Biochem. J. (1980) **191**, 791–797
Printed in Great Britain

Fatty acid elongation by a particulate fraction from germinating pea

Brian R. JORDAN and John L. HARWOOD
Department of Biochemistry, University College, P.O. Box 78, Cardiff CF1 1XL, Wales, U.K.

(*Received 27 May 1980/Accepted 26 June 1980*)

The synthesis of fatty acids from [^{14}C]malonyl-CoA was studied with a high-speed particulate fraction from germinating pea (*Pisum sativum*). The variety used (Feltham First) produced mainly saturated fatty acids with palmitate (30–40%) and stearate (40–60%) predominating. Several palmitate-containing lipids stimulated overall synthesis and, in addition, increased the percentage of label in stearate. The production of stearate was severely inhibited by preincubation of the microsomal fraction with snake venom phospholipase A_2 or by incubation with *Rhizopus arrhizus* lipase. Addition of a series of di-saturated phosphatidylcholines, with different acyl constituents, resulted in stimulation of overall fatty acid synthesis as well as an increase in the radiolabelling of the fatty acid two carbon atoms longer than the acyl chain added. This chain lengthening of fatty acids donated from phosphatidylcholine was due to the action of both fatty acid synthetase and palmitate elongase. The latter would utilize dipalmitoyl phosphatidylcholine and was sensitive to arsenite whereas fatty acid synthetase would use dilauroyl phosphatidylcholine and was sensitive to cerulenin. The results are discussed in relation to previous data obtained *in vivo* on plant fatty acid synthesis and current suggestions for the role of phosphatidylcholine in this process.

Fatty acid synthesis has been studied in a number of higher plant tissues and it is clear that a number of elongation and desaturase enzymes are involved as well as fatty acid synthetase (cf. Stumpf, 1976; Harwood, 1979; von Wettstein-Knowles, 1979). After the demonstration by Macey & Stumpf (1968) that the germinating pea would actively synthesize predominantly saturated fatty acids, including the very long chain acids (> C-18), this tissue has been utilized for further work. Arsenite was found to be an inhibitor of plamitate elongation both *in vivo* and *in vitro* whereas fluoride inhibited stearate elongation (Harwood & Stumpf, 1971). Palmitate elongase itself has been found to be a relatively specific enzyme (Jaworski *et al.*, 1974) which is partly present as a soluble protein when it uses palmitoyl-(acyl carrier protein) as a substrate (Bolton & Harwood, 1977*a*). However, a microsomal fraction from germinating pea was found to be completely inactive with exogenously added [^{14}C]palmitoyl-(acyl carrier protein) (Bolton & Harwood, 1977*b*). Furthermore, it was shown that the addition of dipalmitoyl phosphatidylcholine caused, not only an increase in the total incorporation of radioactivity from [^{14}C]malonyl-CoA into fatty acids, but also a marked increase in the radiolabelling of stearic acid. In a similar way the addition of distearoyl phosphatidylcholine as substrate increased the total radiolabelling of fatty acids and the proportion of [^{14}C]arachidic acid (Bolton & Harwood, 1977*b*) but, in contrast with leek (Cassagne & Lessire, 1978) stearoyl-CoA was inactive. Two possible explanations for the difference in substrate specificity for palmitate elongase in the microsomal and soluble fractions from pea are, firstly, that different enzyme proteins are present or, secondly, that the membrane environment of the microsomes causes a modification of the enzyme's properties or accessibility. Part of the results described in this paper were obtained in an attempt to see if modification of the membrane by the addition of exogenous phospholipids could be exerting physical effects on the enzymes synthesizing fatty acids.

Experimental

Materials

Pea (*Pisum sativum* cultivar Feltham First) seeds were purchased from Asmer Seeds, Leicester, U.K. and were germinated as previously described (Bolton & Harwood, 1977*b*). [2-^{14}C]Malonyl-CoA (53 Ci/mol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. and [1,3-^{14}C]malonyl-CoA (22.4 Ci/mol) from New England Nuclear Corp., Boston, MA, U.S.A. Malonyl-CoA,

0306-3275/80/120791-07$01.50/1

NADH, ATP, phosphatidylcholines and fatty acid standards were purchased from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey KT2 7BH, U.K. and NADPH, *Rhizopus arrhizus* lipase and snake (*Crotalus adamenteus*) venom phospholipase A_2 from Boehringer Corp. (London), Lewes, Sussex, U.K. Phosphatidylcholines and fatty acid standards were also obtained from Bast, DK-1705 Copenhagen V, Denmark. Sodium arsenite and Triton X-100 were purchased from BDH Chemicals, Poole, Dorset, U.K. Cerulenin, isolated from *Cephalosporin cerulens*, was a gift from Dr. N. J. Russell of this department. Organic solvents were obtained and treated as previously (Bolton & Harwood, 1977*b*). Other chemicals were of the best available grades and were purchased from Sigma or BDH.

