Biochem. J. (1978) **170**, 421–433
Printed in Great Britain

Labelling of Glycerolipids in the Cotyledons of Developing Oilseeds by [1-^{14}C]Acetate and [2-^{3}H]Glycerol

By C. ROGER SLACK, P. GRATTAN ROUGHAN and NATHAN BALASINGHAM
Plant Physiology Division, Department of Scientific and Industrial Research, Private Bag, Palmerston North, New Zealand

(*Received* 1 *July* 1977)

1. 3-*sn*-Phosphatidylcholine was identified as the major lipid in cotyledons from the developing seeds of soya bean, linseed and safflower when tissue was steamed before lipid extraction. The proportion of oleate in this lipid decreased markedly and that of the polyunsaturated C_{18} fatty acids increased when detached developing cotyledons were incubated for up to 3 h. Similar but less pronounced changes occurred in diacylglycerol, which had a fatty acid composition resembling that of the 3-*sn*-phosphatidylcholine from cotyledons of the same species. 2. [1-^{14}C]Acetate supplied to detached cotyledons was incorporated into the acyl moieties of mainly 3-*sn*-phosphatidylcholine, 1,2-diacylglycerol and triacylglycerol. Initially label was predominantly in oleate, but subsequently entered at accelerating rates the linoleoyl moieties of the above lipids in soya-bean and safflower cotyledons and the linoleoyl and linolenyl moieties of these lipids in linseed cotyledons. In pulse–chase experiments label was rapidly lost from the oleate of 3-*sn*-phosphatidylcholine and accumulated in the linoleoyl and linolenoyl moieties of this phospholipid and of the di- and triacylglycerols. 3. [2-^{3}H]Glycerol was incorporated into the glycerol moieties of mainly 3-*sn*-phosphatidylcholine and di- and tri-acylglycerols of developing linseed and soya-bean cotyledons. The label entered the phospholipid and diacylglycerol at rates essentially linear with time from the moment the substrate was supplied, and entered the triacylglycerol at an accelerating rate. With linseed cotyledons the labelled glycerol was incorporated initially mainly into species of 3-*sn*-phosphatidylcholine and diacylglycerol that contained oleate, but accumulated with time in more highly unsaturated species. In pulse–chase experiments with linseed cotyledons, label was lost from both 3-*sn*-phosphatidylcholine and diacylglycerol, preferentially from the dioleoyl species, and accumulated in triacylglycerol, mainly in species containing two molecules of linolenate. 4. The results suggest a rapid turnover of 3-*sn*-phosphatidylcholine during triacylglycerol accumulation in developing oilseeds, and are consistent with the operation of a biosynthetic route whereby oleate initially esterified to the phospholipid is first desaturated, then polyunsaturated fatty acids transferred to triacylglycerol, via diacylglycerol. The possible role of oleoyl phosphatidylcholine as a substrate for oleate desaturation is discussed.

It is generally accepted that the triacylglycerols, which comprise the major lipid in oilseeds, are formed by the acylation of diacylglycerols (Hitchcock & Nichols, 1971). Both acyl-(acyl-carrier protein) and acyl-CoA have been shown to act as acyl donors in this reaction in preparations from the mesocarp of developing avocado (*Persea americana*) fruit (Shine *et al.*, 1976). The origin of the diacylglycerol pool that serves as the acyl acceptor, however, remains unclear. It has been suggested that this pool in the developing seed cotyledons of crambé (*Crambé abyssinica*) (Gurr *et al.*, 1974) and safflower (Stumpf, 1975) could be derived directly from phosphatidate formed by the sequential acylation of glycerol 3-phosphate from acyl-CoA species, as in liver- (Kennedy, 1961). Since the triacylglycerols in the seeds of many species are composed principally of polyunsaturated C_{18} fatty acids (Downey & McGregor, 1975) it follows, if the above route is operative, that the diacylglycerol pool must be formed largely from the CoA esters of polyunsaturated fatty acids. However, the only study to date *in vitro* on polyunsaturated fatty acid formation by developing seed tissue, that of Vijay & Stumpf (1971) on the desaturation of oleoyl-CoA by safflower seed microsomal fractions, showed that the linoleate produced was incorporated exclusively into 3-*sn*-phosphatidylcholine, rather than into phosphatidate or diacylglycerols.

Labelling studies with developing seed cotyledons, during accumulation of triacylglycerols, have indicated that both the acyl and glycerol moieties of the phospholipid fraction are labelled initially more rapidly than those of the di- and tri-acylglycerols

Abbreviation used: Mes, 4-morpholine-ethanesulphonic acid.

(Dybing & Craig, 1970; Gurr *et al.*, 1974; Wilson & Rinne, 1976*b*), and further, the kinetics of labelling of the fatty acid moiety of these lipids in linseed cotyledons (Dybing & Craig, 1970) appear consistent with the view that the fatty acids of triacylglycerols may be derived from phospholipid via diacylglycerol. There is, however, little agreement as to the particular phospholipid that is most rapidly metabolized in developing seed cotyledons. Both phosphatidate (Privett *et al.*, 1973) and *N*-acylphosphatidylethanolamine (Wilson & Rinne, 1974) have been reported as the major phospholipid in developing soya-bean cotyledons, and both lipids were rapidly labelled in cotyledons supplied with [^{14}C]acetate (Singh & Privett, 1970; Wilson & Rinne, 1976*a*,*b*). Neither of these lipids, on the other hand, was a major component of the phospholipids, nor did they contain a high proportion of the incorporated radioactivity from [^{14}C]acetate, in linseed (Dybing & Craig, 1970) or crambé (Gurr *et al.*, 1974). In cotyledons of the latter species, 3-*sn*-phosphatidylcholine was the major labelled phospholipid.

We have been impressed by the close similarity between the kinetics of labelling of the individual fatty acids of 3-*sn*-phosphatidylcholine observed by Dybing & Craig (1970) in developing linseed cotyledons and by Slack & Roughan (1975) in developing leaf tissue. Slack *et al.* (1976) have suggested that this phospholipid could serve as the substrate for oleate desaturation in leaves and as a donor of the polyunsaturated diacylglycerols incorporated into diacylmonogalactosylglycerol (Slack *et al.*, 1977). To determine whether this lipid could function in an analogous manner during triacylglycerol accumulation in seeds, we have studied the patterns of labelling of glycerolipids with [^{14}C]acetate and [^{3}H]glycerol in the developing cotyledons of linseed, soya bean and safflower.

Experimental

Materials

Plants of linseed (*Linum usitatissimum*, cultivar Punjab), soya bean (*Glycine max*, cultivar Amsoy) and safflower (*Carthamus tinctorious*, cultivar O-22, from seed supplied by Crop Research Division, D.S.I.R., Palmerston North, New Zealand, and cultivars UC-1 and intermediate linoleate from seed supplied by Dr. D. Davia, Department of Agronomy and Range Science, University of California, Davis, CA 95616, U.S.A.) were grown in pumice peat in a heated naturally lit glasshouse during summer and watered with nutrient solution (Hoagland & Arnon, 1938) daily.

Sodium methoxide was prepared as described (Slack *et al.*, 1976). Sodium [1-^{14}C]acetate and [2-^{3}H]glycerol were from The Radiochemical Centre, Amersham, Bucks., U.K.

