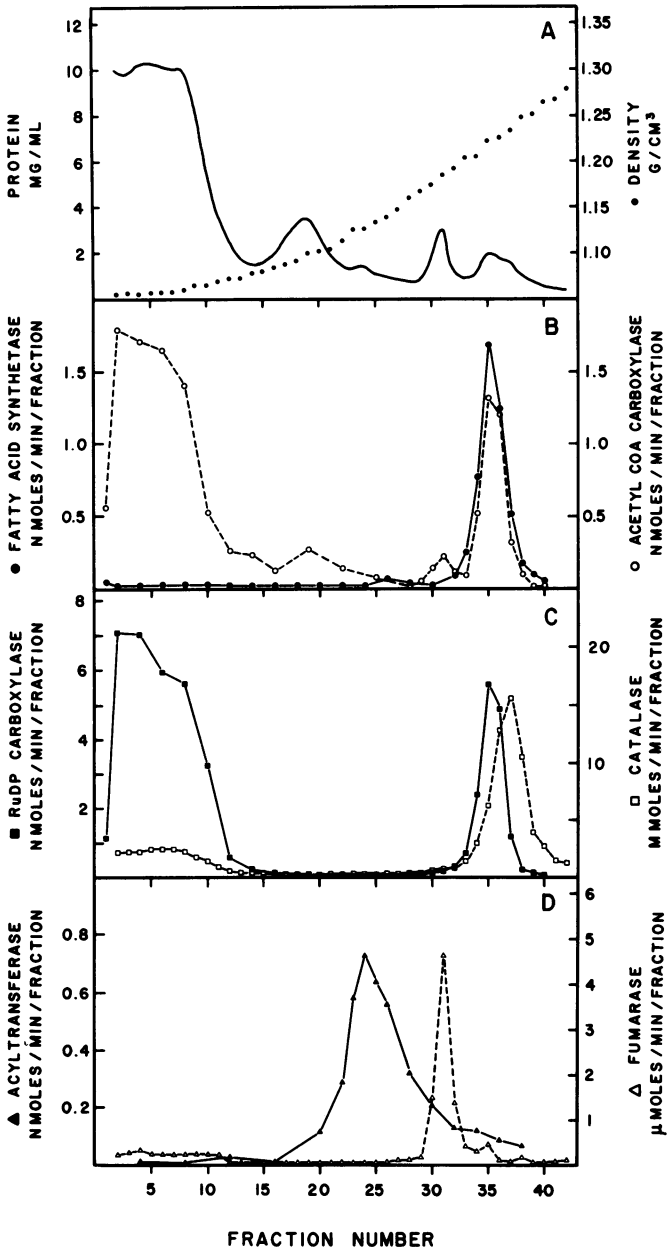

FIG. 1. Distribution of protein and enzyme from 4-day castor bean endosperm on a continuous sucrose density gradient. A: protein (——) and density (· · · · ·); B: fatty acid synthetase (●) and acetyl-CoA carboxylase (○); C: plastid marker RuDP carboxylase (■) and glyoxysome marker catalase (□); D: ER marker acyltransferase (▲) and mitochondria marker fumarase (△).

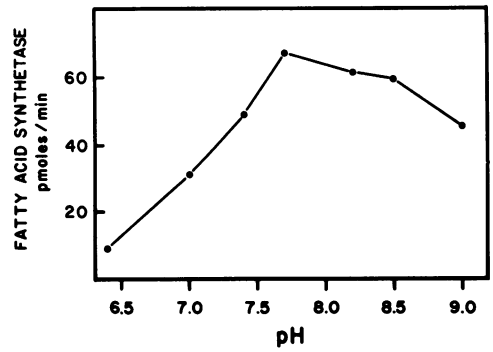

FIG. 2. Fatty acid synthesis activity as a function of pH. Reaction conditions were those described under "Materials and Methods" except where varied as indicated. Plastids isolated from 4-day castor bean endosperm were the enzyme source.

