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Incorporation of Choline and Ethanolamine into Phospholipids in Germinating Soya Bean

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1. Incorporation of [*Me*-^{14}C]choline and [2-^{14}C]ethanolamine into lipids was studied in germinating soya bean (*Glycine max* L.) seeds. The precursors are only incorporated into phosphatidylcholine and into phosphatidylethanolamine respectively. 2. Base-labelling via a phospholipase-D type of reaction was eliminated as a significant factor. 3. Cycloheximide inhibited labelling of phosphatidylcholine from [*Me*-^{14}C]choline but did not affect labelling of the aqueous choline pool. It had no effect on [2-^{14}C]ethanolamine uptake or incorporation into phosphatidylethanolamine. 4. Hemicholinium-15 at 10mM concentrations decreased uptake and lipid labelling from both bases. 5. There was no evidence for base competition. 6. The endogenous pool of choline was much larger than that of ethanolamine, which resulted in higher specific radioactivities for phosphatidylethanolamine than for phosphatidylcholine. 7. The results can be interpreted as indicating that the kinase and phosphoryltransferase enzymes of the CDP-base pathways are separate for each phospholipid.

Phosphatidylcholine is the major phospholipid in almost every plant tissue that has been examined (Galliard, 1973). In non-photosynthetic tissues phosphatidylethanolamine is the second most prevalent phospholipid, whereas in green leaves phosphatidylglycerol is present in amounts often comparable with those of phosphatidylcholine (Hitchcock & Nichols, 1971; Galliard, 1973). Not only are these phospholipids important as structural components of cellular membranes, but they may play important metabolic roles. For example, phosphatidylcholine has been implicated as acceptor (Abdelkader *et al.*, 1973; Vijay & Stumpf, 1971) or substrate during fatty acid desaturation (Gurr *et al.*, 1969; Roughan, 1970; Pugh & Kates, 1973; Willemot & Verret, 1973).

Two major pathways are known for the biosynthesis of phosphatidylcholine *de novo*. These are the CDP-base pathway (Kennedy, 1962) and the methylation pathway from phosphatidylethanolamine (Bremer & Greenberg, 1961). Individual enzymes of the former pathway have been demonstrated in a number of photosynthetic and non-photosynthetic plant tissues. Choline kinase has been found in rape seed (Ramasarma & Wetter, 1957) and in leaves from a number of plants (Tanaka *et al.*, 1966). The presence of all three enzymes in the pathway has been shown in fractions from onion stem (Morre *et al.*, 1970) and castor bean (Moore *et al.*, 1973). Study of some properties of phosphorylcholine glyceride transferase (EC 2.7.8.2) has been made with a microsomal fraction prepared from spinach leaves (Devor & Mudd, 1971; Marshall & Kates, 1974) and from castor bean (Lord, 1975). Indirect evidence from [^{14}C]ethanolamine-incorporation studies pointed to the existence of the methylation pathway for phosphatidylcholine synthesis in tomato root (Willemot & Boll, 1967) and in spinach leaves (Marshall & Kates, 1974). Attempts to measure individual enzymes have, however, been somewhat unconvincing (Kates & Marshall, 1975). In a study using germinating castor bean, Moore (1976) concluded that, although the *S*-adenosylmethionine methyltransferase was present, it had an activity only one-twentieth that of phosphorylcholine glyceride transferase.

Because of the importance of phosphatidylcholine in higher-plant tissues we wished to clarify details of its biosynthesis with respect to (*a*) the relative importance of the various synthetic pathways, and (*b*) differences from enzymes involved in forming other phospholipids. Initially we chose the germinating soya bean as the experimental system and examined incorporation of radioactive base precursors. This enabled experiments to be performed with an active system *in vivo*. Similar techniques have yielded much valuable information on phospholipid synthesis in plants (e.g. Willemot & Boll, 1967; Douce & Dupont, 1969; Willemot & Verret, 1973; Marshall & Kates, 1974). Soya bean was used because it is a convenient experimental seed, has great commercial importance and has been shown to have high rates of phospholipid synthesis (Harwood, 1975). The complex lipid content (Wagner & Wolff,

1964; Privett *et al.*, 1973), fatty acid composition (Craig & Murti, 1959) and distribution (Brockerhoff & Yurkowski, 1966) have been determined by using developing and mature soya-bean seeds. Further, studies on choline in germinating soya beans also provided useful background information (Toyosawa, 1970; Toyosawa *et al.*, 1970).