Methods

Analysis of phospholipids. Seed heads of safflower and capsules of linseed that had flowered 18–25 days before and pods of soya bean 25–30 days after flowering were harvested and placed on ice. On the basis of previous studies of seed development in linseed (Dybing & Craig, 1970), soya bean (Rubel *et al.*, 1972) and safflower (Stumpf, 1975), it was assumed that the seed selected would contain cotyledons in which triacylglycerol synthesis was rapid. The fresh weight of cotyledons from the three species was about one-half that of cotyledons from mature seed. Those from linseed and soya bean were bright green. Cotyledons were removed from the safflower and soya-bean seeds individually. To obtain the cotyledons from linseed capsules the bases were cut off to expose the cotyledons and these squeezed into water at 0°C. Cotyledons were weighed and a sample (about 2.0g) was steamed for 10min as described by Roughan & Boardman (1972), then homogenized in 50ml of chloroform/methanol (2:1, v/v) at room temperature (20°C). The homogenate was kept for 30min, then filtered, 10ml of 0.9% NaCl added, and lipids were partitioned into the chloroform phase (Folch *et al.*, 1957). This was dried under reduced pressure, and the lipids were redissolved in chloroform and stored at −20°C. Lipids from samples of the extracts equivalent to 0.1g fresh weight of tissue were separated by t.l.c. on silica-gel G plates in chloroform/methanol/15M-NH_3 (65:25:2, by vol.) or in chloroform/methanol/acetic acid/water (85:15:10:3, by vol.). and made visible by staining lightly with I_2 vapour. The phospholipid zones were scraped from the chromatograms and their phosphorus content was determined as described by Rouser *et al.* (1966).

3-*sn*-Phosphatidylcholine was also determined by the amounts of choline and phosphatidate plus phosphatidylmethanol released after treatment with phospholipase D. Phosphatidylcholine regions of chromatograms, developed as above, were eluted by adding 5.3ml of methanol/water (1.7:1, v/v), then 6.6ml of chloroform, and the lipid was extracted into the chloroform phase by shaking. This phase was dried under reduced pressure and lipid dissolved in methanol. Samples (25μl) containing 0.1–0.25μmol of phospholipid were added to 0.225ml of 50mM-sodium acetate buffer, pH5.5, containing 40mM-$CaCl_2$ and 0.2mM-sodium dodecyl sulphate. Freshly prepared extract of cabbage leaf (5μl, containing 10–12μg of protein) was added as a source of phospholipase D (Davidson & Long, 1958) and the mixture incubated at 30°C for 10min. Chloroform/methanol (2:1, v/v; 2.5ml) and water (0.25ml) were added, and the lipid was extracted into the chloroform phase. The choline content of the aq.-methanol phase was measured by the method

of Long *et al.* (1967). The chloroform phase was dried and lipids were chromatographed as above in the chloroform / methanol / NH_3 solvent and chromatograms stained with I_2 vapour. The phosphorus contents of the 3-*sn*-phosphatidylcholine and phosphatidate plus phosphatidylmethanol regions were measured as above.

Labelling of cotyledons. Cotyledons harvested as above were used. Soya-bean and safflower cotyledons were cut transversely into slices (approx. 1 mm thick). Linseed cotyledons were not sliced. In time-course studies 0.4 g samples of tissue were each placed in a 25 ml conical flask with 3.0 ml of 50 mM-Mes/NaOH buffer, pH 5.0, containing either 0.14 mM-sodium [1-^{14}C]acetate (60 mCi/mmol) and unlabelled 0.4 mM-glycerol or 0.02 mM-[2-^{3}H]glycerol (560 mCi/mmol) and unlabelled 0.5 mM-sodium acetate, then incubated at 25°C and shaken at 80 strokes/min. In pulse–chase experiments, bulk samples of tissue (about 4.0 g) were incubated with 10 ml of one of the above solutions in a 250 ml conical flask, then washed with water at 0°C and stood on ice while 0.4 g samples were transferred to 25 ml flasks containing 3.0 ml of 50 mM-Mes/NaOH buffer, pH 5.0, unlabelled 0.4 mM-glycerol and unlabelled 0.5 mM-sodium acetate, then the tissue was incubated as above. In experiments with linseed and soya-bean cotyledons, flasks were illuminated at 100 W/m^2. Incubations were commenced within 30 min of harvesting seed heads.

Lipid extraction and analysis. Tissue was washed with ice-cold water to remove incubation media, then steamed as above and homogenized in a TenBroek homogenizer with 15 ml of chloroform/methanol (2:1, v/v). After 30 min, lipids were partitioned into the chloroform phase on addition of 2.8 ml of 0.9% NaCl (Folch *et al.*, 1957), and this was evaporated to dryness under reduced pressure and lipids were redissolved in chloroform. Distribution of radioactivity among individual lipid samples was determined as a routine by chromatography on thin-layer plates of silica gel G with light petroleum (b.p. 60–80°C)/diethyl ether/acetic acid (75:25:1, by vol.) to separate individual neutral lipids from polar lipids and in chloroform/methanol/15 mM-NH_3 (65:25:1, by vol.) to separate individual phospho- and glycolipids from neutral lipids. Samples were also chromatographed on silica gel G containing 5% (w/w) H_3BO_4 in chloroform/acetone (47:3, v/v) (Thomas *et al.*, 1965) to separate 1,2-diacylglycerols from 1,3-diacylglycerols. Radioautographs of chromatograms of lipids labelled with ^{14}C were compared with the chromatograms stained with I_2 vapour. Each lipid zone was scraped into a scintillation vial containing 10 ml of either 0.5% *p*-terphenyl in xylene/Triton X-100/water (6.75:2.25:1, by vol.) for ^{14}C-labelled lipids or the scintillation fluid described by Fricke (1975) containing 10% (v/v) water for ^{3}H-labelled lipids and counted for radioactivity at efficiencies of 40% and 12% for ^{14}C and ^{3}H respectively. To measure the proportion of ^{3}H radioactivity derived from supplied [^{3}H]glycerol, in the acyl and non-acyl moieties of the total lipid extract, samples were transmethylated with sodium methoxide, then neutralized with H_2SO_4, and fatty acid methyl esters extracted into light petroleum (b.p. 40–60°C) essentially as described previously (Slack *et al.*, 1977). Samples of the light-petroleum and aq.-methanol phases were counted for radioactivity individually in scintillation fluid (Fricke, 1975).

Fatty acid methyl esters were prepared from 3-*sn*-phosphatidylcholine and di- and tri-acylglycerols, purified by t.l.c. in the above solvents from lipid extracts equivalent to 0.1 g fresh weight of tissue, and the mass of each was determined by g.l.c. (Slack *et al.*, 1977). Individual ^{14}C-labelled esters from the phospholipid and diacylglycerol were collected from the gas–liquid chromatograph fitted with an effluent-stream-splitter and counted for radioactivity as described (Slack *et al.*, 1977). This method could not be used to measure radioactivity in the fatty acid methyl esters from triacylglycerols as their specific radioactivities were too low. These methyl esters were separated into saturated, mono-, di- and tri-enoic esters by argentation t.l.c. (Morris, 1966), made visible under u.v. light after spraying plates with 0.2% dichlorofluoroscein in ethanol, and individual bands scraped directly into scintillation vials and counted for radioactivity as described above.

To determine the distribution of [^{3}H]glycerol in different molecular species of 3-*sn*-phosphatidylcholine and diacylglycerol, the lipids were first purified by preparative t.l.c. from lipid extracts equivalent to 0.2 g fresh weight of tissue. The 3-*sn*-phosphatidylcholine was eluted, incubated with phospholipase C, the diacylglycerol produced purified by t.l.c., then eluted and separated into different molecular species by argentation chromatography (Gurr & Brawn, 1970), and individual bands were eluted as described previously (Slack *et al.*, 1977). The individual diacylglycerol bands were rechromatographed on thin-layer plates of silica gel G in the light petroleum/diethyl ether/acetic acid solvent, stained lightly with I_2 vapour, then silica gel containing diacylglycerol was transferred to sodium methoxide, the sodium methoxide was neutralized with H_2SO_4 and the fatty acid methyl esters were extracted into light petroleum as previously described (Slack *et al.*, 1977). Samples of the aq.-methanol phase were counted for radioactivity in scintillation fluid (Fricke, 1975) and the fatty acid composition of the different molecular species was determined by g.l.c. of the methyl esters. Triacylglycerols were purified by preparative t.l.c., then separated into bands of different molecular species by argentation chromatography as described by Gurr *et al.* (1972). Each band was eluted and analysed as above.

