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# A Preliminary Analysis of Fatty Acid Synthesis in Pea Roots[1]

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## ABSTRACT

**Subcellular fractions from pea (*Pisum sativum* L.) roots have been prepared by differential centrifugation techniques. Greater than 50% of the recovered plastids can be isolated by centrifugation at 500*g* for 5 minutes. Plastids of this fraction are largely free from mitochondrial and microsomal contamination as judged by marker enzyme analysis. *De novo* fatty acid biosynthesis in pea roots occurs in the plastids. Isolated pea root plastids are capable of fatty acid synthesis from acetate at rates up to 4.3 nanomoles per hour per milligram protein. ATP, bicarbonate, and either $Mg^{2+}$ or $Mn^{2+}$ are all absolutely required for activity. Coenzyme A at 0.5 millimolar improved activity by 60%. Reduced nucleotides were not essential but activity was greatest in the presence of 0.5 millimolar of both NADH and NADPH. The addition of 0.5 millimolar glycerol-3-phosphate increased activity by 25%. The *in vitro* and *in vivo* products of fatty acid synthesis from acetate were primarily palmitate, stearate, and oleate, the proportions of which were dependent on experimental treatments. Fatty acids synthesized by pea root plastids were recovered in primarily phosphatidic acid and diacylglycerol or as water soluble derivatives and the free acids. Lesser amounts were found in phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and monogalactosyldiacylglycerol.**

Fatty acid biosynthesis and glycerolipid metabolism have been extensively studied in a variety of plant tissues and organelles, particularly leaves and developing or germinating oilseeds. Roots, however, have largely been ignored as a tissue for such studies. A single noteworthy work by Mazliak *et al.* (6) investigated fatty acid biosynthesis in mitochondrial preparations from a variety of nonphotosynthetic tissues. Among these were the roots of broad bean (*Vicia faba*) and wolf bean (*Lupinus albus*). Although contrary to what is now generally accepted, these workers reported that plant mitochondria were capable of fatty acid biosynthesis and that such activity was dependent on the presence of suitable respiratory substrates and the usual cofactors for fatty acid biosynthesis (*e.g.* ATP, NADPH, CoA, etc.). However, no mention was made regarding the role of the plastid in the biosynthesis of fatty acids in roots. We report here our findings on the subcellular localization, cofactor requirements, and products of fatty acid biosynthesis in pea root organelles and tissues.

## MATERIALS AND METHODS

**Pea Germination.** Pea seeds (*Pisum sativum*, cv Improved Laxton's Progress; Stokes Seed Co., St. Catharines, Ontario) were soaked overnight in running tap water. Imbibed seeds were surface-sterilized for 5 min in 5% (v/v) commercially obtained hypochlorite solution ('Javex'), thoroughly rinsed with sterile water, and allowed to germinate at 25°C in complete darkness under sterile conditions on filter paper moistened with 5–7 ml water in glass Petri dishes. Germination periods were 3 d for *in vivo* studies and 7 d for organelle isolations.

**Tissue Homogenization and Cellular Fractionation.** Ten to 20 g fresh weight of root tissue (from 80 g dry seed) varying from 2 to 5 cm in length were thoroughly homogenized in a chilled mortar and pestle until no intact tissue remained (approximately 5 min) in a homogenization medium consisting of 0.1 M Tricine buffer (pH 7.9), 0.46 M sucrose, and 1 mM dithiothreitol. The ratio of tissue fresh weight to volume of homogenization medium was 1 g to 2 ml. The homogenate was filtered through 2 layers of fine nylon cloth (0.25 × 0.25 mm openings) by gravity only. The filtrate thus obtained was essentially free from intact tissues and cells but did contain some cell wall and nuclear debris as judged by light microscopy. The crude filtrate was centrifuged at 500*g* for 5 min in a Sorvall SS-34 rotor. The 500*g* supernatant was carefully removed and centrifuged at 10,000*g* for 30 min in the same rotor. The 10,000*g* supernatant was then centrifuged at 100,000*g* in a Beckman L65 rotor for 1 h. Pellets of subcellular fractions were resuspended in a suitable volume (0.8–2.4 ml) of fresh homogenization buffer. All manipulations were performed on ice baths or at 4°C. The crude filtrate, resuspended pellets, and 100,000*g* supernatant were all used for enzymic analyses.