Subcellular fractionation

After the appropriate germination period, pea seeds were homogenized and the microsomal and particle-free supernatant fractions were prepared as described by Bolton & Harwood (1977*b*). Protein was determined by the Lowry method (Lowry *et al.*, 1951) in the presence of 0.1% sodium deoxycholate with bovine serum albumin as standard.

Incubations and fatty acid analysis

Routine incubation conditions consisted of 20mM-potassium phosphate buffer (pH 7.0), 0.5μmol of NADPH, 0.5μmol of NADH, 2μmol of ATP, 0.5mg of acyl carrier protein, 0.01μmol of [^{14}C]malonyl-CoA and 1–2mg of microsomal protein in a total volume of 1ml. Incubations were allowed to proceed at 25°C for periods of up to 4h. These cofactor concentrations had been previously shown to be optimal (Bolton & Harwood, 1977*b*).

Exogenous phosphatidylcholines and other lipids for additional experiments were prepared by sonication as previously (Bolton & Harwood, 1977*b*). Since these authors had shown that preincubation of pea fractions with sodium arsenite did not affect its inhibitory action, the reagent was added directly to the incubation medium as required.

Sonication of the microsomal fraction was carried out at 4°C with 5s bursts at 20kHz. For phospholipase degradation, microsomal preparations were incubated with 0.04–0.1 unit (1 unit causes the hydrolysis of 1μmol of phosphatidylcholine/min at 25°C) of phospholipase A_2/mg of microsomal protein for a period of 30min at 30°C. The treated microsomal fraction was then diluted with 20vol. of grinding buffer and centrifuged at 105000*g* for 60min to re-isolate the microsomal fraction. *Rhizopus arrhizus* lipase was added to the incubation medium at a concentration of 6 units (1 unit causes the hydrolysis of 1μmol of olive oil/min at 25°C)/mg of microsomal protein.

After incubation with [^{14}C]malonyl-CoA, the reaction was terminated by the addition of 0.1ml of KOH (60%, w/v). Hydrolysis, extraction and methylation were carried out as described by Bolton & Harwood (1977*b*). Aliquots of the fatty acid methyl esters were taken for estimation of radioactivity and for g.l.c. analysis. The methyl esters were separated in 15% (w/w) EGSS-X on Supelcoport (100–120 mesh; Supelco Inc., Bellefont, PA 16823, U.S.A.) at 185°C. Glass columns (1.5cm × 4mm internal diam.) were used. Analysis was with a Pye 104 or a Pye GCD gas chromatograph coupled to a Panax radioactivity detector. Pentadecanoate was used as an internal standard. α-Oxidation was carried out by the method of Harris *et al.* (1965).

Radioactivity measurements were made as previously reported (Bolton & Harwood, 1977*b*).

Results and discussion

Previous data with the microsomal fraction from germinating pea (*Pisum sativum* cultivar Kelvedon Wonder) had shown that a range of saturated fatty acids in the range C-16–C-22 were the major products (Bolton & Harwood, 1977*b*). However, we have obtained appreciably higher incorporation of radioactivity from [^{14}C]malonyl-CoA with *Pisum sativum* cultivar Feltham First and, accordingly, this has been used in the current work. The latter variety differs from cultivar Kelvedon Wonder in that very long chain fatty acids are not made at the period of germination used. In addition, a small amount of radioactivity was incorporated into a polar fatty acid, probably a hydroxy derivative previously reported in pea (Jordan & Harwood, 1979).

It was found that individual microsomal preparations differed considerably in the ratio of [^{14}C]palmitate to [^{14}C]stearate in the final products. Values between 1 and 3 were obtained and this variability has tended to decrease the statistical significance of some of the data shown later. However, in all cases where a difference was seen between control and test samples, this trend was observed in each individual experiment.