Results

Phospholipid composition of developing cotyledons from seeds of safflower, linseed and soya bean

In these studies as a routine we inactivated lipases in the tissue by steaming before lipid extraction. With this technique, 3-*sn*-phosphatidylcholine was the predominant phospholipid in all the cotyledons analysed (Table 1). Generally, phospholipids were separated by one-dimensional t.l.c. in the chloroform/methanol/NH_3 solvent described above. In this solvent, phosphatidate remains at the origin. Small amounts of phosphatidate were found in extracts of the three species by two-dimensional t.l.c., and hence part at least of the phosphate present on the chromatogram origin (Table 1) represents phosphatidate. When lipid was eluted from the 3-*sn*-phosphatidylcholine region of thin-layer chromatograms and samples of the eluate in aq. methanol were incubated with phospholipase D, the choline and phosphatidylmethanol plus phosphatidate liberated represented between 95% and 98% of the phospholipid present. We conclude, therefore, that this region of the chromatograms contained only phosphatidylcholine.

Our finding that 3-*sn*-phosphatidylcholine was the major phospholipid in developing soya-bean cotyledons differs from those of Privett *et al.* (1973) and Wilson & Rinne (1974), who respectively found phosphatidate and *N*-acylphosphatidylethanolamine to constitute more than 50% of the phospholipid in extracts from this tissue. Wilson & Rinne (1976*a*) suggested that the high concentration of phosphatidate observed in extracts of soya-bean cotyledons resulted from the hydrolysis of other phospholipids subsequent to harvesting the tissue. However, by using the extraction procedure described above, we did not find significant quantities of a phospholipid with a high mobility on t.l.c. corresponding to that of *N*-acylphosphatidylethanolamine.

To determine whether our inability to detect this phospholipid had resulted from the extraction procedure used, we compared the distribution of radioactivity among phospholipids extracted by different methods from ^{14}C-labelled soya-bean slices (Table 2). When tissue was extracted without prior steaming, and particularly when tissue was ground initially in boiling methanol, a radioactive phospholipid with a high mobility was obtained. This lipid co-chromatographed with the lipid product obtained after treating a methanolic solution of 3-*sn*-phosphatidylcholine with phospholipase D, and hence we suggest that the lipid is 3-*sn*-phosphatidylmethanol derived from 3-*sn*-phosphatidylcholine during extraction. In this regard, it is noteworthy that the sum of the radioactivity and of the amounts of 3-*sn*-phosphatidylcholine and 3-*sn*-phosphatidylmethanol were similar in the extracts prepared by the different methods (Table 2). No other highly mobile phospholipid was detected, and, since Wilson & Rinne (1976*a*,*b*) used hot methanol as an initial extractant in many of their experiments, we suggest that the large amounts of lipid identified by them as *N*-acylphosphatidylethanolamine may have been phosphatidylmethanol.

Labelling of lipid fatty acids in developing seed cotyledons with [^{14}C]acetate

During the 130 min that cotyledons from developing linseed seeds were incubated in [^{14}C]acetate (Fig. 1), radioactivity entered the lipid fraction at a rate that was linear with time. Throughout the experiment 3-*sn*-phosphatidylcholine plus 1,2-diacylglycerols and triacylglycerols contained between 85% and 90% of the total radioactivity in lipids,

Table 1. *Phospholipid content and composition of developing seed cotyledons*

Cotyledons (approx. 1 g) were removed from developing seeds, weighed, steamed and the lipids extracted. The phospholipids were separated by t.l.c. and the phosphorus content of individual lipids was measured as described under 'Methods'. Measurements were made on two batches of cotyledons from each species.

Species	Phospholipid content (μmol/g fresh wt.)	Relative amounts of individual phospholipids (mol/100 mol)				
		Origin	Phosphatidyl-inositol	Phosphatidyl-choline	Phosphatidyl-ethanolamine	Phosphatidyl-glycerol
Safflower (cultivar O-22)	6.6	13	13	47	20	6
	8.1	4	18	55	20	2
Linseed	5.1	6	17	53	19	5
	5.3	7	17	56	16	4
Soya bean	8.4	8	16	55	18	4
	8.1	4	16	55	21	4

Table 2. *Effect of extraction procedure on the radioactivity in, and the mass of, different phospholipids from developing soya-bean cotyledons labelled with* [^{14}C]*acetate*

Slices of cotyledons (5g) were incubated with [^{14}C]acetate for 60min, then washed and divided into samples (1.2g). Each was extracted by one of the following methods: 1, steamed then extracted with 25ml of chloroform/methanol (2:1, v/v) as described under 'Methods'; 2, as for 1 but without steaming; 3, rapidly homogenized in 8ml of methanol, then 16ml of chloroform added; 4, as for 3 but with boiling methanol. Details of other procedures are given under 'Methods'.

Extraction method	Radioactivity (% of total ^{14}C in phospholipids) and amount (μmol/g fresh wt., in parentheses) of phospholipids in extracts obtained by different procedures					
	Origin	Phosphatidyl-inositol	Phosphatidyl-choline	Phosphatidyl-ethanolamine	Phosphatidyl-glycerol	Phosphatidyl-methanol
Steamed then chloroform/ methanol	4.4 (0.4)	7.1 (1.2)	78.3 (4.4)	6.1 (1.7)	3.5 (0.3)	0.3 (0)
Chloroform/ methanol	1.9	6.8	74.4	6.0	3.1	7.6
Methanol	2.2	7.1	58.1	5.5	3.1	23.9
Boiling methanol	2.5 (0.4)	7.4 (1.5)	43.5 (3.2)	4.6 (1.5)	2.9 (0.3)	39.0 (1.6)

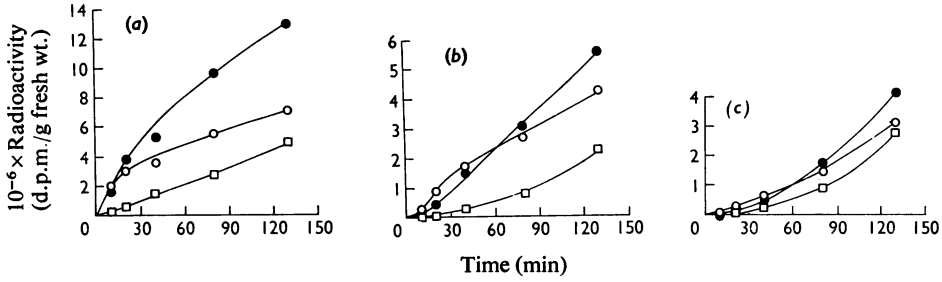

Fig. 1. *Time course of labelling of unsaturated C_{18} fatty acids of 3-sn-phosphatidylcholine and di- and tri-acylglycerols of developing linseed cotyledons supplied with* [1-^{14}C]*acetate*

Batches (0.25g) of developing linseed cotyledons were incubated with [^{14}C]acetate. At each of the times shown after supplying the radioactive substrate a batch of cotyledons was harvested and lipids were extracted and analysed as described under 'Methods'. Radioactivity in (*a*) oleate, (*b*) linoleate and (*c*) linolenate of 3-*sn*-phosphatidylcholine (○), diacylglycerols (●) and triacylglycerols (□) was determined.

and 3-*sn*-phosphatidylinositol and 3-*sn*-phosphatidylethanolamine most of the remainder. Radioactivity in the oleoyl, linoleoyl and linolenoyl moieties of the heavily labelled lipids represented about 90% of the radioactivity present. Initially, oleate in each of these lipids was apparently labelled at a linear rate (Fig. 1*a*), whereas radioactivity entered linoleate and linolenate at accelerating rates (Figs. 1*b* and 1*c*). Similar differences between the initial rates of labelling of oleate and linoleate in 3-*sn*-phosphatidylcholine and di- and tri-acylglycerols of soya-bean cotyledons (Figs. 2*a* and 2*b*) and in safflower cotyledons (C. R. Slack, P. G. Roughan & N. Balasingham, unpublished work) were observed. Linolenate, a minor component of these lipids in soya-bean cotyledons, accumulated little activity.

