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Fat metabolism in higher plants: XIII. phosphatidic acid synthesis and diglyceride phosphokinase activity in mitochondria from peanut cotyledons*

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SUMMARY

A phosphatidic acid is the major lipid to become labeled when Pi^{32} (inorganic orthophosphate labeled with P^{32}), under conditions of oxidative phosphorylation, or ATP^{32} is fed to mitocholine and phosphatidylethanolamine in the mitochondria obtained a much higher level of P^{32}, the only cofactor required was Mg^{++}. The stimulation of phosphatidic acid synthesis by an α,β-diglyceride provided support for the view that this synthesis is due to diglyceride phosphokinase activity in the mitochondria. Evidence is also presented that this enzyme preparation is capable of phosphorylating α-monoglycerides with the formation of monoacyl phosphatidic acids. When Pi^{32} was added to slices of peanut cotyledons, the phosphatidylcholine and phosphatidylethanolamine in the mitochondria obtained a much higher level of radioactivity than that observed in experiments with the isolated mitochondria.

Mazelis and Stumpf (1) have shown that fresh mitochondria from the cotyledons of germinating peanut seedlings are capable of incorporating Pi^{32} (inorganic orthophosphate labeled with P^{32}) into a phospholipid fraction. The labeled phospholipid was not identified, but it was established that the first step on the pathway of Pi^{32} incorporation was the esterification of Pi^{32} into ATP^{32}.‡ Fresh, frozen, or lyophilized mitochondria were able to incorporate label from ATP^{32} into the phospholipid fraction and the only cofactor required by these systems was Mg^{++}. Evidence is presented in the present paper to show that the labeled phospholipid is a phosphatidic acid and that the incorporation of label from ATP^{32} into this lipid is due to diglyceride phosphokinase activity in the mitochondria.

MATERIAL AND METHODS

Preparation of ATP^{32}. ATP, terminally labeled with P^{32}, was prepared from ADP and Pi^{32} by means of the photophosphorylative activity of isolated spinach chloroplasts. The isolation of the chloroplasts and the reaction conditions used were closely similar to the methods described by Arnon *et al.* (2).[1] Each reaction mixture contained 12 μmoles ADP, 10 μmoles Pi^{32} (1 to 2 mc.), 80 μmoles Tris buffer pH 8.1, 10 μmoles sodium ascorbate, 10 μmoles $MgCl_2$, 0.3 μmoles menadione, and chloroplasts (containing 0.5 to 1 mg. chlorophyll) in a total volume of 3 ml. The reaction mixtures were illuminated for 30 minutes at 15°C. Over 95 per cent of the Pi^{32} was incorporated into ATP^{32} under these conditions. The 10 μmoles of ATP^{32}, containing 1 to 2 mc. of P^{32}, was usually diluted with 50 μmoles of nonradioactive ATP prior to the following isolation steps. The removal of the chloroplasts from the reaction mixture by high speed centrifugation and all the subsequent preparative steps

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‡ The following abbreviations have been used: ATP, adenosine triphosphate; ADP, adenosine diphosphate; CTP, cytidine triphosphate; CMP, cytidine monophosphate; FAD, flavin adenine dinucleotide; DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; Co A, coenzyme A; GSH, reduced glutathione; GP, glycerophosphate; GPE, glyceryl phosphoryl ethanolamine; GPC, glyceryl phosphoryl choline; Pi^{32}, inorganic orthophosphate labeled with P^{32} (Pi^{32} solutions were obtained from the Department of Radiation Safety, University of California, Berkeley); ATP^{32}, adenosine triphosphate labeled in the terminal phosphate group with P^{32}.

[1] Acknowledgment is due to Drs. J. Bove and F. R. Whatley for instruction in the preparation of ATP^{32} using spinach chloroplasts.

were carried out at 0°C. The ATP^{32} was precipitated, at neutral pH, as its dibarium salt by the addition of a suitable excess of barium acetate to the chloroplast-free supernatant solution. After two washings with demineralized water, the precipitate was dissolved in the minimum volume of 0.5 N hydrochloric acid and barium ions were removed by the addition of sufficient Dowex 50 (H^+ form). The resin was removed by centrifugation and the ATP^{32} solution was carefully neutralized to pH 7 with M sodium hydroxide. No attempt was made to remove the slight excess of unlabeled ADP or the traces of unchanged Pi^{32} from the preparation. The ATP^{32} was stored at —15°C until required.