Because of the differences in fatty acyl products for the two pea varieties, we re-tested the effects of exogenous lipids on microsomal fatty acid synthesis. As previously reported (Bolton & Harwood, 1977*b*), overall stimulation of incorporation of radioactivity from [^{14}C]malonyl-CoA was obtained with palmitic acid, glycerol tripalmitate and dipalmitoyl phosphatidylcholine. In contrast, dipalmitoyl phosphatidylethanolamine was ineffective. The difference between the two phospholipids may be related to the overall negative charge on phosphatidylethanolamine, which might make it less suitable for fusion with microsomal membranes. As

expected, the most consistent stimulatory results were obtained with dipalmitoyl phosphatidylcholine, whose addition significantly increased the proportion of [^{14}C]stearate. As previously observed (Bolton & Harwood, 1977*b*) there was no change in the pattern of ^{14}C-labelled products when phosphatidylethanolamine was added to the incubation system.

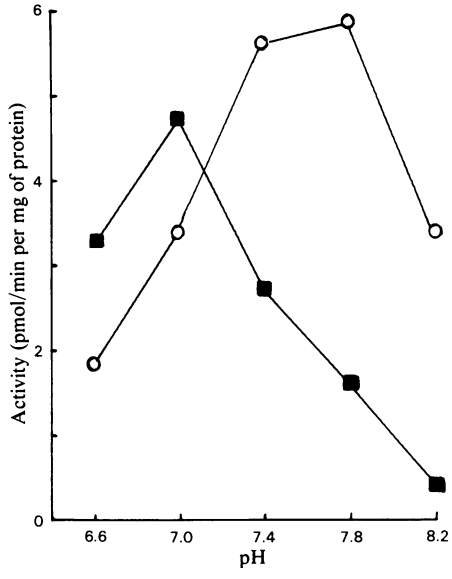

Fig. 1. *Influence of pH on the activities of fatty acid synthetase* (■) *and palmitate elongase* (○)
For the conditions of incubation see the Experimental section. The pH of the medium was altered with potassium phosphate buffers. Activities were calculated from the incorporation of radioactivity from [^{14}C]malonyl-CoA into stearate (palmitate elongase) and into palmitate and shorter-chain fatty acids (fatty acid synthetase). These activities were corrected for the small amount of [^{14}C]palmitate that was elongated by isolating samples of the products and carrying out α-oxidation (Harris *et al.*, 1965). Results indicated that between 84 and 95% of the [^{14}C]stearate was exclusively labelled at the carboxyl moiety, in agreement with previous results (Bolton & Harwood, 1977*b*).

The characteristics of fatty acid synthesis by the microsomal fraction prepared from cultivar Feltham First were also checked. Optimal cofactor concentrations were similar to those previously observed for cultivar Kelvedon Wonder (Bolton & Harwood, 1977*b*). The effect of pH was also examined; in agreement with the experiments of Jaworski *et al.* (1974) palmitate elongase has a higher pH optimum than does fatty acid synthetase (Fig. 1). Since both enzymes had good and rather similar activity at pH 7.0, experiments were conducted under these conditions. The particulate fraction used was highly enriched with the endoplasmic reticulum of the pea seed as estimated by the distribution of glucose 6-phosphatase, UDPase and unspecific esterase but was also contaminated by membranes from other organelles. The subcellular site(s) of the membrane-localized activity measured is, therefore, unclear.

In a previous paper (Bolton & Harwood, 1977*b*) it was suggested that one explanation for the effects of exogenous lipid additions could be due to their alteration of the membrane environment. Several methods of altering the microsomal preparations were tested. Firstly, ultrasonication was employed. This resulted in an impairment of total fatty acid synthesis but no alteration in the nature of the ^{14}C-labelled products (Table 1). Furthermore, the stimulatory effect and increased labelling of stearate caused by dipalmitoyl phosphatidylcholine addition was still seen in the sonicated preparations (Table 1).

Since previous data had indicated that the source of acyl chain to be elongated was likely to be endogenous microsomal phospholipid, the action of phospholipases was tested. The lipolysis caused by these enzymes was thought likely to, firstly, affect the overall membrane environment of the fatty acid-synthesizing enzymes. In addition, the amount

Table 1. *Effect of sonication of microsomal fatty acid synthesis*
For details of incubation see the Experimental section. Control values for fatty acid synthesis were in the range 10–19 pmol of malonyl-CoA incorporated/min per mg of protein. Means ± S.D. are shown and significance was estimated by the paired *t* test: $*P < 0.025$, $**P < 0.001$ when compared with the respective samples without added lipid. The amount of phospholipid added was 0.5 mg.