The decline with time in the rate of labelling of the oleate of 3-*sn*-phosphatidylcholine (Figs. 1*a* and 2*a*) is probably attributable, at least in part, to a pronounced decrease in the mass of oleate within the phospholipid during the incubation of cotyledon tissue (Table 3, Figs. 3*a* and 4*a*). It is noteworthy that in the safflower and soya-bean cotyledons (Table 3) the decrease in oleate was largely compensated for by an increase in linoleate, but in linseed cotyledons by an increase in linolenate. Similar but less pronounced changes occurred in the fatty acid composition of the diacylglycerols. The inclusion of glucose or sucrose in the incubation medium at concentrations up to 80mM did not prevent the loss of oleate from the phospholipid. In studies with detached but photosynthetically active maize laminae (Slack & Roughan, 1975; Slack

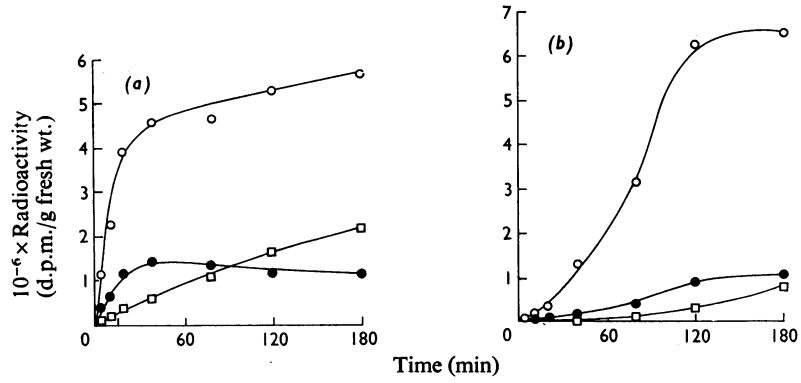

Fig. 2. *Time course of labelling of oleate and linoleate of 3-sn-phosphatidylcholine and di- and tri-acylglycerols in developing soya-bean cotyledons supplied with [1-^{14}C]acetate*

Batches (0.6g) of cotyledon slices from developing soya-bean seeds were supplied with [^{14}C]acetate and analysed at intervals as described in Fig. 1. Radioactivity in (*a*) oleate and (*b*) linoleate of 3-*sn*-phosphatidylcholine (○), diacylglycerols (●) and triacylglycerols (□) was determined.

Table 3. *Changes in the fatty acid composition of 3-sn-phosphatidylcholine in developing seed cotyledons incubated in vitro*

3-*sn*-Phosphatidylcholine from the first and last harvests of the experiments described in Figs. 1 and 2 and from slices of safflower cotyledons incubated for 10 and 75min were analysed as described under 'Methods'.

Species	Incubation time (min)	Fatty acid composition of 3-*sn*-phosphatidylcholine (g/mol)				
		Palmitate	Stearate	Oleate	Linoleate	Linolenate
Safflower (cultivar O-22)	10	65.9	20.7	44.1	428.0	
	75	65.9	21.2	26.3	445.9	
Soya bean	5	80.2	27.7	201.1	208.5	35.4
	180	76.9	25.5	112.3	292.7	45.9
Linseed	10	52.2	58.9	201.6	74.4	167.8
	130	42.5	46.1	134.8	92.5	239.5

et al., 1977) no change occurred in the fatty acid composition of 3-*sn*-phosphatidylcholine over a 24h period. However, when laminae were held under unphysiological conditions in darkness and under N_2, a similar loss in oleate from the phospholipid was observed. It would appear therefore that a substantial part of the oleate esterified to 3-*sn*-phosphatidylcholine in developing leaves and cotyledons may have a high turnover rate and that under certain abnormal conditions the rate of oleate incorporation into the lipid can be decreased more than the subsequent metabolism of the fatty acid.

One explanation for the more rapid initial rate of labelling of the linoleoyl moiety of 3-*sn*-phosphatidylcholine than of the linoleate of the di- and tri-acylglycerols during the above time-course experiments (Figs. 1*b* and 2*b*) was that the linoleate of these neutral lipids may have been derived from oleate esterified to the phospholipid. Consequently, we examined the redistribution of label among glycerolipids subsequent to the transfer of cotyledons from [^{14}C]acetate to unlabelled media. With both linseed and soya-bean cotyledons (Table 4) and with safflower cotyledons (C. R. Slack, P. G. Roughan & N. Balasingham, unpublished work) the proportion of the total radioactivity in 3-*sn*-phosphatidylcholine declined and that in triacylglycerols increased after the transfer; concomitant changes in the proportion of the radioactivity in diacylglycerols were small. Since the total radioactivity in glycerolipids remained relatively constant during the experiments, the redistribution of radioactivity suggests a flow of fatty acid from 3-*sn*-phosphatidylcholine to triacylglycerols.

The major loss of radioactivity from 3-*sn*-phosphatidylcholine was from the oleoyl moiety and was accompanied by a decrease in the amount of oleate in the lipid (Figs. 3*a* and 4*a*). Much of the radio-

Table 4. *Total radioactivity in lipids and the proportion of the total radioactivity in 3-sn-phosphatidylcholine and di- and triacylglycerols after transferring cotyledons of linseed and soya beans from solution containing [^{14}C]acetate to unlabelled solution*

Slices of soya-bean cotyledons and linseed cotyledons were each incubated with [^{14}C]acetate for 15 min, then transferred to unlabelled solutions and samples assayed at the time of transfer and at times thereafter. Conditions during the incubation and the methods of lipid analysis were as described under 'Methods'.

Species	10^{-6} × Total radioactivity in lipids (d.p.m./g fresh wt.) and proportion of radioactivity in individual lipids (%)					
Linseed						
Time in unlabelled solution (min) ...	0	12	24	43	95	140
Total radioactivity	12.1	12.1	13.4	13.1	14.0	12.5
3-*sn*-Phosphatidylcholine	40.4	34.3	29.8	28.6	20.9	21.6
Diacylglycerol	36.0	41.6	44.1	42.3	45.9	43.8
Triacylglycerol	7.5	10.0	12.9	15.0	19.3	22.1
Soya bean						
Time in unlabelled solution (min) ...	0	60	120	180	240	300
Total radioactivity	4.6	4.2	4.6	4.3	4.5	4.6
3-*sn*-Phosphatidylcholine	64.7	62.6	59.7	56.1	53.1	50.2
Diacylglycerol	13.3	8.3	8.3	7.1	6.5	7.7
Triacylglycerol	8.7	11.9	15.5	17.3	21.5	21.4