Hoelzl & Wagner (1966, 1971), during a study of phospholipid synthesis in germinating soya beans, showed that [^{14}C]choline was incorporated into phosphatidylcholine but that [*Me*-^{14}C]methionine was not. Our results, some of which have been reported in a preliminary form (Harwood, 1976*a*), also showed that during the first 48h of germination, there is no synthesis of phosphatidylcholine by the methylation pathway. They also indicated differences in the enzymes catalysing incorporation of bases into phosphatidylcholine and phosphatidylethanolamine.

Experimental

Materials

[*Me*-^{14}C]Choline (59mCi/mmol), [2-^{14}C]ethanolamine (30mCi/mmol), [1-^{14}C]acetate (40mCi/mmol), [U-^{14}C]glycerol (18mCi/mmol), CDP-[*Me*-^{14}C]-choline (60mCi/mmol) and CDP-[2-^{14}C]ethanolamine (28mCi/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K., and [^{33}P]orthophosphate (100mCi/mmol) was from New England Nuclear Corp., Frankfurt, Germany.

CDP-ethanolamine, chloramphenicol, cycloheximide, choline chloride, ammonium reineckate and lipid standards were obtained from Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey KT2 7BH, U.K. Ethanolamine was from BDH Chemicals Ltd., Poole, Dorset, U.K., hemicholinium-15 from Aldrich Chemical Co., Milwaukee, WI 53211, U.S.A., and CDP-choline from Boehringer (London) Corp., Lewes, Sussex BN7 1LG, U.K. Other reagents were of the highest available grades and were purchased from BDH, Boehringer or Sigma.

Phosphatidylcholine and phosphatidylethanolamine were extracted from soya beans and purified as previously described (Harwood, 1975).

Seeds, germination and extraction

Soya bean (*Glycine max* L., var. Fiskeby V) seeds were obtained from Thompson and Morgan Ltd., Ipswich, U.K. They were surface-sterilized with 2.5% (w/v) sodium hypochlorite (12% available chlorine) and germinated in test tubes in sterile water at 25°C in an environmental chamber (Gallenkamp and Co. Ltd., London EC2P 2ER, U.K.). Appropriate additions of radioisotopes and chemicals were made in sterile water and 1ml of solution/seed was used. After the germination period, seeds were removed and thoroughly rinsed with three changes of water. They were then homogenized and extracted by the method of Garbus *et al.* (1963). Samples of the aqueous phase were taken for radioactivity counting, the rest of the phase was removed and the lower phase was washed twice with 'synthetic upper phase'. Samples of the lower phase were then taken for chromatography or radioactivity counting. The method was found to give 100% partition of ^{14}C-labelled bases into the upper phase and 100% recovery of ^{14}C-labelled phospholipids in the lower.

Lipid separation and identification

Extracted lipids were separated by t.l.c. and identified as described by Harwood *et al.* (1975).

Radioactivity determinations

Radioactive samples were counted in a scintillant consisting of PCS (Amersham–Searle, The Radiochemical Centre, Amersham, U.K.)–xylene (2:1, v/v) in a Beckman LS-100 liquid-scintillation counter. The internal-standard method of quench correction was used. T.l.c. plates were also examined by using a Panax radiochromatogram scanner (Panax Equipment Ltd., Redhill, Surrey, U.K.) or a spark-chamber radioautograph (Birchover Instruments Ltd., Hitchin, Herts., U.K.).