Treatment	Addition	Total fatty acid synthesis (% of control)	No. of expts.	Fatty acid ...	Distribution of radioactivity (% of total [^{14}C]fatty acids) 14:0	16:0	18:0	Others
None	None	100	3		3 ± 4	39 ± 7	56 ± 3	2 ± 1
Sonication	None	68 ± 6	3		2 ± 2	39 ± 3	58 ± 3	1 ± 1
None	Dipalmitoyl phosphatidylcholine	117 ± 10	3		1 ± 2	24 ± 2*	74 ± 2**	1 ± 1
Sonication	Dipalmitoyl phosphatidylcholine	86 ± 4	3		2 ± 1	27 ± 5*	70 ± 3*	1 ± 1

of potential phospholipid donors for elongation would be depleted. Two enzymes were tested, phospholipase A_2 from snake venom and the lipase from *Rhizopus arrhizus*, which cleaves the 1-position of phospholipids as well as other acyl lipids (Fisher *et al.*, 1973). Preincubation of the microsomal fraction with phospholipase A_2 or the addition of *Rhizopus* lipase to the incubation system resulted in an impairment of the total incorporation of radioactivity from [^{14}C]malonyl-CoA (Table 2). Interestingly, the pattern of fatty acids made was drastically altered, with a severe decrease in labelling of stearate. This could have been caused either because the elongase was more sensitive or alternatively because it was more accessible to lipase action than was fatty acid synthetase.

A third type of experiment to alter the membrane environment involved the addition of phosphatidylcholines that differed in the nature of their acyl constituents. The results are shown in Table 3. The data show that phosphatidylcholines containing even-chain saturated fatty acids in the range C-8–C-16 all stimulated total fatty acid synthesis and, moreover, in each case particularly increased the formation of the fatty acid two carbon atoms longer in chain length. It thus appeared that each species of phosphatidylcholine was able to donate its respective acyl groups for chain lengthening. Since the microsomal fractions of the variety of pea used did not synthesize very long chain fatty acids, the lack of effect of distearoyl phosphatidylcholine (Table 3) and dioleoyl phosphatidylcholine was expected. One mixed-acid molecular species was tested, 1-palmitoyl, 2-oleoyl phosphatidylcholine, and it had a moderate effect in stimulating stearate synthesis. These results showed clearly that the effect of phosphatidylcholine addition were not related to alterations in membrane fluidity. Such a conclusion was also reached in experiments on the stimulation of microsomal acyl-CoA–cholesterol acyltransferase activity by phosphatidylcholine (Hashimoto & Dayton, 1978).

The results of phosphatidylcholine addition are more interesting since this particular lipid has been suggested to be a substrate for fatty acid desaturation in plants (cf. Harwood, 1979). However, unlike the use of phosphatidylcholine in oleate desaturation (Gurr *et al.*, 1969; Pugh & Kates, 1973, 1975; Slack & Roughan, 1975; Stymne & Appelqvist, 1978; Slack *et al.*, 1979), elongation of its acyl chains cannot take place on the intact lipid because the two-carbon unit is added at the carboxyl end

Table 2. *Influence of lipases on the fatty acids synthesized from [2-^{14}C]malonyl-CoA by pea microsomes*
For details see legend to Table 1 and Experimental. Significance: n.s., not significant; $*P < 0.01$; $**P < 0.005$.

Treatment	Total fatty acid synthesis (% of control)	No. of expts.	Fatty acid ...	Distribution of radioactivity (% of total [^{14}C]fatty acids) 12:0	14:0	16:0	18:0
None	100	3		trace	3 ± 3	41 ± 8	53 ± 12
Preincubation with phospholipase A_2	35 ± 7	3		6 ± 4*	17 ± 5**	64 ± 8**	14 ± 11**
None	100	2		1 ± 1	3 ± 2	40 ± 6	56 ± 4
Incubation with *Rhizopus* lipase	60 ± 5	2		15 ± 8**	34 ± 4**	39 ± 9	5 ± 2**

Table 3. *Effect of the addition of phosphatidylcholines of different acyl chain length on microsomal fatty acid synthesis*
For details see the legend to Table 1 and the Experimental section. The amount of each lipid used was 0.5 mg. Significance: $*P < 0.05$; $**P < 0.001$; n.d., not detected.