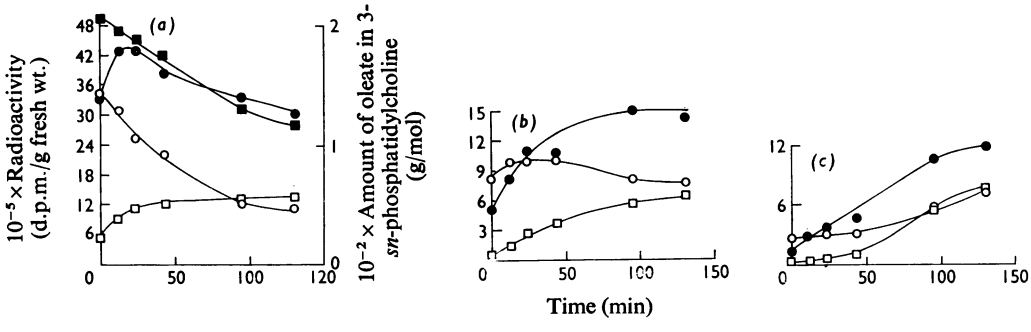

Fig. 3. *Redistribution of radioactivity among the unsaturated C_{18} fatty acids of 3-sn-phosphatidylcholine, diacylglycerols and triacylglycerols, and changes in the amount of oleate present in 3-sn-phosphatidylcholine, after the transfer of developing linseed cotyledons from [1-^{14}C]acetate to unlabelled media*

Developing linseed cotyledons (4g) were incubated with [^{14}C]acetate for 15 min, then transferred to ice, washed and samples (0.5 g) transferred to unlabelled media and returned to the incubation bath. A sample was harvested at the time of transfer and at each of the times shown thereafter and the lipids were extracted and analysed as described under 'Methods'. Radioactivity in (*a*) oleate, (*b*) linoleate and (*c*) linolenate of 3-*sn*-phosphatidylcholine (○), diacylglycerols (●) and triacylglycerols (□) and the amount of oleate in 3-*sn*-phosphatidylcholine (■) were determined.

activity lost from this fatty acid in the soya-bean cotyledons (Fig. 4*a*) accumulated in the linoleoyl moiety of the phospholipid (Fig. 4*b*), but throughout the experiment there was also a continuous increase in the amount of radioactivity in the oleate and linoleate of the triacylglycerols (Fig. 4*a* and *b*). In linseed cotyledons, on the other hand, the radioactivity in the linoleoyl moiety of 3-*sn*-phosphatidylcholine remained relatively constant (Fig. 3*b*), and the radioactivity lost from the oleate of this phospholipid accumulated in the linoleate of the di- and triacylglycerols (Fig. 3*b*) and in the linolenate of these neutral lipids and 3-*sn*-phosphatidylcholine (Fig. 3*c*). In the above experiments, the proportion of the total radioactivity in diacylglycerol compared with that in 3-*sn*-phosphatidylcholine was considerably greater in linseed than soya-bean cotyledons. This difference appeared to reflect the relative amounts of these lipids

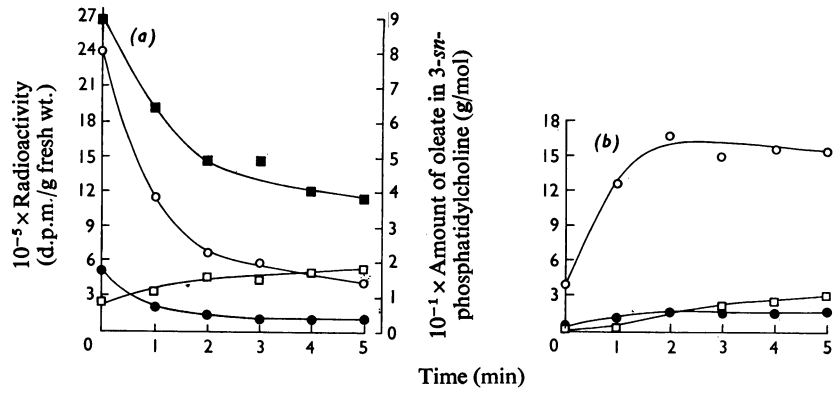

Fig. 4. *Redistribution of radioactivity between oleate and linoleate of 3-sn-phosphatidylcholine, di- and tri-acylglycerols, and changes in the amount of oleate present in 3-sn-phosphatidylcholine, after the transfer of developing soya-bean cotyledons from [1-^{14}C]acetate to unlabelled media*

Cotyledon slices of developing soya-bean seeds were incubated with [1-^{14}C]acetate, washed, subsampled, transferred to unlabelled media and analysed at intervals as described in Fig. 3. Radioactivity in (*a*) oleate and (*b*) linoleate of 3-*sn*-phosphatidylcholine (○), diacylglycerols (●) and triacylglycerols (□) and the amount of oleate in 3-*sn*-phosphatidylcholine (■) were determined.

Table 5. *Fatty acid composition of 3-sn-phosphatidylcholine and diacylglycerol from developing seed cotyledons of various species*

Cotyledons were steamed, then lipids extracted and these purified by t.l.c. The fatty acids of each were analysed by g.l.c. as described under 'Methods'. Abbreviations: PC, 3-*sn*-phosphatidylcholine; DiAG, diacylglycerol.

		Amounts of individual fatty acids in each lipid (g/mol)				
Species	Lipid	Palmitate	Stearate	Oleate	Linoleate	Linolenate
Linseed	PC	50	41	193	90	175
	DiAG	35	35	215	77	188
Soya bean	PC	81	28	200	210	35
	DiAG	78	26	184	212	55
Safflower	PC	65	20	43	420	
Cultivar O-22	DiAG	69	20	51	405	
Cultivar UC-1	PC	33	4	371	152	
	DiAG	27	3	378	152	
Intermediate	PC	69	11	106	362	
linoleate	DiAG	53	10	115	370	

in the two species, since the ratios of the amount (μmol/g fresh wt.) of this phospholipid to that of diacylglycerol were about 0.6 and 2.0 for the linseed and soya-bean cotyledons respectively. Moreover, interspecific differences in the relative amounts of radioactivity incorporated into the individual C_{18} fatty acids of these lipids appeared to reflect the amounts of the individual fatty acids in the lipids (Table 5). It is noteworthy in this regard that the fatty acid compositions of 3-*sn*-phosphatidylcholine and diacylglycerol isolated from the same batch of cotyledons were similar. The safflower cultivars O-22, intermediate linoleate and UC-1 possess high, intermediate and low amounts of linoleate respectively in the triacylglycerols of mature seed (Knowles, 1969).

Incorporation of [2-^{3}H]glycerol into the glycerolipids of developing seed cotyledons

[^{3}H]Glycerol was also incorporated mainly into 3-*sn*-phosphatidylcholine and di- and tri-acylglycerols by cotyledons, and radioactivity was found only in the glycerol moieties of these lipids by procedures described under 'Methods'. With linseed cotyledons

(Fig. 5) and also with the soya-bean cotyledons, [^{3}H]-glycerol entered the 3-*sn*-phosphatidylcholine and diacylglycerol at rates essentially linear with time from zero time, and the triacylglycerols at an accelerating rate. An initially slow rate of labelling of the triacylglycerols of developing crambé-seed cotyledons by [U-^{14}C]glycerol has also been observed (Gurr *et al.*, 1974).

Although [^{3}H]glycerol entered the total diacylglycerol pool in the linseed cotyledons at a rate essentially linear with time, the rates of labelling of individual diacylglycerol species, separated by argentation chromatography (Table 6), differed considerably (Fig. 6). Initially the label appeared most rapidly in those species that contained oleate and entered the more unsaturated species at accelerating rates. It is noteworthy that the initial rates of labelling of the different species bore no relationship to the relative amounts of the individual species present in the tissue (Table 6). The rates of labelling of individual species of 3-*sn*-phosphatidylcholine were similar to those obtained with diacylglycerols.