***In Vitro* Fatty Acid Synthesis and Product Analysis.** Fatty acid synthesis from [$^{14}$C] acetate was measured at 25°C by incubating 0.24 ml of each subcellular fraction for 1 h in a final reaction volume of 0.5 ml containing the following: 10 mM $KHCO_3$, 4 mM each of ATP, $MgCl_2$, and $MnCl_2$, 0.5 mM each of NADPH, NADH, CoA, glycerol-3-phosphate, and 0.14 mM sodium[1-$^{14}$C] acetate (1 $\mu$Ci, 14.7 $\mu$Ci/$\mu$mol). For total fatty acid analysis, reactions were terminated by the addition of 0.1 ml 8 N NaOH and then heated at 80°C for 1 h. The mixture was cooled, 0.1 ml 12 N HCl was added, and the free fatty acids were extracted as described by Mudd and DeZacks (10). For analysis of polar lipids synthesized from radioactive fatty acids, reactions were terminated by the addition of 3 ml chloroform/methanol/acetic acid (1:2:0.1, v/v) and the lipids extracted as indicated above. The radioactivity of hydrolyzable water soluble fatty acid derivatives (presumably acyl-CoAs and acyl-ACPs) of the initially partitioned aqueous phase of the extraction was determined as follows. One hundred $\mu$g of carrier palmitic acid in 100 $\mu$l benzene was added to the aqueous phase which was then treated with 8 N NaOH and 12 N HCl in volumes proportional to those described for total fatty acid analyses. Fatty acids released were then extracted three times with hexane. Radioactive polar lipids of the chloroform phase were separated by thin layer chromatography using a double solvent system similar to that of Sparace and Mudd (14), except that the second solvent system was chloroform/acetone/methanol/acetic acid/water (50:20:10:15:5, v/v). Regions of radioactivity were localized by autoradiography, and the radioactivity was measured. When necessary, methyl esters of fatty acids were prepared with $BF_3$ in methanol as described

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by Bishop and Smillie (2) and analyzed by GLC using a Varian model 3400 gas chromatograph equipped with a 1.8 m × 4 mm glass column packed with 10% CSP-509 on Chromosorb W operated isothermally at 185°C. A stream splitter diverted approximately 75% of the effluent prior to a flame ionization detector to a Packard model 894 gas proportional counterradioactivity detector. Radioactivity and mass were quantified with a Varian model 4290 dual channel integrator. All other measurements of sample radioactivity were made using Beckman HP/b scintillation cocktail counted with a LKB model 1219 Rackbeta scintillation counter.

***In Vivo* Fatty Acid Biosynthesis.** One cm tips of 3 d old pea roots were excised and cut into segments of 2 to 3 mm. Segments from 10 root tips were transferred to flat-bottomed vials of 1 cm diameter each containing 2.2 $\mu$Ci sodium[1-$^{14}$C]acetate (40.6 $\mu$Ci/$\mu$mol) in 150 $\mu$l of 10 mM $KH_2PO_4$ buffer (pH 6.5). Under these conditions, root tissue segments were one layer thick and only partially submerged to allow for aeration. Tissues were incubated at room temperature (22–23°C) for an appropriate length of time, after which the bathing medium was removed and the root tissue homogenized in 3 ml of chloroform/methanol/acetic acid (1:2:0.1, v/v) in a Potter ground glass homogenizer. Lipids were extracted and methyl esters prepared and analyzed as described above.

**Marker Enzymes, Protein Measurement, and Reagents.** Marker enzymes used for identification of subcellular fractions were particulate triose phosphate isomerase for the plastids (1), fumarase for mitochondria (12), and NADPH-Cyt *c* reductase for the endoplasmic reticulum (4). Marker enzymes for other subcellular organelles such as the plasma membrane, tonoplast, golgi bodies, and microbodies were not performed since these organelles are not commonly associated with fatty acid synthesis and lipid metabolism. Protein measurements were made according to Lowry *et al.* (5). Organic cofactors were obtained from the Sigma Chemical Co. (St. Louis, MO). Organic solvents and salts were of ACS-approved analytical reagent grade or better. TLC plates were purchased from Brinkmann Instruments (Rexdale, Ontario).

All experiments were performed at least twice, and all analyses within experiments were generally performed in triplicate. Standard deviations of replicates were normally within 5% of means.