Determinations

Choline was measured by the method of Glick (1944). For ethanolamine determinations, seeds were extracted by the method of Katayama & Funahashi (1968) and ethanolamine was determined by reaction with ninhydrin after separation by ion-exchange chromatography on a Locarte amino acid analyser (Locarte Co. Ltd., London W12 9RT, U.K.). Samples were taken up in 0.1M-sodium citrate buffer, pH2.2 (21g of citric acid monohydrate; 8.4g of NaOH; 16ml of concentrated HCl per litre), and loaded manually on to a column (23cm×1cm) of sulphonated polystyrene resin (10% cross-linked; 8–10μm diam.; Locarte 24 cut) equilibrated at 50°C with 0.117M-citrate buffer, pH5.28 (24.6g of citric acid monohydrate; 14.4g of NaOH; 6.8ml of concentrated HCl per litre). Elution was continued with the same buffer, which effected complete resolution of ethanolamine between histidine and NH_4^+. Recoveries (>95%) were checked by assaying samples with and without added ethanolamine.

Enzyme assays

CDP-choline phosphorylcholine glyceride transferase (EC 2.7.8.2) and CDP-ethanolamine–diacylglycerol ethanolaminephosphotransferase (EC

2.7.8.1) were measured by an assay based on those of Devor & Mudd (1971) and Marshall & Kates (1974). Diglyceride was added at 100 μg/ml and CDP-base substrates were used at 0.4 mM. Both these concentrations had been found to be optimal for soya bean (J. L. Harwood, unpublished work).

Phospholipase D activity was measured by the methods of Dawson (1967) and Yang *et al.* (1967). ^{14}C-labelled phospholipids isolated from soya bean were used as substrates. Both whole-seed homogenates (in 0.32 M-sucrose/5 mM-Tris/HCl, pH 7.0) and $6\times10^6 g$-min supernatants were used. Transphosphatidylation was measured by using [*Me*-^{14}C]-choline or [2-^{14}C]ethanolamine as substrate by the method of Vandor & Richardson (1968) or under two conditions described for phospholipase D activity (Dawson, 1967; Yang *et al.*, 1967).

Results and Discussion

Initial incorporation studies revealed that [*Me*-^{14}C]choline was only incorporated into phosphatidylcholine and that [2-^{14}C]ethanolamine was only incorporated into phosphatidylethanolamine of the total lipids during the first 48 h of germination (Harwood, 1976*a*). This was thus in agreement with the data of Hoelzl & Wagner (1971), who failed to detect significant labelling of phosphatidylcholine after incubation of [*Me*-^{14}C]methionine with soya-bean seeds. We can therefore eliminate the methylation pathway as a source of phosphatidylcholine biosynthesis during the first 2 days of soya-bean germination. Another lipid-rich seed, castor bean, was shown (Moore, 1976) to have the capacity for the formation of phosphatidylcholine by the methylation pathway *in vitro*. It would therefore be interesting to determine whether castor bean, in contrast with soya bean, shows evidence for the methylation pathway *in vivo*.

There remain two possible methods by which the two phospholipids of soya bean can become labelled from their base precursors during germination: via the CDP-base pathway or by means of some form of base-exchange. Although base-exchange has been discussed by many authors as having little physiological importance for phospholipid synthesis within plant tissue (e.g. Hitchcock & Nichols, 1971; Galliard, 1973; Kates & Marshall, 1975), the reported Ca^{2+}-stimulated exchange in pea seedlings (Vandor & Richardson, 1968) and the well-known ability of phospholipase D to catalyse similar reactions (Yang *et al.*, 1967; Dawson, 1967) meant that it had to be considered as a possible source of labelling. In Fig. 1, a time-course of phospholipase D activity is plotted during germination, along with the uptake of ^{14}C-labelled bases and phospholipid labelling, showing clearly that phospholipase D activity is high throughout germination, whereas phospholipid labelling, in