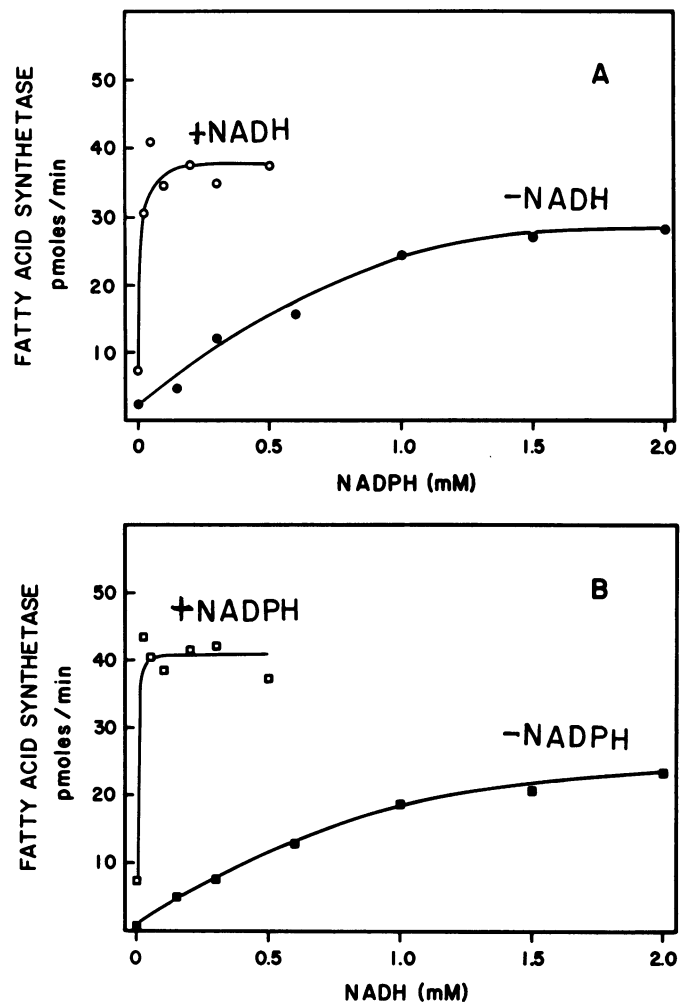

FIG. 3. A: effect of NADPH concentration on fatty acid synthesis activity in the presence (○) of 0.3 mM NADH or in the absence (●) of NADH. B: effect of NADH concentration on fatty acid synthesis activity in the presence (□) of 0.3 mM NADPH or in the absence (■) of NADPH. Reaction conditions were those described under "Materials and Methods" except where varied as indicated. Plastids isolated from 4-day castor bean endosperm were the enzyme source.

NADH, the maximum rate of fatty acid synthesis was already attained when the concentration of NADPH was 0.05 mM. When NADH was absent (lower curve), the reaction proceeded very slowly. The rate could be increased by higher concentrations of NADPH, but the high rate of synthesis obtained with both NADH and NADPH present could not be achieved by NADPH alone. Similarly, Figure 3B shows the effect of increasing NADH concentration in the presence or absence of NADPH. Again, the upper curve (0.3 mM NADPH present) shows that a high rate of fatty acid synthesis occurred when both NADPH and NADH were present, but the highest rate of synthesis which could be achieved with NADH alone (lower curve) was only half the rate obtained when both reductants were present. Thus, in germinating castor bean endosperm both NADPH and NADH are required as direct reductants for fatty acid synthesis. Similar observations were reported by Drennan and Canvin (6) and Nakamura and Yamada (20) with developing castor bean endosperm.