## RESULTS

The distributions of the total activities of fatty acid biosynthesis and marker enzymes among the subcellular fractions of pea root homogenates are shown in Table I. The patterns shown were essentially as expected. Seventy-eight percent of the total recovered activity for fatty acid synthesis occurs in the 500*g* pellet followed by 16% in the 10,000*g* pellet which corresponds to the recovery of particulate or pelletable triose phosphate isomerase activity (plastid marker). The large amount of triose phosphate isomerase activity associated with the 100,000*g* supernatant represents the cytosolic location of the same enzyme of the glycolytic pathway, which thus precludes any assessment of plastid intactness. Collectively, the data of Table I indicate that the 500*g* pellet consists primarily of plastids, the 100,000*g* pellet consists largely of endoplasmic reticulum, and the 10,000*g* pellet contains greater than 87% of the recovered mitochondria but is heavily contaminated with both plastics and endoplasmic reticulum. These results thus indicate that fatty acid synthesis in pea roots is localized primarily in the plastid.

The products of fatty acid synthesis by the crude homogenate and the 500 and 10,000*g* subcellular fractions of pea root homogenates are summarized in Table II. These fractions all synthesize primarily palmitate and oleate. The 10,000*g* fraction also synthesized a large proportion (40%) of stearate. Fatty acids synthesized by each of the subcellular fractions were recovered primarily as hydrolyzable, water soluble derivatives of the aqueous phase of the extraction (presumably acyl CoAs and acyl-ACPs), free fatty acids, and phospholipids (Table III) The principal phospholipids synthesized were phosphatidic acid, phosphatidylcholine, and phosphatidylethanolamine, with somewhat smaller amounts of phosphatidylinositol and phosphatidylglycerol.

The cofactor requirements for fatty acid synthesis by pea root plastids were also investigated. The results of these investigations are summarized in Table IV. ATP, bicarbonate, and either $MgCl_2$ or $MnCl_2$ were absolute requirements for activity, with $Mn^{2+}$ strongly preferred as the divalent cation. Fatty acid synthesis was only partially dependent on exogenously added reduced nucleotides. Omission of either NADPH or NADH from the standard reaction mixture resulted in slightly diminished rates of fatty acid synthesis with only a slight preference for NADH demonstrated. The omission of both nucleotides further diminished rates of fatty acid synthesis to approximately 58% of the complete control activity. In the absence of glycerol-3-phosphate or CoA, fatty acid synthesis was reduced by 26 and 61%, respectively.

When measurable, the omission of various cofactors had little effect on the proportions of fatty acids synthesized (Table IV). Most notably, however, the omission of either $MgCl_2$ or $MnCl_2$ both reduced the proportion of palmitate synthesized and increased the proportions of stearate and oleate synthesized, respectively. The omission of glycerol-3-phosphate also reduced the proportion of palmitate synthesized while increasing the proportion of oleate synthesized. In the absence of CoA, the amounts of palmitate synthesized were increased while the amounts of primarily stearate, and to a lesser extent oleate, were reduced. The effects of omitting reduced nucleotides on fatty acid synthesis were generally small except that the omission of NADH greatly reduced the levels of stearate accumulated. Last, the omission of NADH and glycerol-3-phosphate resulted in the synthesis of small amounts of a substance co-chromatographing in the region of palmitolenate.

Table I. *Recovery of Total Activities for Fatty Acid Synthesis and Marker Enzymes and Protein Among Subcellular Fractions Isolated from Pea Roots*

Data shown represent the means of triplicate determinations and are in terms of total yield per 12 g of initial root tissue fresh weight.

| | Subcellular Fraction | | | | |
|---|---|---|---|---|---|
| | Crude homogenate | 500*g* pellet | 10,000*g* pellet | 100,000*g* pellet | 100,000*g* supernatant |
| Fatty acid synthesis (nmol/h) | 0.52 | 4.32 | 0.88 | 0 | 0.36 |
| Triose phosphate isomerase ($\mu$mol/min) | 124.8 | 21.8 | 19.3 | 9.0 | 104.0 |
| Fumarase ($\mu$mol/min) | 5.79 | 0.14 | 3.17 | 0.35 | 0.51 |
| NADPH:Cyt *c* reductase ($\mu$mol/min) | 0.152 | 0.023 | 0.152 | 0.137 | 0 |
| Protein (mg) | 21.94 | 1.80 | 5.86 | 5.64 | 14.88 |

Table II. *Products of Fatty Acid Synthesis from [$^{14}$C] Acetate in Subcellular Fractions of Pea Root Homogenates*