Plant Material. Peanuts were germinated in moist vermiculite in the dark at 25°C. Mitochondria were prepared, as described by Stumpf (3), from the cotyledons obtained from 3- to 7-day-old seedlings. The mitochondria, suspended in 0.1 M Tris, 0.5 M sucrose, pH 7.2, were either used immediately or were stored at —15°C until needed.

Reaction Conditions. The mitochondria were usually incubated at 30°C for 60 minutes in a reaction mixture containing 0.1 M Tris, 0.5 M sucrose buffer, of pH 7.2. When ATP^{32} was employed as the source of P^{32}, the only cofactor required was Mg^{++}. In some experiments with freshly prepared mitochondria, Pi^{32} was used and these systems required, in addition, an adenine nucleotide, oxygen, and a tricarboxylic acid cycle intermediate. The precise reaction mixtures used are shown in the appropriate tables. Potassium fluoride, in a final concentration of 0.1 M, was commonly included since it was found to increase the incorporation of label into phospholipid, presumably by means of its inhibition of adenosine triphosphatase.

Lipid Extraction and Analysis. At the end of the reaction period the mitochondria were precipitated by the addition of 2 ml. of 10 per cent trichloroacetic acid. After centrifugation, the precipitate was washed twice with aliquots of 5 per cent trichloroacetic acid and the lipids were then extracted and washed by means of the methods of Kennedy (4), as modified by Mazelis and Stumpf (1).

Aliquots of the lipid extract were counted with a Nuclear-Chicago gas-flow counter fitted with a micromil window.

Portions of the lipid extracts were chromatographed on Whatman 3MM paper impregnated with silicic acid, according to the method of Lea *et al.* (5). The developing solvent was diisobutyl ketone:acetic acid:water (40:30:7, v/v/v) (6).

Deacylation of the phospholipids was carried out by reaction with 0.1 N methanolic potassium hydroxide at 37°C for 15 minutes, according to the method of Dawson (7) as modified by Benson and Maruo (8). After removal of the excess potassium ions by Dowex 50 (H^+ form) and the subsequent removal of the resin, the phosphate esters were chromatographed in two dimensions on acid-washed Whatman No. 1 paper. The developing solvents were water-saturated phenol and *n*-butanol : propionic acid : water (142 : 71 : 100, v/v/v). In addition, methyl cellosolve : methyl ethyl ketone : 3 N ammonium hydroxide (7 : 2 : 3, v/v/v) and isobutanol : picric acid : water (80 ml. : 2 g. : 20 ml.) were occasionally used for one-dimensional chromatography.

Phosphates were detected on paper by the modification of the Hanes and Isherwood procedure (9) described by Bandurski and Axelrod (10). Lipids were demonstrated with 0.001 per cent aqueous Rhodamine B (11) and compounds with an amino group were located with 0.2 per cent ninhydrin in acetone.

Radioactive compounds were detected on paper by radioautography with Kodak "No Screen" X-ray film.

RESULTS

In some preliminary experiments the results of Mazelis and Stumpf (1), concerning the necessary conditions for the incorporation of Pi^{32} and ATP^{32} into phospholipid by peanut mitochondria, were confirmed. The stimulatory effect of Mg^{++} upon the incorporation of label from ATP^{32} into phospholipid by frozen peanut mitochondria was again demonstrated. There was some residual activity in the absence of added Mg^{++} and this was probably due to Mg^{++} contained in the mitochondria. Mazelis and Stumpf (1) showed that this residual activity could be abolished by the addition of versene to the enzyme system and that activity could be restored by the subsequent addition of either Mg^{++} or Mn^{++}. The effect of high concentrations of Mg^{++} in the reaction mixture was not investigated. Mg^{++} was the only cofactor required for the incorporation of label from ATP^{32} into the phospholipid by fresh or frozen mitochondria. The incorporation of Pi^{32}, on the other hand, only occurred under conditions favorable for oxidative phosphorylation, i.e., with fresh mitochondria, Mg^{++}, an adenine nucleotide, a tricarboxylic acid cycle intermediate, and oxygen.