Species of phosphatidylcholine added	Total fatty acid synthesis (% of control)	No. of expts.	Fatty acid ...	Distribution of radioactivity (% of total [^{14}C] fatty acids) 10:0	12:0	14:0	16:0	18:0	Other
None	100	10		n.d.	2 ± 5	10 ± 6	38 ± 10	47 ± 11	3 ± 3
Dioctanoyl	119 ± 18	2		2 ± 3	7 ± 7	16 ± 6**	38 ± 5	37 ± 4	trace
Didecanoyl	119 ± 18	3		trace	6 ± 2**	14 ± 8*	39 ± 14	40 ± 19	1 ± 1
Dilauroyl	144 ± 24	6		n.d.	1 ± 3	25 ± 14*	34 ± 5	39 ± 12	1 ± trace
Dimyristoyl	120 ± 5	4		n.d.	n.d.	6 ± 3	51 ± 16	42 ± 10	1 ± 1
Dipalmitoyl	128 ± 19	10		n.d.	3 ± 4	8 ± 6	21 ± 6**	67 ± 13**	1 ± trace
Distearoyl	92 ± 16	3		n.d.	2 ± 3	7 ± 7	35 ± 5	54 ± 7	2 ± 1
1-Palmitoyl 2-oleoyl	111	1		n.d.	trace	trace	39	62	1

of the acyl chain (Bolton & Harwood, 1977*b*; Harwood & Stumpf, 1970).

It was of interest to determine whether fatty acid synthetase or palmitate elongase was responsible for chain-lengthening of the exogenous phosphatidylcholines. Jaworski *et al.* (1974) indicated that palmitate elongase was a relatively specific enzyme but the results shown in Table 3 raised the possibility that the reason that elongation of medium-chain fatty acids was not normally observed was simply because these acids are not normally available in any quantity for elongation. Cerulenin, an antibiotic produced by *Cephalosporim cerulens*, inhibits *Escherichia coli* fatty acid synthetase at the level of the condensing enzyme (D'Agnolo *et al.*, 1973). The compound is also active in plants (Packter & Stumpf, 1975). On the other hand, sodium arsenite will inhibit palmitate elongase at concentrations where fatty acid synthetase is unaffected (Harwood & Stumpf, 1971; Bolton & Harwood, 1977*a*,*b*). Accordingly, we used these two compounds to try to define more precisely the nature of the chain-lengthening reactions.

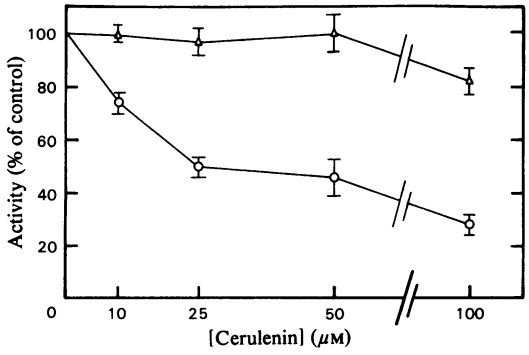

Fig. 2. *Inhibition of fatty acid synthesis in the microsomal fraction by cerulenin*

For experimental details see the Experimental section and the legend to Fig. 1. Means ± S.D. are shown. ○, Fatty acid synthetase; △, palmitate elongase.