Amounts of protein per reaction were 1.04, 0.32, and 1.42 mg of each of the crude homogenate filtrate and the 500 and 10,000*g* pellets. Values indicated represent the means of three determinations.

| | Crude Homogenate | 500*g* Pellet | 10,000*g* Pellet |
|---|---|---|---|
| Acetate incorporation (pmol/h/mg protein) | 116.5 | 2204.0 | 423.5 |
| DPM for analysis | 2794 | 16422 | 13912 |
| | | % | |
| 16:0[a] | +[b] | 49.3 | 37.6 |
| 16:1 | 0 | 5.1 | 2.5 |
| 18:0 | 0 | 2.6 | 39.9 |
| 18:1 | + | 42.3 | 18.8 |

[a] 16:0, 16:1, 18:0, 18:1 each correspond to the methyl esters of palmitic, palmitoleic, stearic, and oleic acids, respectively. [b] + = Insufficient radioactivity for precise analysis, detected in approximately equal amounts.

Table III. *Products of Radioactive Fatty Acid Utilization in Subcellular Fractions of Pea Root Homogenates*

| | Crude Homogenate | 500 *g* Pellet | 10,000 *g* Pellet |
|---|---|---|---|
| Acetate incorporation (pmol/h/mg protein)[a] | 122.2[b] | 2202.9 | 453.5 |
| Analysis (dpm) | 2794 | 16422 | 13912 |
| Products of fatty-acid utilization (%) | | | |
| Hydrolyzable aqueous | 38.1 | 16.4 | 15.1 |
| Triacylglycerol | 0 | 2.3 | 2.8 |
| Diacylglycerol | 0 | 14.2 | 10.1 |
| Free fatty acid | 10.6 | 12.3 | 33.8 |
| Monogalactosyldiacylglycerol[c] | 18.2 | 7.2 | 8.2 |
| Phosphatidic acid | 8.6 | 25.0 | 11.2 |
| Phosphatidylethanolamine | 9.4 | 5.4 | 7.7 |
| Phosphatidylglycerol | 2.4 | 3.2 | 2.1 |
| Phosphatidylcholine | 4.2 | 9.0 | 4.0 |
| Phosphatidylinositol | 2.7 | 1.2 | 2.3 |
| Origin | 5.8 | 3.7 | 2.7 |

[a]Amounts of protein per reaction as indicated in Table II. [b]Values shown represent the means of three replicates. [c]Tentative identification.

In order to determine how closely *in vitro* fatty acid synthesis from [$^{14}$C]acetate corresponds to that of *in vivo* synthesis, segments of pea root tissue were incubated in the presence of [$^{14}$C]acetate for 1, 2, and 3 h. The results obtained, shown in Table V, indicate that relatively intact pea roots rapidly incorporate exogenously supplied acetate into virtually the same fatty acids and in the same proportions as those synthesized by subcellular fractions of root homogenates. These were primarily palmitate, stearate, and oleate. Intact tissues also synthesized small amounts of longer chain fatty acids (20:0 and 22:0) but did not synthesize palmitoleate. There was little difference in the radioactive fatty acid compositions measured at the 1, 2, and 3 h incubation intervals except that the levels of stearate gradually declined from approximately 25 to 18%. In other studies (data to be reported elsewhere), fatty acids synthesized *in vivo* were associated with primarily phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, and triacylglycerol.

## DISCUSSION

We report here for the first time the role of root plastids as the principal organelle of this tissue involved in fatty acid biosynthesis. Fatty acid synthesis in pea root plastids resembles that of virtually all other tissues studied (9, 15–18). ATP, bicarbonate, and either $Mg^{2+}$ or $Mn^{2+}$ are absolute requirements for activity, while CoA, reduced nucleotides, and glycerol-3-phosphate all improve activity but are not essential. The products of *in vitro* fatty acid synthesis were primarily palmitate, oleate, and stearate. The amounts of stearate accumulated, particularly in *in vivo* incubations, were generally higher than described for other systems (18). This may reflect a somewhat decreased or

Table IV. *Effects of Cofactor Deletion on the Rate of Fatty Acid Synthesis and Proportions of Radioactive Fatty Acids Synthesized by Pea Root Plastids*

Activity of complete was 1.16 nmol/h/mg/protein. Each reaction contained plastids equivalent to 0.14 mg protein from a 500*g* pellet. Values represent the means of triplicate determinations.