Identification of the Labeled Phospholipid. The main aim of the present study was to identify the phospholipid(s) which became labeled in peanut mitochondria under the experimental conditions described by Mazelis and Stumpf (1).

When portions of the lipid extract were chromatographed on silicic acid-impregnated paper with diisobutyl ketone-acetic acid-water as the developing solvent, approximately 80 to 90 per cent of the radioactivity ran with an R_f value of 0.78. This mobility most closely resembles that of a diacyl phosphatidic acid, for which R_f values of 0.72 (6) and 0.84 (12) have been reported. The remaining 10 to 20 per cent of the radioactivity had an R_f of 0.52.

Aliquots of the lipid extract were deacylated by the method described and the residual phosphate esters were chromatographed both in one and in two dimensions on acid-washed Whatman No. 1 paper. Between 96 and 99 per cent of the radioactivity was located in a single spot, which was identified as α-glyerophosphate by cochromatography with authentic L,α-glycerophosphate[2] in the four solvents used. This compound was finally proved to be α-glycerophosphate by periodate oxidation according to the method of Baddiley *et al.* (13). Chromatography of the oxidation products on Whatman No. 1 paper, with isobutanol : picric acid : water as the developing solvent, showed that the radioactivity had entirely disappeared from the α-glycerophosphate position (R_f 0.23) and now cochromatographed precisely with the glycolaldehyde phosphate (R_f 0.51), which was formed by the simultaneous periodate oxidation of the standard α-glycerophosphate. This identification of α-glycerophosphate as the product of deacylation identifies the original radioactive lipid as being a phosphatidic acid. This finding is consistent with the observed mobility of the lipid on silicic acid-impregnated paper, as described earlier.

It should be noted that the methods used would clearly differentiate a phosphatidic acid from bisphosphatidic acids and from phosphatidyl glycerols. Deacylation of the two latter groups of compounds would yield, in both cases, glycerol phosphoryl glycerol, which would be readily separated from α-glycerophosphate under the paper chromatographic conditions employed.

The fact that over 96 per cent of the label of the deacylated phosphate esters was in α-glycerophosphate indicates that the lipid, which contained 10 to 20 per cent of the radioactivity in the original lipid extract and which chromatographed with an R_f of 0.52 in the silicic acid system, must also give rise to some α-glycerophosphate upon deacylation. These facts have been taken to indicate that part of the radioactivity at this position on the silicic acid-impregnated chromatogram is due to a monoacyl phosphatidic acid. Further evidence in support of this suggestion is presented below.

Small amounts of radioactivity (less than 4 per cent) in the deacylated extract were found in a compound which was identified as glyceryl phosphoryl ethanolamine by cochromatography with the authentic substance. From the known mobility of the parent phosphatidylethanolamine, from which this phosphate ester is derived, it has been concluded that labeled phosphatidylethanolamine contributes some radioactivity to the radioactive lipid spot which has an R_f of 0.52 on the silicic acid-impregnated paper. This lipid spot would then consist of a mixture of a monoacyl phosphatidic acid and phosphatidylethanolamine. Attempts were made to stimulate the incorporation of label from ATP^{32} into phosphatidylethanolamine by the inclusion of CTP, CMP, α,β-diglyceride, ethanolamine, and a supernatant protein fraction (obtained by 70 per cent ammonium sulfate saturation of the supernatant solution which remained after removal of the mitochondria from the peanut cotyledon homogenate) in the reaction mixtures. The incorporation of the radiophosphorus into the phosphatidylethanolamine remained low in all cases, as shown in Table 1.

Traces of radioactivity were occasionally observed in other areas on the two-dimensional chromatograms of the deacylated lipid extract, but these spots did not correspond in position to any well-known phosphate esters, and were not identified.

The evidence indicates, however, that when ATP^{32} is fed to mitochondria from peanut cotyledons, the main lipid which becomes labeled is a phosphatidic acid. There is also a small amount of radioactivity in phosphatidylethanolamine.