When ACP from *E. coli* was omitted from the reaction mixture, the reaction rate decreased by 30%. We took this as evidence that

the ACP thioester of malonate was the true substrate for the fatty acid synthetase reaction, and that a malonyl-CoA transacylase was present in the plastids to convert malonyl-CoA to malonyl-ACP. Native ACP was apparently present in the plastids, however, since 70% of the fatty acid-synthesizing activity remained in the absence of added ACP. Maximum fatty acid synthesis was also dependent upon the presence of acetyl-CoA, for synthesis was decreased by 47% in its absence. The acetyl-CoA was presumably converted to acetyl-ACP by a transacylase and utilized in the initial condensing reaction with malonyl-CoA to yield acetoacetyl-ACP and CO_2.

Analysis of Products. When the products of [2-^{14}C]malonyl-CoA incorporation were analyzed for the distribution of ^{14}C among acyl-ACP, acyl-CoA, and neutral and polar lipid, more than 99% of the ^{14}C was found in the neutral and polar lipid fraction and virtually none was found in acyl-ACP or acyl-CoA. The nature of the labeled fraction was examined further in another experiment. Unsaponified reaction products were extracted into chloroform and separated by TLC (petroleum ether-ethyl ether-acetic acid, 70:30:1, v/v). The results showed that 83% of the ^{14}C corresponded to free fatty acids, suggesting that an active thioesterase was present in the plastids which hydrolyzed acylthioesters to free fatty acids. Shine *et al.* (25) likewise observed the synthesis of free fatty acids, and demonstrated that extracts of maturing safflower seeds, avocado mesocarp, and spinach stroma contained highly active thioesterases which converted acyl-ACPs to free fatty acids.

In one experiment [1-^{14}C]acetyl-CoA was substituted for [2-^{14}C]malonyl-CoA and tested as a substrate for fatty acid synthesis. Cofactors for the acetyl-CoA carboxylase reaction, HCO_3^-, ATP, Mg^{2+}, and Mn^{2+}, were also included. The total incorporation of [1-^{14}C]acetyl-CoA into chloroform-soluble products after 2 hr was only 30% of that when [2-^{14}C]malonyl-CoA was used. In addition, only 44% of the ^{14}C was found in free fatty acids. Most of the ^{14}C was in a mixture of unsaponifiable products. For this reason [2-^{14}C]malonyl-CoA was used in all other experiments.

Table I shows the distribution of fatty acids synthesized from [2-^{14}C]malonyl-CoA by the plastids. Under optimum synthesizing conditions (both NADPH and NADH present) the major products were palmitic acid and a C-18 monounsaturated fatty acid, with the latter predominating. The C-18 monounsaturated acid was identified as oleic acid after isolating the methyl ester by argentation TLC and subjecting it to oxidative ozonolysis and reesterification. The resulting products had retention times on a Silar 10C gas chromatography column which were equivalent to the retention times of a C-9 monocarboxylic and a C-9 dicarboxylic acid, confirming the position of the double bond at carbon 9.

When NADH was omitted from the reaction and the NADPH concentration was increased (Table I), there was a slight increase in oleic acid synthesis, but when NADPH was omitted and NADH was increased, there was a sharp reduction in oleic acid synthesis and an accumulation of stearic acid. Oleic acid synthesis was also altered by lowering the O_2 concentration. Table II shows that when O_2 was removed from the reaction, the total incorporation of [2-^{14}C]malonyl-CoA into lipid was lowered, but the proportion of stearic acid was increased, at the expense of oleic acid. Similar effects of NADH, NADPH, and O_2 were observed by Drennan and Canvin (6) in their study of fatty acid synthesis in developing (ripening) castor bean endosperm. However, in the present experiments, the anaerobic treatment also led to some loss of total fatty acid synthesis, an effect also observed by Yamada (31).