On transferring linseed cotyledons from [^{3}H]-glycerol-labelled to unlabelled media there was a considerable redistribution of label between the different lipids concomitant with relatively small changes in the total radioactivity present in lipids (Table 7). The decrease in radioactivity in 3-*sn*-phosphatidylcholine and diacylglycerol and the increase in radioactivity in triacylglycerol were consistent with the view that a net transfer of the glycerol moieties from the two former lipids to the latter had occurred. Similar results were obtained with soya-bean cotyledon tissue. The triacylglycerols from linseed cotyledons were separated by argentation chromatography into seven major bands; the largest, and that into which most of the label was accumulated during the 'chase', was apparently composed of a series of triacylglycerol species that contained two molecules of linolenate (Table 8). In view of the preferential movement of [^{3}H]glycerol from 3-*sn*-phosphatidylcholine and diacylglycerol into these highly unsaturated species, it was noteworthy that the major loss of [^{3}H]glycerol from both the phospholipid and diacylglycerol occurred from dioleoyl species (Table 9).

Discussion

It is difficult to reconcile the considerable delay observed in the present studies before the polyunsaturated C_{18} fatty acids and glycerol of triacylglycerols accumulated significant amounts of radioactivity with the view (Hichcock & Nichols, 1971; Gurr *et al.*, 1974; Stumpf, 1975) that triacylglycerols

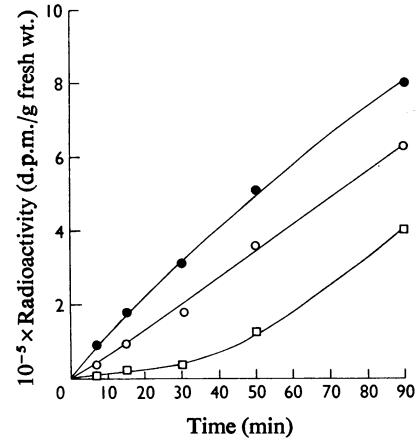

Fig. 5. *Time course of labelling the 3-sn-phosphatidylcholine, di- and tri-acylglycerols of developing linseed cotyledons with [2-^{3}H]glycerol*

Batches (0.5g) of developing linseed cotyledons were incubated with [^{3}H]glycerol and a batch was harvested at each of the times shown. Lipids were extracted and analysed as described under 'Methods'. ○, 3-*sn*-Phosphatidylcholine; ●, diacylglycerols; □, triacylglycerols.

Table 6. *Fatty acid composition of different molecular species of diacylglycerols from linseed cotyledons*

Diacylglycerols were purified from the lipid extracts of each of the harvests of the time-course labelling experiment described in Fig. 5 and separated into eight different bands by argentation chromatography. Individual bands were eluted, repurified by t.l.c., then transmethylated. Radioactivity in individual bands was determined (Fig. 6). Methyl esters from corresponding bands of the different harvests were pooled and analysed by g.l.c.

	Amount of individual fatty acids in different bands (μg/g fresh wt. of tissue)							
Band ...	1	2	3	4	5	6	7	8
Fatty acid								
$C_{16:0}$	2.4	1.3	2.4	0.7	5.9	1.0	1.0	—
$C_{18:0}$	1.5	0.9	1.4	0.6	6.1	1.0	1.1	0.4
$C_{18:1}$	4.3	11.9	2.0	3.6	1.8	11.2	1.7	0.4
$C_{18:2}$	—	1.1	4.4	4.2	1.6	1.0	9.9	0.6
$C_{18:3}$	—	—	—	—	12.6	11.7	10.5	18.3

Table 7. *Changes in the distribution of radioactivity in developing seed cotyledons after transfer from [^{3}H]glycerol to unlabelled glycerol*

Samples (0.5g) of linseed cotyledons were incubated with [^{3}H]glycerol for 30min then washed and transferred to unlabelled glycerol. Two batches of cotyledons were harvested at the time of transfer and at 2 and 5h thereafter and the distribution of radioactivity among individual lipids was determined as described under 'Methods'.

Time after transfer from [^{3}H]glycerol (h)	$10^{-6}\times$ Radioactivity in lipid extracts (d.p.m./g fresh wt.)	Radioactivity in individual lipids (% of total radioactivity in lipid extracts)		
		Phosphatidylcholine	Diacylglycerol	Triacylglycerol
0	3.8	34.0	45.2	5.9
	4.4	34.6	46.7	7.0
2	3.8	23.8	41.6	23.1
	3.8	27.2	34.8	21.2
5	4.8	18.8	28.0	41.7
	5.6	17.3	33.8	38.6

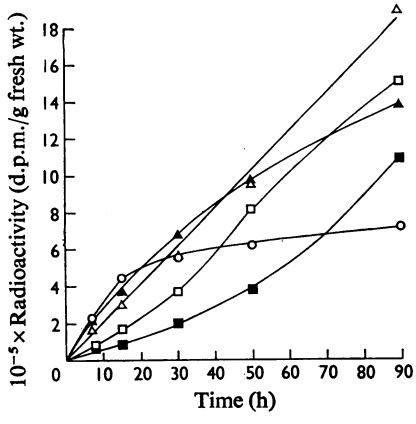

Fig. 6. *Time course of labelling of different molecular species of diacylglycerols from linseed cotyledons supplied with [2-^{3}H]glycerol*

Diacylglycerols were purified from lipid extracts of each harvest of the experiment shown in Fig. 5 and separated by argentation chromatography into different bands as described in Table 6. Radioactivity in dioleoylglycerol, band 2 (○); saturated fatty acid-linolenylglycerol, band 5 (△); oleoyl-linolenylglycerol, band 6 (▲); linoleoyl-linolenylglycerol, band 7 (□); dilinolenoylglycerol, band 8 (■).

are synthesized in developing seed cotyledons by a 'simple form' of the glycerol 3-phosphate pathway (Kennedy, 1961). For instance, if labelled triacylglycerols had been derived directly from a pool of phosphatidate, produced by the acylation of [^{3}H]glycerol 3-phosphate, then one would have to assume that the [^{3}H]glycerol equilibrated much more slowly with this pool than with phosphatidate from which the rapidly labelled diacylglycerols and 3-*sn*-phosphatidylcholine were formed. Rather we interpret the higher rates of entry of ^{14}C-labelled fatty acids and [^{3}H]glycerol into these latter lipids and the transfer of label from them to triacylglycerols as indicating that the metabolism of 3-*sn*-phosphatidylcholine is associated with triacylglycerol synthesis. This phospholipid has been similarly implicated in the supply of diacylglycerols for triacylglycerol synthesis in rat liver (Kanoh & Ohno, 1973*b*) and the lactating mammary gland (Patton & Jensen, 1975).