Since cerulenin, at high concentrations, will also inhibit palmitate elongase (Jaworski *et al.*, 1974) it was necessary, in the first place, to determine effective concentrations for use with pea microsomes. The effect of cerulenin on fatty acid synthesis by the microsomal fraction is shown in Fig. 2. It will be seen that cerulenin inhibits synthesis of palmitate, but not that of stearate, at concentrations of up to 50 μM. At 50 μM-cerulenin, with lower concentrations of microsomal protein, occasional inhibition of stearate formation was seen. Since the ratio of inhibitor to condensing enzyme would be expected to be critical, then the above effect might have been predicted. Because fatty acid synthesis was measured with freshly prepared fractions (before protein concentrations could be determined) cerulenin was used at 10 or 15 μM concentrations to exclude any possibility of its inhibiting stearate synthesis. In Table 4, the effect of cerulenin on the alterations of fatty acid synthesis by exogenous phosphatidylcholines is shown. Cerulenin alone caused an inhibition of palmitate synthesis without effect on the amounts of [^{14}C]stearate. Because of the 28% inhibition of total synthesis, the proportion of [^{14}C]stearate in the products increased. Addition of dilauroyl phosphatidylcholine alone produced the expected total increase in synthesis and a larger percentage of radioactivity in myristate (cf. Table 3). In the presence of 15 μM-cerulenin, this increase was reduced appreciably. On the other hand, the increase in [^{14}C]stearate formation induced by addition of dipalmitoyl phosphatidylcholine was raised and was still seen in the presence of cerulenin. These results are most easily interpreted by proposing that the chain-lengthening of C-12 fatty acids donated by phosphatidylcholine is carried out by fatty acid synthetase, whereas the chain lengthening of C-16 fatty acids is not.

In confirmation of the above proposal, the

Table 4. *Effect of cerulenin on the alteration of microsomal fatty acid synthesis by exogenous phosphatidylcholines*

For details see the legend to Table 1 and the Experimental section. Means ± S.D. are shown. Cerulenin was used at 15 μM and 0.5 mg of phosphatidylcholine was used.

Addition	Total fatty acid synthesis (% of control)	No. of expts.	Distribution of radioactivity (% of total [^{14}C] fatty acids) Fatty acid ...	14:0	16:0	18:0
None	100	10		3 ± 2	32 ± 7	53 ± 8
Cerulenin	72 ± 12	5		1 ± 1	11 ± 7	88 ± 14
Dilauroyl phosphatidylcholine	142 ± 16	6		23 ± 9	30 ± 4	47 ± 8
Cerulenin + dilauroyl phosphatidylcholine	78 ± 8	7		11 ± 5	19 ± 3	70 ± 14
Dipalmitoyl phosphatidylcholine	133 ± 6	7		4 ± 3	21 ± 6	75 ± 10
Cerulenin + dipalmitoyl phosphatidylcholine	123 ± 9	7		4 ± 4	14 ± 2	82 ± 3

Table 5. *Ability of sodium arsenite to prevent changes in microsomal fatty acid synthesis by exogenous phosphatidylcholines*

Sodium arsenite was used at 1 mM and 0.5 mg of each lipid was added. For details see the legend to Table 1 and the Experimental section. Means ± S.D. are shown.

Addition	Total fatty acid synthesis (% of control)	No. of expts.	Distribution of radioactivity (% of total [^{14}C]fatty acids) Fatty acid ... 14:0	16:0	18:0	Other
None	100	6	7±2	41±12	47±9	5±3
Dilauroyl phosphatidylcholine	142±19	4	22±11	35±7	42±15	1±1
Dilauroyl phosphatidylcholine + arsenite	106±10	3	39±17	40±11	20±11	1±trace
1-Palmitoyl, 2-oleoyl phosphatidylcholine	112	1	trace	38	62	trace
1-Palmitoyl, 2-oleoyl phosphatidylcholine + arsenite	88	1	7	47	45	1
None	100	2	8±1	33±2	50±7	9±4
Dipalmitoyl phosphatidylcholine	123±2	2	8±1	27±4	60±5	5±3
Dipalmitoyl phosphatidylcholine + arsenite	68±18	2	13±1	44±6	36±3	7±2

addition of sodium arsenite to the incubation system enhanced the effect of dilauroyl phosphatidylcholine on [^{14}C]myristate formation but blocked the effects of dipalmitoyl phosphatidylcholine on fatty acid patterns (Table 5).