From these results and from the pattern established by previous workers (6, 11, 20, 25, 29, 35), we concluded that the plastids from the endosperm of germinating castor bean readily synthesized C_{16} and C_{18} acyl-ACPs which were rapidly hydrolyzed to free fatty acids. Stearyl-ACP was converted to oleoyl-ACP by an active desaturase reaction in the plastids in which NADPH and O_2 were intimately involved. Although NADPH was the preferred reductant, some oleic acid was synthesized in the presence of NADH alone, indicating that NADH could substitute, but less effectively, for NADPH in the desaturation of stearyl-ACP to oleoyl-ACP.

Table I. Incorporation of [2-^{14}C]Malonyl-CoA into Fatty Acids by Plastids from Germinating Castor Bean Endosperm and the Effect of NADPH and NADH.

Reaction conditions were those described in Materials and Methods except where varied as indicated. The plastids were obtained from 4-day old castor bean endosperm.

Cofactors present	Concentration (mM)	[2-^{14}C]Malonyl-CoA Incorporated (nmoles/2 hr)	Distribution of ^{14}C in newly synthesized fatty acids (%)				
			14:0	16:0	18:0	18:1	18:2
NADPH, NADH	0.3 0.3	1.19	<1	22	2	74	2
NADPH	1.5	1.10	<1	18	3	79	0
NADH	1.5	1.05	<1	27	22	51	0

Table II. Effect of O_2 on the Incorporation of [2-^{14}C]Malonyl-CoA into Fatty Acids by Plastids from Germinating Castor Bean Endosperm.

Reaction conditions were those described in Materials and Methods. The plastids were obtained from 3-day old castor bean endosperms.

Reaction Conditions	[2-^{14}C]Malonyl-CoA Incorporated (nmoles/2 hr)	Distribution of ^{14}C in newly synthesized fatty acids			
		14:0	16:0	18:0	18:1
Complete	0.85	<1	16	2	82
-O_2 [a]	0.14	<1	16	63	21

[a] N_2 was bubbled through the reaction mixture for 30 min prior to the addition of enzyme.

Developmental Changes in Fatty Acid Synthesis Activity. Figure 4A shows the changes in the capacity to synthesize fatty acids during early stages of growth. The crude endosperm homogenate, containing intact organelles, was used for the enzyme assays. However, an additional experiment, in which the organelles were separated from 2-day endosperm, showed that the ability to bring about synthesis was restricted to the plastid fraction, as it is at 4 days. Clearly the rate of fatty acid synthesis in the endosperm is highest during the early stages of development (1–3 days). Independent studies by Glew (8) and Donaldson (4) had shown previously that the incorporation of [^{14}C]acetate *in vivo* was highest during the 2nd day of growth.

By contrast, the rapid development of mitochondria and glyoxysomes, measured by fumarase and catalase, respectively (Fig. 4B), did not begin until the 3rd day. Hence, the fatty acids synthesized by the plastids could have been used for the synthesis of the membranes of the mitochondria and glyoxysomes. Figure 4A also shows that various plastid enzymes did not develop concurrently. Fatty acid synthesis was most active in the less mature plastids, but RuDP carboxylase activity developed only after 3 days. Figure 4C shows the average weight of an endosperm on each day of germination and was used as a guide to the physiological age of the seedling.

DISCUSSION

Previous work with organelles isolated from the endosperm of germinating castor bean seedlings has shown that during the massive utilization of stored fat, β-oxidation of fatty acids occurs in glyoxysomes (3, 12). In the present experiments with organelles from the same tissue we have investigated the synthesis of long chain fatty acids from malonyl-CoA. Yamada (31) has previously shown that 3,000*g* particles from germinating castor bean endosperm could incorporate [1-^{14}C]acetate into fatty acids. Our results extend the work of Yamada by showing that fatty acids were synthesized exclusively in the plastids, and that maximum activity occurred during the first 3 days of germination, prior to the maximum development of mitochondria and glyoxysomes. It is also during this period that the phospholipid-synthesizing capacity of the ER is at a maximum (2, 27). The order in which the various enzymes appear makes it reasonable to propose a general scheme of events in the germinating castor bean endosperm. Immediately