The observed sequence of labelling of unsaturated C_{18} fatty acids agreed with the numerous previous findings (Simmons & Quackenbush, 1954; Dutton & Mounts, 1966; Canvin, 1965*a*,*b*; Rinne & Canvin, 1971; Dybing & Craig, 1970) that in developing cotyledons linolenate is formed from oleate by sequential desaturation via linoleate. Further, the more rapid appearance of ^{14}C radioactivity in the linoleoyl moiety of 3-*sn*-phosphatidylcholine than in the corresponding fatty acid of diacylglycerol in linseed and soya-bean cotyledons together with the movement of label from the oleoyl to linoleoyl moiety of soya-bean 3-*sn*-phosphatidylcholine, during pulse–chase labelling, suggests that this phospholipid may be intimately involved in the initial desaturation of oleate to linoleate in a manner analogous to that in leaves (Slack *et al.*, 1976). We find it difficult to accommodate this redistribution of label between the oleoyl and linoleoyl moieties of 3-*sn*-phosphatidylcholine, and the concomitant large decrease in the mass of the oleoyl moiety, indicative of a rapid turnover of a substantial portion of this fatty acid in the phospholipid, with the view that the major oleate desaturase in seed cotyledons utilizes oleoyl-CoA as substrate (Vijay & Stumpf, 1971; Stumpf, 1975; Stumpf & Porra, 1976). If an oleoyl-CoA desaturase were involved, one would have to envisage a rapid exchange of oleate and linoleate between CoA and 3-*sn*-phosphatidylcholine. Such a hypothesis is favoured by Heinz & Harwood (1977) to explain the kinetics of labelling of fatty

Table 9. *Distribution of radioactivity among the different molecular species of diacylglycerol and 3-sn-phosphatidylcholine of linseed cotyledons during a pulse–chase with [^{3}H]glycerol*

Diacylglycerols and 3-*sn*-phosphatidylcholine were isolated by t.l.c. from lipid extracts of the linseed cotyledons harvested at 0, 2 and 5h after pulse-labelling with [^{3}H]glycerol in the experiment described in Table 7. The purified diacylglycerols and diacylglycerols liberated from the 3-*sn*-phosphatidylcholine samples by treatment with phospholipase C were each separated into eight bands by argentation chromatography. The radioactivity in each band and the fatty acid composition of bands with corresponding mobilities were measured as described under 'Methods'. The major molecular species in each band was determined from the proportion of the individual fatty acids present.

Lipid	Time after transfer from [^{3}H]glycerol (h)	Distribution of radioactivity among different molecular species (%)							
		$C_{16:0}$+ $C_{18:0}$/ $C_{18:1}$	$C_{18:1_2}$	$C_{16:0}$+ $C_{18:0}$/ $C_{18:2}$	$C_{18:1}$/ $C_{18:2}$	$C_{16:0}$+ $C_{18:0}$/ $C_{18:3}$	$C_{18:1}$/ $C_{18:3}$	$C_{18:2}$/ $C_{18:3}$	$C_{18:3_2}$
Diacylglycerol	0	9.7	26.3	8.6	10.8	9.7	21.4	8.9	4.6
	2	6.8	11.5	9.1	8.4	15.8	23.0	13.8	11.5
	5	9.1	11.0	7.5	9.1	14.0	21.4	15.8	12.1
3-*sn*-Phosphatidylcholine	0	11.9	18.6	12.2	10.3	12.6	12.6	14.0	7.2
	2	15.6	11.6	11.4	10.3	16.0	14.9	10.0	10.1
	5	16.1	9.0	11.0	10.4	15.8	15.4	9.8	12.2

acids in leaves of *Vicia faba*. However, although there is now good evidence that in plants stearoyl-(acyl-carrier protein) is the substrate for stearate desaturation (Nagai & Bloch, 1968; Stumpf & Porra, 1976), the present evidence that an oleoyl-thiol ester, such as oleoyl-CoA, is the substrate for oleate desaturation is much less compelling. The formation of [^{14}C]linoleate from [^{14}C]oleoyl-CoA by microsomal fractions of *Neurospora crassa* (Baker & Lynen, 1971), safflower (*Carthamus tinctorius*) seed cotyledons (Vijay & Stumpf, 1971), potato tubers (Ben Abdelkader *et al.*, 1973; Kader, 1977), *Torulopsis utilis* (Talamo *et al.*, 1973), *Candida lipolytica* (Pugh & Kates, 1973, 1975) and pea leaves (Slack *et al.*, 1976; Dubacq *et al.*, 1976) has been demonstrated, but in none of these studies has linoleoyl-CoA been identified conclusively as the product. Where the form of the labelled linoleate has been investigated by chromatography it has, with one exception (Pugh & Kates, 1973), been shown to be esterified in phospholipids, largely phosphatidylcholine (Baker & Lynen, 1971; Ben Abdelkader *et al.*, 1973; Talamo *et al.*, 1973; Slack *et al.*, 1976; Dubacq *et al.*, 1976). Further, investigations of the metabolism of oleoyl-CoA by microsomal fractions (Baker & Lynen, 1971; Talamo *et al.*, 1973; Slack *et al.*, 1976) indicated that fatty acid was incorporated into 3-*sn*-phosphatidylcholine rapidly, then desaturated. These studies, together with the direct demonstration that oleoyl-phosphatidylcholine can serve as a substrate for oleate desaturation (Gurr *et al.*, 1969; Talamo *et al.*, 1973; Pugh & Kates, 1975), lead us to believe that the studies *in vivo* described above indicate that oleoyl phosphatidylcholine is very probably the substrate for oleate desaturation in oil seeds. The nature of the linoleate that is desaturated to form linolenate is not clear from our present studies. However, since ^{14}C radioactivity entered the linolenate of 3-*sn*-phosphatidylcholine and diacylglycerol more rapidly than that of triacylglycerol, and [^{3}H]glycerol originally in the dioleoyl species of these lipids accumulated in triacylglycerols containing two molecules of linolenate during pulse-chase labelling, it would appear that linoleate esterified to one or other of these former lipids could be the immediate precursor of linolenate.

In developing maize leaf the re-distribution of label from the acyl and glycerol moieties of 3-*sn*-phosphatidylcholine and diacylmonogalactosylglycerol was very similar and consistent with the transfer of the diacylglycerol moiety from the phospholipid to other lipids (Slack *et al.*, 1977). Although the present studies suggest that this phospholipid serves a similar role during triacylglycerol synthesis, differences were observed in the proportions of ^{14}C-labelled fatty acids and [^{3}H]glycerol that entered triacylglycerol from the phospholipid. Whereas the labelled fatty acids gained by triacylglycerol in pulse–chase studies represented a net loss principally from 3-*sn*-phos-

Table 8. *Distribution of (a) radioactivity and (b) fatty acids among different molecular species of the triacylglycerol of linseed cotyledons during a pulse–chase with [3H]glycerol*

Triacylglycerol was isolated by t.l.c. from lipid extracts of linseed cotyledons harvested 0, 2 and 5 h after pulse-labelling with [^{3}H]glycerol in the experiment described in Table 7, and separated into different bands by argentation chromatography. Individual bands were eluted and, after repurification by t.l.c., transmethylated with sodium methoxide. In Expt. (*a*) the distribution of radioactivity in the glycerol moiety and in Expt. (*b*) the fatty acid composition of each band were determined as described under 'Methods'. The distribution of radioactivity given is the means of the duplicate samples at each harvest. The fatty acid composition was determined on a pooled sample of each band from all harvests.

Expt. (*a*)

Time after transfer from [^{3}H]glycerol (h)	Distribution of radioactivity among the different bands (%)						
Band ...	1	2	3	4	5	6	7
0	8.0	4.1	3.8	12.0	22.3	16.0	33.8
2	4.8	1.6	2.4	9.3	17.0	19.9	44.9
5	3.3	1.5	3.2	9.1	11.2	17.2	54.9

Expt. (*b*)

Fatty acids	10^{-2} × Amount of individual fatty acids in the different bands (μg/g fresh wt. of tissue)						
Band ...	1	2	3	4	5	6	7
$C_{16:0}$	4.0	1.6	0.5	3.9	0.9	2.0	4.0
$C_{18:0}$	4.8	1.5	0.4	4.7	0.8	2.0	4.2
$C_{18:1}$	25.9	5.2	5.5	10.0	21.0	10.4	16.1
$C_{18:2}$	0.6	2.1	2.3	2.1	0.7	9.3	10.1
$C_{18:3}$	—	—	—	9.1	10.9	12.8	74.4

phatidylcholine, the labelled glycerol accumulated by triacylglycerol was derived from both the phospholipid and diacylglycerol. One explanation for this observation could be that labelled fatty acids from the phospholipid were transferred to the triacylglycerol by deacylation then insertion into the C-3 position of the triacylglycerol as well as via diacylglycerol. The joint action of these two mechanism would also account for the greater proportion of labelled fatty acids, compared with [^{3}H]glycerol, incorporated into 3-*sn*-phosphatidylcholine (Tables 4 and 7, Figs. 1 and 5), since fatty acids could enter the lipid by the acylation of lysophosphatidylcholine and from labelled diacylglycerol, whereas [^{3}H]glycerol could enter the lipid only via diacylglycerols.