| | Activity, % of Complete | Radioactive Fatty Acid | | | |
|---|---|---|---|---|---|
| | | 16:0 | 16:2[a] | 18:0 | 18:1 |
| | | | % | | |
| Complete | 100.0 | 42.0 | | 16.3 | 41.7 |
| Boiled | 1.4[b] | | | | |
| −ATP | 1.1[b] | | | | |
| $-MgCl_2$ | 118.2 | 28.5 | | 28.9 | 42.5 |
| $-MnCl_2$ | 58.3 | 31.1 | | 11.3 | 57.6 |
| $-MgCl_2$, $-MnCl_2$ | 5.9[b] | | | | |
| −NADPH | 85.7 | 44.9 | | 13.6 | 41.5 |
| −NADH | 79.6 | 49.1 | 3.8 | 3.0 | 44.1 |
| −NADPH/−NADH | 57.5 | 44.6 | | 14.4 | 41.1 |
| −G3P | 74.2 | 33.2 | 4.6 | 10.0 | 52.2 |
| −CoA | 39.4 | 58.5 | | 3.6 | 37.9 |
| $-HCO_3^-$ | 12.0[b] | | | | |
| | | Average Mass Distribution,[c] % | | | |
| | | 18.6 | | 6.0 | 6.2 |

[a] Tentative identification. [b] Treatment was too low for radioactive fatty acid analysis. [c] Including 53.1 and 16.1% of 18:2 and 18:3, respectively. Mass compositions did not change for each treatment.

Table V. *Fatty Acids Synthesized From Exogenously Supplied $^{14}C$-Acetate by Intact Pea Root Segments*

| Incubation Incorporation Rate | | Radioactive Fatty Acid | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 16:0 | 18:0 | 18:1 | 18:2 | 18:3[a] | 20:0 | 22:0 |
| | | Mass Distribution, %[b] | | | | | | |
| | | 10.3 | 3.6 | 16.6 | 56.2 | 12.6 | <1 | <1 |
| *Time (h)* | *nmol/g fresh wt* | Radioactivity Distribution, % | | | | | | |
| 1 | 11.9 | 30.8 | 25.6 | 41.3 | | | 2.2 | <1 |
| 2 | 14.1 | 38.4 | 20.8 | 35.6 | | | 2.2 | 3.1 |
| 3 | 27.7 | 32.3 | 18.3 | 38.8 | | | 3.3 | 5.0 |

[a] The radioactivity of 18:3 and 20:0 was not completely resolved; however, most was associated with 20:0 and indicated as such. [b] Mass distributions did not vary. Values shown represent the means of all analyses. Radioactivity values represent the mean of three determinations.

suboptimal activity of stearoy-ACP desaturase under the conditions used here, perhaps due to limiting oxygen levels. The proportions of these fatty acids synthesized showed only small changes with each of the various treatments. The most notable effects were observed when divalent cations, CoA, or glycerol-3-phosphate were omitted from the standard reaction mixture or when plastids contaminated with mitochondria (10,000*g* fraction) were tested. The omission of $Mg^{2+}$ or $Mn^{2+}$ resulted in reduced amounts of palmitate synthesized while increasing the levels of $C_{18}$ acids, suggesting that the concentrations of these ions tested might interfere with fatty acid elongation. The absence of CoA also increased the proportions of palmitate and decreased the proportions of $C_{18}$ acids synthesized. This observation suggests the possible involvement of a 'CoA-track' elongation system as described by Vance and Stumpf (19) for spinach chloroplasts. The omission of glycerol-3-phosphate increased the levels of oleate synthesized which is a common observation shown by other workers also using spinach chloroplasts (7, 13). Last, plastids heavily contaminated with mitochondria synthesized large amounts of stearate at the expense of decreased oleate accumulation. These results are very likely due to the depletion of available oxygen by mitochondrial respiration which is required for the desaturation of stearoyl-ACP to oleoyl-ACP (8, 11). Fatty acids synthesized by excised root segments were similar to those of isolated root plastids except that small amounts of $C_{20}$ and $C_{22}$ fatty acids were also synthesized by root segments. However, in no case did the composition of radioactive fatty acids ever reach the same proportions as the mass of these fatty acids. The recovery of the products of fatty acid synthesis primarily in such glycerolipids as phosphatidic acid, diacylglycerol, phosphatidylcholine, and others suggests that pea root plastids are, at least in part, somewhat autonomous with respect to glycerolipid metabolism. Similar observations were made by Journet and Douce (3) for cauliflower bud plastids. In contrast to the work of Mazliak *et al.* (6) but in agreement with other studies (3, 20), it appears that the mitochondria of roots and other nonphotosynthetic tissues have, if any, a relatively minor role in fatty acid biosynthesis. Further work is necessary to fully characterize pea root plastids for their capacity for fatty acid synthesis and glycerolipid metabolism.