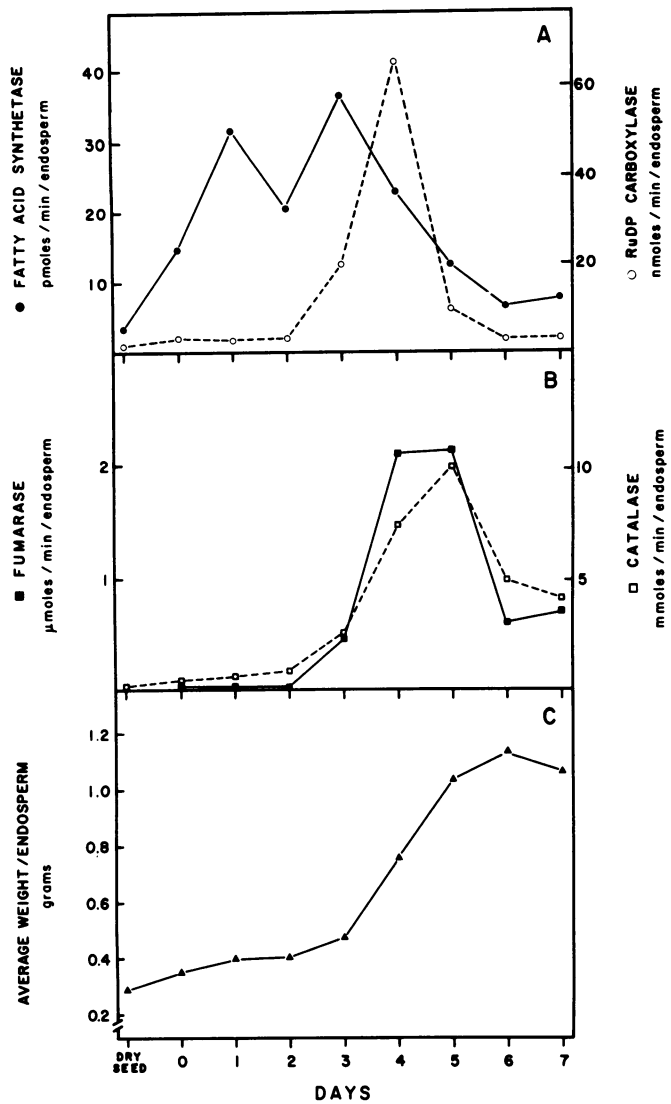

FIG. 4. Effect of age of castor bean endosperm on the activity of various enzymes in the crude homogenate. A: fatty acid synthetase (●) and plastid enzyme RuDP carboxylase (○); B: mitochondrial enzyme fumarase (■) and glyoxysomal enzyme catalase (□); C: variation in average weight (▲) of a castor bean endosperm with age. Reaction conditions were those described under "Materials and Methods."

after inhibition, the plastids present in the dry seed begin to synthesize free fatty acids, mainly palmitic and oleic. The fatty acids are transported out of the plastids to the ER where they are activated to acyl-CoA thioesters by a thiokinase enzyme (32). An acyltransferase enzyme in the ER (27) catalyzes the esterification of the acyl-CoA thioesters to glycerol-3-P to form phosphatidic acid, which is subsequently converted by enzymes in the ER (2, 13, 17, 18, 19) to all of the other membrane phospholipids. Oleate is most likely further desaturated in the ER (4), but whether the substrate for desaturation is oleoyl-CoA (29) or a β-oleoyl phospholipid (9) is a subject of current discussion. The phospholipids thus formed in the ER are available for incorporation into the membranes of the developing mitochondria and glyoxysomes, which then function in the conversion of storage lipid to carbohydrate.