Diglyceride and Monoglyceride Phosphokinase Activity. Having identified the labeled phospholipid as being predominantly a phosphatidic acid, the next step was to identify the step or steps by which P^{32} is incorporated into this compound. The first possibility to be considered was that the phosphatidic acid had arisen via an initial phosphorylation and subsequent acylation of glycerol, as has been described by Kornberg and Pricer (14) and by Kennedy (15). However, from isotope dilution experiments with unlabeled glycerophosphate and from experiments with C^{14}-labeled glycerol, Mazelis and Stumpf (1) showed that glycerophosphate was not an intermediate in the formation of their labeled phospholipid. The ob-

[2] Thanks are due to Dr. C. E. Ballou for samples of α- and β-glycerophosphate; to Dr. E. Baer for samples of dipalmitoyl L,α-glycerophosphoric acid and L,α-glyceryl phosphoryl ethanolamine; to Dr. D. J. Hanahan for samples of glyceryl phosphoryl choline, α,β-diglyceride (C_{18} unsaturated) and phosphatidylinositol; and to Dr. J. G. Marr for a sample of α-mono palmitin.

TABLE 1. ATTEMPTS TO INCREASE P^{32} INCORPORATION INTO CEPHALIN BY PEANUT MITOCHONDRIA *

	Source of P^{32}			
	Pi^{32}		ATP^{32}	
Tube Number	1	2	3	4
cpm. in lipid fraction	63,618	69,184	72,811	91,114
Proportions of P^{32} in GP and GPE after deacylation of lipid extract: GP	100	100	100	100
GPE	4	4	10	1

* Tubes 1 and 2 contained 10 ml. fresh mitochondria, $MgCl_2$ (10 μmoles), GSH (10 μmoles), 1 mg. each of FAD, DPN, TPN, ATP, Co A, cytochrome c, ADP (10 μmoles), succinate (20 μmoles), Pi^{32} (4 μmoles), and KF (200 μmoles). In addition, tube 2 also contained dipalmitolein (5 μmoles), ethanolamine (10 μmoles), serine (10 μmoles), choline (10 μmoles), CTP (0.5 μmoles), CMP (0.5 μmoles) in a total volume of 4.3 ml. Tubes 3 and 4 contained 1 ml. fresh mitochondria, $MgCl_2$ (10 μmoles), GSH (10 μmoles), ATP^{32} (4 μmoles), KF (200 μmoles), dipalmitolein (5 μmoles), ethanolamine (10 μmoles), serine (10 μmoles), choline (10 μmoles), CTP (0.5 μmoles), CMP (0.5 μmoles) in a total volume of 3.7 ml. Tube 4 also contained 1.5 ml. of the supernatant solution which remained after removal of the mitochondria from the homogenized cotyledons. All tubes incubated at 30°C for 60 minutes.

TABLE 2. THE EFFECTS OF DI- AND TRI-GLYCERIDE UPON THE INCORPORATION OF P^{32} FROM ATP^{32} INTO LIPID BY PEANUT MITOCHONDRIA *

Tube Number	Additions		Lipid Extract
		μmoles	cpm.
1		0	43,740
2	α, β-dipalmitolein	7	82,660
3		0	22,722
4	α, β-dipalmitolein	5	40,695
5	Triolein	5	36,057
6	Triolein	5	31,027

* Tubes 1 and 2 contained 1 ml. mitochondrial preparation, $MgCl_2$ (2 μmoles), and ATP^{32} (8 μmoles, 0.1 mc.) in a total volume of 1.8 ml. Tubes 3, 4, 5, and 6 contained 0.5 ml. mitochondrial preparation, ATP^{32} (4 μmoles, 40 μc.), $MgCl_2$ (10 μmoles), KF (150 μmoles), and GSH (10 μmoles) in a total volume of 1.6 ml. All tubes incubated at 30°C for 60 minutes.