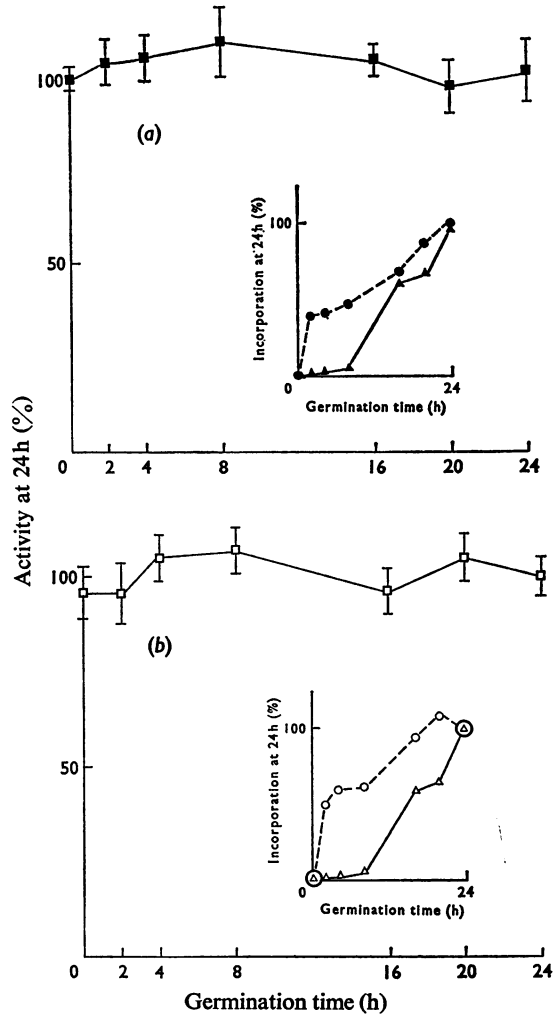

Fig. 1. *Phospholipase D activity of soya bean during germination*

Results are the means ± S.E.M. for two experiments each with homogenates and with 'high-speed' supernatant fractions (see the Experimental section). Average activities at 24 h were respectively 82 nmol of phosphatidylcholine hydrolysed/min per mg of homogenate protein, 135 nmol of phosphatidylcholine hydrolysed/min per mg of supernatant protein, 66 nmol of phosphatidylethanolamine hydrolysed/min per mg of homogenate protein and 86 nmol of phosphatidylethanolamine hydrolysed/min per mg of supernatant protein. ■, Phosphatidylcholine hydrolysis. □, Phosphatidylethanolamine hydrolysis. The insets show: (*a*) ●, [^{14}C]choline uptake; ▲, [^{14}C]choline incorporation into phosphatidylcholine; (*b*) ○, [^{14}C]-ethanolamine uptake; △, [^{14}C]ethanolamine incorporation into phosphatidylethanolamine (data taken from Harwood, 1976*a*).

Table 1. *Distribution of radioactively labelled phosphatidylcholine and phosphatidylethanolamine within the germinating seed*

Seeds were germinated for 24h at 25°C. For further details, see the Experimental section.

	Phosphatidyl[^{14}C]choline			Phosphatidyl[^{14}C]ethanolamine		
	$10^{-3}\times$Radioactivity (d.p.m.)	% of total	d.p.m./mg wet wt. of seed	$10^{-3}\times$Radioactivity (d.p.m.)	% of total	d.p.m./mg wet wt. of seed
Testa	0.011	0.3	0.8	0.189	0.1	3.0
Radicle	1.080	32.8	43.2	4.668	25.5	186.7
Cotyledon	2.215	66.9	3.2	13.500	75.4	19.4

spite of rapid uptake of ^{14}C-labelled base precursors, begins after a distinct lag phase and then proceeds in bursts corresponding to definite physiological stages (Harwood, 1976*a*). The distribution of newly synthesized ^{14}C-labelled phospholipids showed particularly high specific radioactivities in the radicle (Table 1) as opposed to the cotyledons, in spite of the fact that phospholipase D activity was higher in the latter (J. L. Harwood, unpublished work). Although these data appear clear-cut, experiments by Roughan & Slack (1976) have emphasized that some caution must be exercised when extrapolating phospholipase D activity *in vitro* to a situation *in vivo*.

Measurement of base exchange *in vitro* by any of the three methods described in the Experimental section gave maximal values of 0.091 pmol/min per mg of protein. Under most conditions, base-exchange *in vitro* was negligible. The maximal rates obtained were clearly insufficient to account for the minimum synthetic rates *in vivo*, which can be calculated from previous data (Harwood, 1975) to be 82pmol/min per mg of protein for phosphatidylcholine and 27pmol/min per mg of protein for phosphatidylethanolamine. Similar low values for exchange *in vitro* were also obtained by Vandor & Richardson (1968) and Kates & Marshall (1975), using plant tissues. All of these results therefore favour the CDP-base pathway rather than base-exchange. The terminal enzymes of the CDP-base pathway gave maximal values of 1.6mmol/min per mg of protein, clearly several orders of magnitude higher than base exchange *in vitro*. Such results therefore agree with the conclusions of Kates & Marshall (1975), who considered that the CDP-base pathways were the most important in spinach leaves.