The plastids have now been shown to be active sites of fatty acid synthesis in both developing (20, 35) and germinating castor bean endosperm. In the endosperm of germinating seeds synthesis is confined to the plastids. In developing endosperm tissue, where massive amounts of fat are produced, particulate inclusions are apparently an important site of fatty acid synthesis (10, 11) but the fat fraction from the endosperm of germinating seeds did not show this activity.

The source of carbon for fatty acid synthesis in the developing endosperm is sucrose transported from the leaves as a photosynthetic product, and Yamada has shown that the isolated plastids were able to convert sucrose to fatty acids (33, 34). Simcox *et al.* (26) have measured the subcellular distribution of the enzymes in the developing endosperm which convert sucrose to pyruvate. They concluded that sucrose was converted to hexose-P in the cytoplasm and the pathway from hexose-P to pyruvate was in the plastids. They proposed that NADPH was generated in the plastids in the pentose-P pathway. Pyruvate dehydrogenase, which gives acetyl-CoA as a product, has also been demonstrated in the plastids (23). Thus, the glycolytic and pentose-P pathways could account for the NADH, NADPH, ATP, and acetyl-CoA needed for fatty acid synthesis in the developing endosperm. Similar information is now needed regarding the source of these precursors in the endosperm of the germinating castor bean.

LITERATURE CITED

1. BENEDICT CR 1973 The presence of ribulose-1,5-diphosphate carboxylase in the non-photosynthetic endosperm of germinating castor beans. Plant Physiol 51: 755–759
2. BOWDEN L, JM LORD 1975 Development of phospholipid synthesizing enzymes in castor bean endosperm. FEBS Lett 49: 369–371
3. COOPER TG, H BEEVERS 1969 β-Oxidation in glyoxysomes from castor bean endosperm. J Biol Chem 244: 3514–3520
4. DONALDSON RP 1976 Membrane lipid metabolism in germinating castor bean endosperm. Plant Physiol 57: 510–515
5. DONALDSON RP, H BEEVERS 1977 Lipid composition of organelles from germinating castor bean endosperm. Plant Physiol. 59: 259–263
6. DRENNAN CH, DT CANVIN 1969 Oleic acid synthesis by a particulate preparation from developing castor oil seeds. Biochim. Biophys. Acta 187: 193–200
7. GERHARDT B, H BEEVERS 1968 Influence of sucrose on protein determination by the Lowry procedure. Anal Biochem 24: 337–339
8. GLEW RH 1968 Developmental aspects of lipid metabolism in the developing and germinating castor bean seed. PhD thesis. University of California, Davis
9. GURR MI 1974 The biosynthesis of unsaturated fatty acids. *In* T Goodwin, ed, MTP International Review of Science, Biochemistry of Lipids, Vol 4 Chap 5. University Park Press, Baltimore, pp 181–235
10. HARWOOD JL, A SODJA, PK STUMPF, AR SPURR 1971 On the origin of oil droplets in maturing castor bean seeds, *Ricinus communis*. Lipids 6: 851–854
11. HARWOOD JL, PK STUMPF 1972 Fat metabolism in higher plants. XLIX. Fatty acid biosynthesis by subcellular fractions of higher plants. Lipids 7: 8–19
12. HUTTON D, PK STUMPF 1969 Fat metabolism in higher plants. XXXVII. Characterization of the β-oxidation systems from maturing and germinating castor bean seeds. Plant Physiol 44: 508–516
13. LORD JM, T KAGAWA, H. BEEVERS 1972 Intracellular distribution of enzymes of the cytidine diphosphate choline pathway in castor bean endosperm. Proc Nat Acad Sci USA 69: 2429–2432
14. LÜCK H 1965 Catalase. *In* HU Bergmeyer, ed, Methods of Enzymatic Analysis. Academic Press, New York, pp 885–894
15. MAJERUS PW, AW ALBERTS, PR VAGELOS 1969 Acyl carrier protein from *Escherichia coli*. Methods Enzymol 14: 43–50
16. MANCHA M, GB STOKES, PK STUMPF 1965 Fat metabolism in higher plants. LXV. The determination of acyl-acyl carrier protein and acyl coenzyme A in a complex lipid mixture. Anal Biochem 68: 600–608
17. MOORE TS JR 1974 Phosphatidylglycerol synthesis in castor bean endosperm. Plant Physiol 54: 164–168
18. MOORE TS JR 1976 Phosphatidylcholine synthesis in castor bean endosperm. Plant Physiol 57: 382–386
19. MOORE TS JR, JM LORD, T KAGAWA, H BEEVERS 1973 Enzymes of phospholipid metabolism in the endoplasmic reticulum of castor bean endosperm. Plant Physiol 52: 50–53
20. NAKAMURA Y, M YAMADA 1974 Long chain fatty acid synthesis in developing castor bean seeds. I. The operation of the path from acetate to long chain fatty acids in a subcellular particulate system. Plant Cell Physiol 15: 37–48
21. OCHOA S 1955 Crystalline condensing enzyme from pig heart. Methods Enzymol 1: 685–694
22. RACKER E 1950 Spectrophotometric measurements of the enzymatic formation of fumaric acid and *cis*-aconitic acids. Biochim Biophys Acta 4: 211–214
23. REID EE, P THOMPSON, CR LYTTLE, DT DENNIS 1977 Pyruvate dehydrogenase complex from higher plant mitochondria and proplastids. Plant Physiol 59: 842–848
24. SCOTT-BURDEN T, DT CANVIN 1975 The effect of Mg^{2+} on density of proplastids from the developing castor bean and the use of acetyl-CoA carboxylase activity for their cytochemical identification. Can J Bot 53: 1371–1376
25. SHINE WE, M MANCHA, PK STUMPF 1976 Fat metabolism in higher plants. LXVI. The function of acyl thioesterase in the metabolism of acyl-coenzymes A and acyl-acyl carrier proteins. Arch Biochem Biophys 172: 110–116
26. SIMCOX PD, EE REID, DT CANVIN, DT DENNIS 1977 Enzymes of the glycolytic and pentose