TABLE 3. EFFECTS OF MONO-, DI-, AND TRI-GLYCERIDES AND OF PHOSPHATIDIC ACID ON THE INCORPORATION OF P^{32} FROM ATP^{32} INTO LIPID BY PEANUT MITOCHONDRIA *

Additions	Lipid Extract	Per Cent of P^{32} in Lipid Fraction on Silicic Acid-impregnated Paper with R_f of:	
		0.78	0.52
	cpm.		
	36,419	87.1	12.9
α, β-dipalmitolein	77,046	86.4	13.6
Dipalmitoyl phosphatidic acid	21,630	58.4	41.6
α-monopalmitin	28,731	17.1	82.9
Triolein	99,416	82.4	17.6

* Each reaction mixture contained 1 ml. mitochondrial preparation, ATP^{32} (3 μmoles), $MgCl_2$ (10 μmoles), and KF (200 μmoles) in a total volume of 2.3 ml. Ten μmoles of emulsified lipids was added appropriately as indicated above. All reaction mixtures incubated at 30°C for 60 minutes.

jection that the glycerophosphate may not have penetrated the mitochondria can be ruled out since Stumpf (3) has shown that glycerophosphate is readily metabolized by these particles. A second possibility is that the phosphatidic acid may have arisen by phosphorylation of a diglyceride by means of a diglyceride phosphokinase such as that which has been demonstarted in particles from guinea pig brain tissue by Hokin and Hokin (16).

The addition of an α,β-diglyceride to the reaction mixtures markedly stimulated the incorporation of label from ATP^{32} into the lipid fraction (Tables 2 and 3). Chromatography of the lipid extract on silicic acid-impregnated paper showed that the distribution of the radioactivity was essentially the same as that obtained in the experiments without added diglyceride, i.e., the bulk of the activity ran to the same place as the component, which has been identified as a diacyl phosphatidic acid, and the remainder chromatographed with the fraction which is believed to consist of a mixture of monoacyl phosphatidic acid and phosphatidylethanolamine. Deacylation of the lipids showed that, as before, almost all of the radioactivity was contained in α-glycerophosphate, thus giving a clear demonstration of the fact that the diglyceride stimulated incorporation of label from ATP^{32} into phosphatidic acid.

Strong evidence that the radioactive lipid of R_f 0.52 on the silicic acid-impregnated paper is mainly a monoacyl phosphatidic acid was obtained when α-

monopalmitin was included in the reaction mixture. Although the monopalmitin caused some inhibition of the over-all P^{32} incorporation into the lipid fraction, over 80 per cent of the incorporated label was now found at the position of the suspected monoacyl phosphatidic acid (i.e., at R_f 0.52) rather than at the position of the diacyl phosphatidic acid (R_f 0.78) (Table 3). Since deacylation of this lipid extract gave α-glycerophosphate as essentially the only radioactive compound, this effect of the monopalmitin can be explained only in terms of monoglyceride phosphokinase activity to give a monoacyl phosphatidic acid.

As shown in Tables 2 and 3, the incorporation of P^{32} from ATP^{32} into phosphatidic acid was also markedly stimulated by triolein and the proportions of label in the diacyl and monoacyl compounds were approximately the same as those obtained when diglyceride was added. It is suggested that the triglyceride is broken down to a diglyceride which is subsequently phosphorylated, although the triolein was not checked for the initial presence of a diglyceride.

Dipalmitoyl phosphatidic acid was found to inhibit the incorporation of label from ATP^{32} into the lipid fraction (Table 3).

Acetone Powders. Acetone powders of the peanut mitochondria were prepared by the method described by Martin and Stumpf (17). Five per cent suspensions of these powders in 0.2 M Tris buffer, pH 7.2, were effective in incorporating label from ATP^{32} into phosphatidic acid, which was identified by the methods described previously. Table 4 shows the stimulation, by diglyceride, of the incorporation of the label into the lipid fraction. Removal of the insoluble matter from the acetone powder suspensions by centrifugation gave water-clear extracts which still possessed some diglyceride phosphokinase activity, although this activity was much less than that of the suspensions. The stimulation of this system by diglyceride is also shown in Table 4.