Further evidence is as follows.

(*a*) The relative rates of formation of the phospholipids from [^{14}C]acetate, [^{32}P]orthophosphate and [^{14}C]glycerol (J. L. Harwood, unpublished work) and the time-course of their uptake (Harwood, 1975) compare with those of the ^{14}C-labelled bases. This is evidence for synthesis *de novo*, and agrees with the conclusions of Hoelzl & Wagner (1971), that exchange is not significant.

(*b*) The large net increase in phospholipid amounts/seed (Harwood, 1975) observed during this period of germination means that there is no source of a large amount of phosphatidyl precursor for exchange reactions.

We consider that the above evidence, taken together with the previous doubts cast on the role of base-exchange as a source of phospholipid formation *in vivo* (Tang & Castelfranco, 1968; Hitchcock & Nichols, 1971; Galliard, 1973; Kates & Marshall, 1975), means that phosphatidylcholine and phosphatidylethanolamine are not subject to any significant base-exchange during soya-bean germination. Hoelzl & Wagner (1971) using soya bean, and Moore (1976), using castor bean, also considered that base-exchange was inoperative.

Effect of protein-synthesis inhibitors

Since both phospholipids were synthesized via the CDP-base pathway, it was decided to determine the characteristics of their formation, especially since any difference observed might be due to the need for separate enzymes to form each lipid. Cycloheximide, which inhibits microsomal protein synthesis (Marcus & Freeley, 1966), and chloroamphenicol, which inhibits chloroplast (protoplast) and mitochondrial protein synthesis (App & Jagendorf, 1963), were reported to have little effect on the labelling of phosphatidylcholine and phosphatidylethanolamine from [1-^{14}C]acetate (Harwood, 1975). The action of the two inhibitors on labelling from ^{14}C-labelled bases is shown in Table 2. Whereas uptake of [2-^{14}C]ethanolamine and its incorporation into lipid was unaffected by either inhibitor, that from [*Me*-^{14}C]choline was decreased in the presence of cycloheximide. The total label in the water-soluble fraction, in the latter case, was not decreased, but phosphatidylcholine labelling was decreased by 40% (Table 2). This would indicate that at least one of the three enzymes required for the incorporation of choline into phosphatidylcholine is synthesized by the ribosomes during the first 48h of germination and must be rate-limiting for the pathway. Further, it obviously indicates one point of difference in the CDP-base pathway enzymes. It is not known how choline and ethanolamine enter germinating seeds. If uptake is due to an extracellular–intracellular concentration gradient

Table 2. *Effect of protein-synthesis inhibitors on incorporation of ^{14}C-labelled bases into phospholipids*

Soya beans were germinated for 48h. For further details, see the Experimental section. Results are corrected to one decimal place. Means±S.D. are given ($n = 2$). * Significant at 2% level (Student's t test).

Precursor	Inhibitor	$10^{-3}\times$Total uptake (d.p.m.)	Lipid ($10^{-3}\times$d.p.m.)	Lipid (% of control)
[*Me*-^{14}C]Choline	None	220.4± 4.9	30.4±1.7	100.0±5.5
	Cycloheximide (10μg/ml)	181.5±12.6	18.1±1.8	*59.7±5.8
	Chloramphenicol (50μg/ml)	208.1± 6.8	29.9±1.0	98.5±3.3
[2-^{14}C]Ethanolamine	None	197.2±13.6	84.5±5.2	100.0±6.2
	Cycloheximide (10μg/ml)	196.0±14.8	85.5±2.5	101.0±3.0
	Chloramphenicol (50μg/ml)	212.6±15.2	84.5±4.8	100.0±5.7

Table 3. *Effect of hemicholinium-15 on phospholipid labelling*

Seeds were germinated for 48h. For further details, see the Experimental section. Means±S.D. are given.