The ability of pea microsomal fatty acid synthetase to elongate laurate but not palmitate fits in well with observations *in vivo* on the metabolism of exogenous fatty acids by leaf tissue (cf. Hitchcock & Nichols, 1971). It would now be of interest to determine how acyl groups are transferred from phosphatidylcholine to fatty acid synthetase and palmitate elongase. The recent demonstration of stearoyl-(acyl carrier protein) formation in a leek microsomal fraction (Lessire & Cassagne, 1979) raises the possibility that the actual substrate for palmitate elongation in the pea microsomal fraction is palmitoyl-(acyl carrier protein) (cf. Bolton & Harwood, 1977*b*). However, phosphatidylcholines would be more effective than exogenously-added palmitoyl-(acyl carrier protein) since they can inset more efficiently into the microsomal membrane. A consistent picture in experiments with subcellular fractions from plants is beginning to emerge in that soluble enzymes work best with thioester derivatives of fatty acids whereas, in many cases, membrane-bound enzymes have maximal activity with intact exogenous lipids. Thus, soluble palmitate elongase (Bolton & Harwood, 1977*a*; Jaworski *et al.*, 1974) and stearate desaturase (Jaworski & Stumpf, 1974) use added acyl carrier protein derivatives whereas microsomal oleate desaturase (Gurr *et al.*, 1969; Stymne & Appelqvist, 1978; Slack *et al.*, 1979) and, in pea, palmitate or stearate elongase (Bolton & Harwood, 1977*b*) appear to use phosphatidylcholine. The exact details of the enzyme mechanisms involved must await the solubilization and purification of the proteins concerned.

The financial support of the Science Research Council is gratefully acknowledged.

References

Bolton, P. & Harwood, J. L. (1977*a*) *Biochim. Biophys. Acta* **489**, 15–24

Bolton, P. & Harwood, J. L. (1977*b*) *Biochem. J.* **168**, 261–269

Cassagne, C. & Lessire, R. (1978) *Arch. Biochem. Biophys.* **191**, 146–152

D'Agnolo, G., Rosenfeld, I. S., Awaya, J., Omura, S. & Vagelos, P. R. (1973) *Biochim. Biophys. Acta* **326**, 155–166

Fisher, W., Heinz, E. & Zeus, M. (1973) *Hoppe-Seyler's Z. Physiol. Chem.* **354**, 1115–1123

Gurr, M. I., Robinson, P. & James, A. T. (1969) *Eur. J. Biochem.* **9**, 70–78

Harris, R. V., Harris, P. & James, A. T. (1965) *Biochim. Biophys. Acta* **106**, 465–473

Harwood, J. L. (1979) *Prog. Lipid Res.* **18**, 55–86

Harwood, J. L. & Stumpf, P. K. (1970) *Plant Physiol.* **46**, 500–508

Harwood, J. L. & Stumpf, P. K. (1971) *Arch. Biochem. Biophys.* **142**, 181–191

Hashimoto, S. & Dayton, S. (1978) *Biochem. Biophys. Res. Commun.* **82**, 1111–1120

Hitchcock, C. & Nichols, B. W. (1971) *Plant Lipid Biochemistry*, Academic Press, London

Jaworski, J. G., Goldschmidt, E. E. & Stumpf, P. K. (1974) *Arch. Biochem. Biophys.* **163**, 769–776

Jaworski, J. G. & Stumpf, P. K. (1974) *Arch. Biochem. Biophys.* **162**, 152–165

Jordan, B. R. & Harwood, J. L. (1979) *Biochim. Biophys. Acta* **573**, 218–221

Kates, M., Wilson, A. C. & De La Roche, A. I. (1979) in *Advances in the Biochemistry and Physiology of Plant Lipids* (Appelqvist, L.-A. & Liljenberg, C., eds.), pp. 329–342, Elsevier, Amsterdam

Lessire, R. & Cassagne, C. (1979) *Plant Sci. Lett.* **14**, 43–48

Lowry, D. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Macey, M. J. K. & Stumpf, P. K. (1968) *Plant Physiol.* **43**, 1637–1647

Packter, N. & Stumpf, P. K. (1975) *Biochim. Biophys. Acta* **409**, 274–282

Pugh, E. L. & Kates, M. (1973) *Biochim. Biophys. Acta* **316**, 305–316

Pugh, E. L. & Kates, M. (1975) *Biochim. Biophys. Acta* **380**, 442–453

Slack, C. R. & Roughan, P. G. (1975) *Biochem. J.* **152**, 217–228

Slack, C. R., Roughan, P. G. & Browse, J. (1979) *Biochem. J.* **179**, 649–656

Stumpf, P. K. (1976) in *Plant Biochemistry* (Bonner, J. & Varner, J. E., eds.), 3rd edn., pp. 427–461, Academic Press, New York

Stymne, S. & Appelqvist, L.-A. (1978) *Eur. J. Biochem.* **90**, 223–229

von Wettstein-Knowles, P. (1979) *Dev. Plant Biol.* **3**, 1–26