Slices of Peanut Cotyledons. Experiments were performed in which Pi^{32} was added to slices of cotyledons obtained from 5- to 6-day-old peanut seedings. Usually about 10 g. fresh weight of plant material was used and the slices were immersed in approximately 12 ml. of 0.1 M Tris, 0.5 M sucrose buffer, pH 7.2 contained in 50 ml. Erlenmeyer flasks. To these systems was added 1 to 2 mc. of Pi^{32} (pH 7.2, 15 to 30 μmoles). The slices were then incubated at 30°C for periods which varied from 6 to 18 hours. At the end of these periods the slices were washed, mixed with 30 to 40 g. of fresh peanut cotyledons, and the mitochondria were separated as described previously. The lipids were extracted from the mitochondria and were subjected to the same analytical procedures as before. Chromatography of the phosphate esters obtained after deacylation of the lipid extract showed five major radioactive areas. The R_f values of these spots in the phenol and butanol-propionic solvents and the proportions of radioactivity which they contained, after a typical experiment, are shown in Table 5. Three of these compounds have been identified as α-glycerophosphate, glyceryl phosphoryl ethanolamine, and glyceryl phosphoryl choline by cochromatography with the authentic substances. Both the unknown esters and the standard compounds chromatographed as discrete spots in this two-dimensional, paper chromatographic system. The unknown I has been tentatively identified as glyceryl phosphoryl inositol because it cochromatographed with the phosphate ester formed by deacylation of a phosphatidyl inositol. The unknown II was not identified but its chromatographic properties are similar to those of glyceryl phosphoryl glycerol as described by Benson and Maruo (8).

DISCUSSION

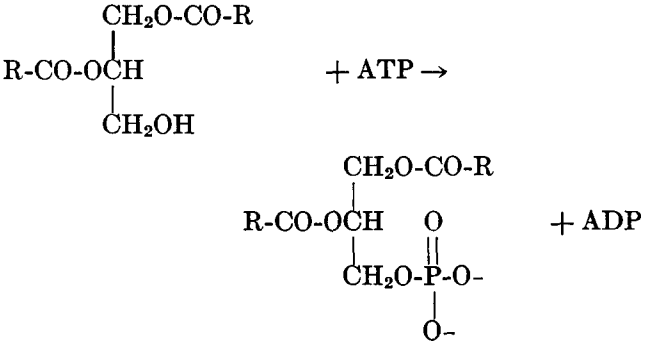
The unknown phospholipid, described by Mazelis and Stumpf (1), and which became labeled when peanut mitochondria were exposed to Pi^{32} or to ATP^{32} under suitable conditions, has been identified as a phosphatidic acid. The results are similar to those which have been described by Hokin and Hokin (18),

TABLE 4. EFFECTS OF DI- AND TRI-GLYCERIDES ON THE INCORPORATION OF P^{32} FROM ATP^{32} INTO LIPID BY ACETONE POWDERS OF PEANUT MITOCHONDRIA *

Acetone Powder					
Suspended			Water-clear Extract		
Additions	*μmoles*	*cpm.*	*Additions*	*μmoles*	*cpm.*
	0	4080		0	294
α, β-dipalmitolein	5	9888	α, β-dipalmitolein	5	1264
Triolein	5	4740			

* With the acetone powder suspension, each reaction mixture contained 0.5 ml. enzyme preparation (50 mg. acetone powder suspended/ml. 0.2 M Tris buffer, pH 7.2), ATP^{32} (40 μmoles, 40 μc.), $MgCl_2$ (10 μmoles), KF (100 μmoles) in a total volume of 1.3 ml. With water-clear extract, each reaction mixture contained 0.35 ml. enzyme preparation (50 mg. acetone powder suspended/ml. 0.2 M Tris buffer, pH 7.2, and insoluble material removed by centrifugation), ATP^{32} (4 μmoles, 40 μc.), $MgCl_2$ (10 μmoles), and KF (100 μmoles) in a total volume of 1 ml. All reaction mixtures incubated at 30°C for 60 minutes.

in whose experiments, under conditions of oxidative phosphorylation, Pi^{32} was readily incorporated into phosphatidic acid by a particulate fraction from guinea pig brain hemispheres. The work of Mazelis and Stumpf (1) has clearly demonstrated that glycerophosphate is not an intermediate in this formation of phosphatidic acid by peanut mitochondria. The stimulation of the phosphatidic acid synthesis by diglyceride has provided evidence that this process takes place by direct phosphorylation of an α,β-diglyceride, as shown in the following reaction:



The only cofactor requirement is for Mg^{++}. The enzyme that catalyzes this reaction has been given the name diglyceride phosphokinase by Hokin and Hokin (16), who showed that such activity was contained in deoxycholate extracts of microsomes obtained from guinea pig brain tissue. The peanut enzyme system is also capable of phosphorylating α-monoglycerides with the formation of monoacyl phosphatidic acids.