Hemicholinium-15 (mM)	Labelling (% of control) [*Me*-^{14}C]Choline Uptake	[*Me*-^{14}C]Choline In lipid	[2-^{14}C]Ethanolamine Uptake	[2-^{14}C]Ethanolamine In lipid
0	100.0±4.9	100.0±4.8	100.0±5.7	100.0±3.9
0.1	101.1±2.9	101.7±0.6	102.7±3.2	103.5±0.2
1	106.2±3.5	102.3±4.5	104.4±5.4	98.0±2.3
10	69.6±2.9	39.4±0.8	70.1±1.4	35.8±3.4

(Broad & Dawson, 1975) and hence is caused indirectly by choline kinase activity, then the absence of an inhibition by cycloheximide of the labelling of the aqueous-soluble pool would place the effect of the inhibitor at either the second or third step of the pathway. It is possible also that transport of choline to the site of phospholipid synthesis is dependent on protein synthesis, and thus the effect of cycloheximide is indirect.

Effects of hemicholinium-15 and base competition

Hemicholinium-15, or its analogue, hemicholinium-3, has been used to inhibit choline kinase and phosphatidylcholine synthesis in a number of systems (Ansell & Spanner, 1974; Bygrave & Dawson, 1975; Bowman *et al.*, 1967; Hemsworth *et al.*, 1971). Concentrations as low as 10μM resulted in complete inhibition of lipid labelling in brain (Bowman *et al.*, 1967). A high concentration (10mM) of hemicholinium-15 was required to produce any effect on either uptake or lipid labelling in soya bean (Table 3). The percentage decreases in total uptake (30%) and lipid labelling (60%) were similar for each ^{14}C-labelled base. The relatively high concentration of hemicholinium-15 needed when compared with other systems (Hemsworth, 1970; Bygrave & Dawson, 1975), together with its known action with membranes (Hemsworth, 1971), meant that the inhibitor could have caused membrane perturbation rather than inhibition of, for example, the base kinases or other enzymes. It was thus essential that we studied the base-kinase step further.

Choline kinase has been demonstrated in a number of plant tissues (Johnson & Kende, 1971; Lord *et al.*, 1972; Morre *et al.*, 1970; Ramasarma & Wetter, 1957; Tanaka *et al.*, 1966). Studies on the analogous ethanolamine kinase have been less well documented, but Marshall & Kates (1974) have reported the enzyme to be present in spinach leaves. Although phosphorylation of ethanolamine may also be carried out by the enzyme responsible for phosphorylcholine synthesis in mammals (Thompson, 1973), Ramasarma & Wetter (1957) stated clearly that choline kinase from rape seed had no activity towards ethanolamine.

Base-competition experiments with soya bean showed that unlabelled choline had no effect on uptake or incorporation of [2-^{14}C]ethanolamine into lipid (Table 4). This was in spite of a 150-fold excess of choline. Similarly, the uptake of [*Me*-^{14}C]choline was not affected by ethanolamine. Broad & Dawson (1975) also found no effect of ethanolamine on [*Me*-^{14}C]choline uptake by *Entodinium caudatum*. The lack of base competition observed with germinating soya bean would again indicate at least one enzyme is specific for each phospholipid. Since the aqueous-

Table 4. *Effect of base competition on uptake and phospholipid labelling*

Seeds were germinated for 48h. For further details, see the Experimental section. Means±S.D. are given.

Precursor	Unlabelled base (mM)	No. of expts.	Total uptake (% of control)	Lipid labelling (% of control)
[*Me*-^{14}C]Choline (3.39 μM)	Ethanolamine (0.00)	3	100.0±8.1	100.0±6.6
	Ethanolamine (0.25)	2	95.5±1.1	95.6±5.3
	Ethanolamine (0.50)	2	93.7±9.0	104.8±9.6
	Ethanolamine (1.00)	3	95.9±4.3	102.6±2.6
[2-^{14}C]Ethanolamine (6.66 μM)	Choline (0.00)	3	100.0±3.2	100.0±5.2
	Choline (1.00)	3	97.5±4.1	95.5±3.5

pool label was constant in the presence or absence of competing base, one can conclude that separate choline kinase and ethanolamine kinase are present.