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## LITERATURE CITED


1. Beisenherz G 1955 Triose phosphate isomerase from calf muscle. Methods Enzymol 1: 427–435
2. Bishop DG, RM Smillie 1970 The effects of chloramphenicol and cycloheximide on lipid synthesis during chloroplast development in *Euglena gracilis*. Arch Biochem Biophys 139: 179–189
3. Journet E-P, R Douce 1985 Enzymic capacities of purified cauliflower bud plastids for lipid synthesis and carbohydrate metabolism. Plant Physiol 79: 458–468
4. Lord JM, T Kagawa, TS Moore, H Beevers 1973 Endoplasmic-reticulum as the site of lecithin formation in castor bean endosperm. J Cell Biol 57: 659–677
5. Lowry OH, NJ Rosebrough, AL Farr, RJ Randall 1951 Protein measurement with the Folin phenol reagent. J. Biol Chem 192: 265–275
6. Mazliak P, A Oursel, A Ben Abdelkader, M Grosbois 1972 Biosynthèse des acides gras dans les mitochondries végétales isolées. Eur J Biochem 28: 399–411
7. McKee JWA, JC Hawke 1979 The incorporation of [$^{14}C$] acetate into the constituent fatty acids of monogalactosyldiglyceride by isolated spinach chloroplasts. Arch Biochem Biophys 197: 322–332
8. McKeon, TA, PK Stumpf 1982 Purification and characterization of the stearoyl-acyl carrier protein desaturase and the acyl-acyl carrier protein thioesterase from maturing seeds of safflower. J Biol Chem 257: 12141–12147
9. Mudd JB, TT McManus 1962 Metabolism of acetate by cell-free preparations from spinach leaves. J Biol Chem 237: 2057–2063
10. Mudd, JB, R DeZacks 1981 Synthesis of phosphatidylglycerol by chloroplasts from leaves of *Spinacia oleracea* L. (spinach). Arch Biochem Biophys 209: 584–591
11. Nagai J, K Bloch 1968 Enzymatic desaturation of stearoyl acyl carrier protein. J Biol Chem 243: 4626–4633
12. Racker E 1950 Spectrophotometric measurements of the enzymatic formation of fumaric and cis-aconitic acids. Biochim Biophys Acta 4: 211–214
13. Roughan PG, R Holland, CR Slack 1979 On the control of long-chain-fatty acid synthesis in isolated intact spinach (*Spinacea oleracea*) chloroplasts. Biochem J 184: 193–202
14. Sparace SA, JB Mudd 1982 Phosphatidylglycerol synthesis in spinach chloroplasts: characterization of the newly synthesized molecule. Plant Physiol 70: 1260–1264
15. Squires CL, PK Stumpf, C Schmid 1958 Effect of bicarbonate on the enzymic synthesis of long chain fatty acids. Plant Physiol 33: 365–366
16. Stumpf PK, AT James 1963 The biosynthesis of long chain fatty acids by lettuce chloroplast preparations. Biochim Biophys Acta 70: 20–32
17. Stumpf PK, GA Barber 1957 Fat metabolism in higher plants. IX. Enzymic synthesis of long chain fatty acids by avocado particles. J Biol Chem 227: 407–417
18. Stumpf PK 1980 Biosynthesis of saturated and unsaturated fatty acids. *In* PK Stumpf, ed, The Biochemistry of Plants, Vol 4, Lipids: Structure and Function. Academic Press, New York, pp 177–204
19. Vance WA, PK Stumpf 1978 Fat metabolism in higher plants: the elongation of saturated and unsaturated acyl-CoAs by a stromal system from isolated spinach chloroplasts. Arch Biochem Biophys 190: 210–220
20. Vick B, H Beevers 1978 Fatty acid synthesis in endosperm of young castor bean seedlings. Plant Physiol 62: 173–178