Further work is required to determine the relationships of phosphatidic acid synthesis in peanut mitochondria to the metabolism of other phospholipids and to the function of the mitochondrion as a whole. From their work with guinea pig brain tissue, Hokin and Hokin (19) have obtained evidence that phosphatidic acid can function as a carrier for sodium ions across intracellular membranes. The similarity of behavior between the peanut mitochondria and the guinea pig particles of Hokin and Hokin, with regard to the formation of phosphatidic acid by means of a diglyceride phosphokinase, might suggest a similar close relationship in the function of the phosphatidic acid.

The lipid extracts from the peanut mitochondrial experiments usually contained small amounts of radioactivity in phosphatidylethanolamine. However, a much higher proportion of label in phosphatidylethanolamine was obtained in the mitochondria when Pi^{32} was added to slices of the peanut cotyledons (Table 5). Kennedy (15) has summarized the evidence that phosphatidylethanolamine is synthesized in animal tissues by means of the steps centered below:

Ethanolamine + ATP → phosphoethanolamine + ADP
Phosphoethanolamine + CTP → CMP-phosphoethanolamine + pyrophosphate
CMP-phosphoethanolamine + α,β-diglyceride → phosphatidylethanolamine + CMP

TABLE 5. RADIOACTIVE PHOSPHATE ESTERS OBTAINED BY DEACYLATION OF THE LIPID EXTRACT FROM MITOCHONDRIA SEPARATED FROM SLICES OF PEANUT COTYLEDONS EXPOSED TO Pi^{32} *

Phosphate Ester	R_f in Butanol/Propionic Acid/Water (40/30/7)	R_f in Phenol	
			cpm.
GP	0.17	0.26	4421
GPE	0.19	0.57	3526
GPC	0.23	0.83	693
I	0.08	0.10	2898
II	0.13	0.38	1684

* Ten g. fresh weight of peanut cotyledon slices incubated in 12 ml. 0.1 M Tris, 0.5 M sucrose, pH 7.2, containing Pi^{32} (1.7 mc., 28 μmoles) at 30°C for 18 hours. Lipids extracted from the mitochondria and then deacylated and residual phosphate esters chromatographed on acid-washed Whatman No. 1 paper. The cpm. given are those obtained from a typical two-dimensional chromatogram of an aliquot of the phosphate esters.

Accordingly, with isolated peanut mitochondria, attempts were made to increase the porportion of label in phosphatidylethanolamine by the incorporation of ethanolamine, CTP, and diglyceride into the reaction mixtures. These attempts were not successful. This may be due to loss of activity during the preparation of the mitochondria, or to the fact that phosphatide synthesis in plants follows a different pathway from that in animal tissues.

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Editorial Correction

Due to a printing error, several lines were incorrectly placed in the Summary to the Bradbeer and Stumpf article, page 214 of the April, 1960, issue of the JOURNAL. The entire Summary is therefore reprinted correctly below:

SUMMARY

A phosphatidic acid is the major lipid to become labeled when Pi^{32} (inorganic orthophosphate labeled with P^{32}), under conditions of oxidative phosphorylation, or ATP^{32} is fed to mitochondria from the cotyledons of germinating peanut seedlings. With ATP^{32} as the source of P^{32}, the only cofactor required was Mg^{++}. The stimulation of phosphatidic acid synthesis by an α,β-diglyceride provided support for the view that this synthesis is due to diglyceride phosphokinase activity in the mitochondria. Evidence is also presented that this enzyme preparation is capable of phosphorylating α-monoglycerides with the formation of monoacyl phosphatidic acids. When Pi^{32} was added to slices of peanut cotyledons, the phosphatidylcholine and phosphatidylethanolamine in the mitochondria obtained a much higher level of radioactivity than that observed in experiments with the isolated mitochondria.