We suggested (Slack *et al.*, 1977) that the liberation of the diacylglycerol moiety from 3-*sn*-phosphatidylcholine could involve the joint action of phospholipase D and phosphatidate phosphatase. An alternative route could be via choline phosphotransferase (EC 2.7.8.2), which catalyses the reversible transfer of choline phosphate between 1,2-diacylglycerol and CMP (Weiss *et al.*, 1958). The CMP-dependent release of CDP-choline and diacylglycerol from 3-*sn*-phosphatidylcholine in rat liver microsomal fractions has been studied in some detail (Kanoh & Ohno, 1973*a*,*b*) and it is noteworthy in regard to the present studies that the liver enzyme could utilize the total 3-*sn*-phosphatidylcholine pool within the microsomal fractions (Kanoh & Ohno, 1973*b*). The cooperative function of an oleoylphosphatidylcholine desaturase, discussed above, and choline phosphotransferase in microsomal fractions of seed cotyledons could maintain a pool of polyunsaturated diacylglycerols. A comparative study of the entry of glycerol and choline into 3-*sn*-phosphatidylcholine of developing seed cotyledons may provide information as to whether or not the phosphotransferase can establish an equilibrium between diacylglycerols and the diacylglycerol moiety of the phospholipid *in vivo*.

We thank Mr. Cecil Tunnicliffe and Mrs. Ngaire Lourie for technical assistance, Miss Jill Bambery for help in preparing the manuscript, and Dr. Martin McEwan and Dr. Dennis Davia for supplying seed of safflower cultivars.

References

Baker, N. & Lynen, F. (1971) *Eur. J. Biochem.* **19**, 200–210
Ben Abdelkader, A., Cherif, A., Demandre, C. & Mazliak, P. (1973) *Eur. J. Biochem.* **32**, 155–165
Canvin, D. T. (1965*a*) *Can. J. Bot.* **43**, 49–62
Canvin, D. T. (1965*b*) *Can. J. Bot.* **43**, 71–74
Davidson, F. M. & Long, C. (1958) *Biochem. J.* **69**, 458–466
Downey, R. K. & McGregor, D. I. (1975) *Curr. Adv. Plant Sci.* **6**, 151–167
Dubacq, J. P., Mazliak, P. & Tremolières, A. (1976) *FEBS Lett.* **66**, 183–186
Dutton, H. J. & Mounts, T. L. (1966) *J. Lipid Res.* **7**, 221–225
Dybing, C. D. & Craig, B. M. (1970) *Lipids* **5**, 422–433

Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497–509
Fricke, V. (1975) *Anal. Biochem.* **65**, 555–558
Gurr, M. I. & Brawn, P. (1970) *Eur. J. Biochem.* **17**, 19–22
Gurr, M. I., Robinson, M. P. & James, A. T. (1969) *Eur. J. Biochem.* **9**, 70–78
Gurr, M. I., Blades, J. & Appleby, R. S. (1972) *Eur. J. Biochem.* **29**, 362–368
Gurr, M. I., Blades, J., Appleby, R. S., Smith, C. G., Robinson, M. P. & Nichols, B. W. (1974) *Eur. J. Biochem.* **43**, 281–290
Heinz, E. & Harwood, J. L. (1977) *Hoppe-Seylers' Z. Physiol. Chem.* **358**, 897–908
Hichcock, C. & Nichols, B. W. (1971) *Plant Lipid Biochemistry*, p. 178, Academic Press, London and New York
Hoagland, D. R. & Arnon, D. I. (1938) *Calif. Agric. Exp. Stn. Circ.* **347**, 36–56
Kader, J. C. (1977) *Biochim. Biophys. Acta* **486**, 429–436
Kanoh, H. & Ohno, K. (1973*a*) *Biochim. Biophys. Acta* **306**, 203–217
Kanoh, H. & Ohno, K. (1973*b*) *Biochim. Biophys. Acta* **326**, 17–25
Kennedy, E. P. (1961) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **20**, 934–940
Knowles, P. F. (1969) *J. Am. Oil Chem. Soc.* **46**, 130–132
Long, C., Odavic, R. & Sargent, E. J. (1967) *Biochem. J.* **102**, 216–220
Morris, L. J. (1966) *J. Lipid Res.* **7**, 717–732
Nagai, J. & Bloch, H. (1968) *J. Biol. Chem.* **243**, 4626–4633
Patton, S. & Jensen, R. G. (1975) in *Progress in Chemistry of Fats and Other Lipids* (Holman, R. T., ed.), vol. 14, Pt. 4, pp. 228, Pergamon Press, Oxford
Privett, O. S., Dougherty, K. A., Erdahl, W. L. & Stolywho, A. (1973) *J. Am. Oil Chem. Soc.* **50**, 516–520
Pugh, E. L. & Kates, M. (1973) *Biochim. Biophys. Acta* **316**, 305–316
Pugh, E. L. & Kates, M. (1975) *Biochim. Biophys. Acta* **380**, 442–453
Rinne, R. W. & Canvin, D. T. (1971) *Plant Cell Physiol.* **12**, 387–393
Roughan, P. G. & Boardman, N. K. (1972) *Plant Physiol.* **50**, 31–34
Rouser, G., Siakatos, A. M. & Fleischer, S. (1966) *Lipids* **1**, 85–86
Rubel, A., Rinne, R. W. & Canvin, D. T. (1972) *Crop Sci.* **12**, 739–741
Shine, W. E., Mancha, M. & Stumpf, P. K. (1976) *Arch. Biochem. Biophys.* **173**, 472–479
Simmons, R. O. & Quackenbush, F. W. (1954) *J. Am. Oil Chem. Soc.* **31**, 441–443
Singh, H. & Privett, O. S. (1970) *Biochim. Biophys. Acta* **202**, 200–202
Slack, C. R. & Roughan, P. G. (1975) *Biochem. J.* **152**, 217–228
Slack, C. R., Roughan, P. G. & Terpstra, J. (1976) *Biochem. J.* **155**, 71–80
Slack, C. R., Roughan, P. G. & Balasingham, N. (1977) *Biochem. J.* **162**, 289–296
Stumpf, P. K. (1975) *J. Am. Oil Chem. Soc.* **52**, 484A–490A
Stumpf, P. K. & Porra, R. J. (1976) *Arch. Biochem. Biophys.* **176**, 63–70
Talamo, B., Chang, N. & Bloch, K. (1973) *J. Biol. Chem.* **248**, 2738–2742
Thomas, A. E., Scharoun, J. E. & Ralston, H. (1965) *J. Am. Oil Chem. Soc.* **42**, 789–792
Vijay, I. K. & Stumpf, P. K. (1971) *J. Biol. Chem.* **246**, 2910–2917
Weiss, S. B., Smith, S. W. & Kennedy, E. P. (1958) *J. Biol. Chem.* **231**, 53–64
Wilson, R. F. & Rinne, R. W. (1974) *Plant Physiol.* **54**, 744–747
Wilson, R. F. & Rinne, R. W. (1976*a*) *Plant Physiol.* **57**, 270–273
Wilson, R. F. & Rinne, R. W. (1976*b*) *Plant Physiol.* **57**, 375–381