phosphate pathways in proplastids from the developing endosperm of *Ricinus communis* L. Plant Physiol 59: 1128–1132

27. VICK B, H BEEVERS 1977 Phosphatidic acid synthesis in castor bean endosperm. Plant Physiol 59: 459–463
28. VIGIL EL 1970 Cytochemical and developmental changes in microbodies (glyoxysomes) and related organelles of castor bean endosperm. J Cell Biol 46: 435–454
29. VIJAY IK, PK STUMPF 1971 Fat metabolism in higher plants. XLVI. Nature of the substrate and the product of oleyl coenzyme A desaturase from *Carthamus tinctorius*. J Biol Chem 246: 2910–2917
30. WEAIRE PJ, RGO KEKWICK 1975 The synthesis of fatty acids in avocado mesocarp and cauliflower bud tissue. Biochem J 146: 425–437
31. YAMADA M 1966 Fatty acid synthesis by particulate fraction from germinating castor bean seeds. Bot Mag Tokyo 79: 619–625
32. YAMADA M, PK STUMPF 1965 Fat metabolism in higher plants. XXIV. Soluble β-oxidative system from germinating seeds of *Ricinus communis*. Plant Physiol 40: 653–658
33. YAMADA M, Q USAMI 1975 Long chain fatty acid synthesis in developing castor bean seeds. IV. The synthetic system in proplastids. Plant Cell Physiol 16: 879–884
34. YAMADA M, Q USAMI, K NAKAJIMA 1974 Long chain fatty acid synthesis in developing castor bean seeds. II. Operation of the path from sucrose to long chain fatty acids in a subcellular particulate system. Plant Cell Physiol 15: 49–58
35. ZILKEY BF, DT CANVIN 1972 Localization of oleic acid biosynthesis enzymes in the proplastids of developing castor endosperm. Can J Bot 50: 323–326