Labelling rates of phosphatidylcholine and phosphatidylethanolamine

From the results given in Table 2, it seems that the amount of ^{14}C-labelled base which was incorporated into lipid, when expressed as a percentage of that imbibed, varied considerably depending on which base was considered. Phosphatidylethanolamine was always more heavily labelled than phosphatidylcholine when comparable counts were added in the form of radioactive bases. This was in contrast with labelling from [1-^{14}C]acetate (Harwood, 1975; Hoelzl & Wagner, 1966, 1971), [^{32}P]orthophosphate or [1-^{14}C]glycerol (Hoelzl & Wagner, 1971). However, as any radioactively labelled precursor imbibed by a seed will be subsequently diluted in the endogenous pool(s), we wished to know if the relative amounts of endogenous choline and ethanolamine could account for our results. One problem with intact tissues is that endogenous pools can often change in size with time. Since most of our incorporation experiments were for 48h, we chose an intermediate time for the chemical determination of seed contents of choline and ethanolamine. Results obtained by these methods are compared with ones by an isotope-dilution procedure in Table 5. There was excellent agreement between the two methods for choline, but the isotope-dilution method for ethanolamine gave a high value for the pool size. Knowing the total amounts of newly synthesized phosphatidylcholine and phosphatidylethanolamine (Harwood, 1975), one can also calculate the relative dilutions of the two ^{14}C-labelled bases that would give the observed incorporations.

Such a calculation gave good agreement with the values for the choline pool, but again yielded a significantly higher value for the ethanolamine pool. Since the isotope-dilution method, which involved high concentrations of ethanolamine, did not give a markedly higher value than the isotope-incorporation

Table 5. *Choline and ethanolamine contents of germinating soya bean*

The methods of measurement are explained in the Results and Discussion section. Results are expressed as means ±S.D. for the numbers of experiments in parentheses. Chemical determinations were carried out at 24h germination. Isotope experiments were for 48h.

Method of measurement	Pool size (μg/g wet wt.) Choline	Ethanolamine
Chemical	130.5± 0.5 (3)	6.88±0.21 (4)
Isotope incorporation	112.5±25.5 (2)	15.5 ±1.5 (3)
Isotope dilution	136.0 (1)	17.4 ±3.2 (2)

method, one can deduce that enzyme rates are not being significantly affected by changing ethanolamine pool size. We consider that the difference between the chemical and radioisotope methods for ethanolamine is mainly due to the fact that the pool is not at a maximum at 24h. However, the results shown in Table 5 provide a good explanation for the relative rates of labelling of the two phospholipids. Thus, even though phosphatidylcholine is synthesized at twice the rate of phosphatidylethanolamine (Harwood, 1975) and is twice as highly labelled as phosphatidylethanolamine from [1-^{14}C]acetate, [^{32}P]-orthophosphate or [1-^{14}C]glycerol (Hoelzl & Wagner, 1971), the large size of the choline pool relative to that of ethanolamine means that the reverse is true for [^{14}C]base labelling. The pool-size estimation for choline in soya bean agrees well with the data of Toyosawa *et al.* (1970), who also showed that free choline was at a maximum after 24h of germination.

Conclusions

The total recoveries of label when either [*Me*-^{14}C]-choline or [2-^{14}C]ethanolamine was used were good (>98%). This meant, for example, that entry of choline into general cell metabolism via betaine (Dekhuijzen & Vonk, 1974) or ethanolamine to CO_2 via glycolaldehyde, glycollate and glyoxylate

(Miedema & Richardson, 1966) can only take place at low rates during the first 48h of soya-bean germination.

The incorporation of choline and ethanolamine into phospholipids in soya bean occurs predominantly (if not exclusively) via the CDP-base pathway. The difference in the effect of cycloheximide on their incorporation and the absence of base competition can be interpreted as showing that there are differences in the enzymes catalysing the CDP-base pathways to phosphatidylcholine and phosphatidylethanolamine. Such a conclusion is in agreement with results obtained from the labelling of molecular species of the two phospholipids (Harwood, 1976*b*). Separation of enzymes that are specific to each CDP-base pathway must be made to prove this unequivocally.

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