Biochem. J. (1972) **130**, 557–567
Printed in Great Britain

The Uptake and Metabolism of Plasma Lysophosphatidylcholine *in vivo* by the Brain of Squirrel Monkeys

By D. ROGER ILLINGWORTH and OSCAR W. PORTMAN
Department of Nutrition and Metabolic Diseases, Oregon Regional Primate Research Center, Beaverton, Oreg. 97005, U.S.A., and the Department of Biochemistry, University of Oregon Medical School, Portland, Oreg. 97201, U.S.A.

(*Received* 3 *July* 1972)

1. Adult squirrel monkeys were injected intravenously with doubly labelled lysophosphatidylcholine (a mixture of 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphorylcholine and 1-acyl-*sn*-glycero-3-phosphoryl[*Me*-^{3}H]choline; ^{3}H:^{14}C ratio 3.75) complexed to albumin, and the incorporation into the brain was studied at times up to 3h. 2. After 20min, 1% of the radioactivity injected as lysophosphatidylcholine had been taken up by the brain. 3. Approx. 70% of the doubly labelled lysophosphatidylcholine taken up by both grey and white matter was converted into phosphatidylcholine, whereas about 30% was hydrolysed. 4. The absence of significant radioactivity in the phosphatidylcholine, free fatty acid and water-soluble fractions of plasma up to 30min after injection of doubly labelled lysophosphatidylcholine rules out the possibility that the rapid labelling of these compounds in brain could be due to uptake from or exchange with their counterparts in plasma. 5. The similarity between the ^{3}H:^{14}C ratios of brain phosphatidylcholine and injected lysophosphatidylcholine demonstrates that formation of the former occurred predominantly via direct acylation. 6. Analysis of the water-soluble products from lysophosphatidylcholine catabolism revealed that appreciable glycerophosphoryl-[*Me*-^{3}H]choline did not accumulate in the brain and that radioactivity was incorporated into choline, acetylcholine, phosphorylcholine and betaine. 7. The role of plasma lysophosphatidylcholine as both a precursor of brain phosphatidylcholine and a source of free choline for the brain is discussed.

Lysophosphatidylcholine is a normal constituent of mammalian plasma, where it constitutes 5–20% of the total phospholipids (Nelson, 1967). In squirrel monkeys (Portman *et al.*, 1970), as in other species (Switzer & Eder, 1965), most of this lysophosphatidylcholine is transported bound to plasma albumin, although lesser amounts are also carried on both the low- and high-density lipoproteins. In all three components the lysophosphatidylcholine appears to be in rapid exchange equilibrium (Illingworth & Portman, 1972*a*). The transesterification of the phosphatidylcholine and cholesterol of high-density lipoproteins by the enzyme phosphatidylcholine–cholesterol acyltransferase is probably the principal source of lysophosphatidylcholine in plasma (Glomset, 1968) although the activity of tissue and plasma phospholipases undoubtedly contributes.

Previous reports from this and other laboratories have demonstrated that after intravenous injection radiolabelled lysophosphatidylcholine is rapidly removed from the plasma of both squirrel monkeys (Portman *et al.*, 1970) and rats (Stein & Stein, 1966). Although both studies showed that many tissues, particularly the liver, were the sites of uptake, neither reported any information about the possible entry of lysophosphatidylcholine into the brain. Since the adult brain is unable to synthesize choline *de novo* (Bremer & Greenberg, 1961; Ansell & Spanner, 1967, 1971; Chida & Arakawa, 1971), it must rely entirely on the blood for its supply. In what form choline enters the brain, whether as the free base or lipid-bound in lysophosphatidylcholine, sphingomyelin or phosphatidylcholine, is, however, uncertain. Several workers have demonstrated the entry of labelled choline, injected intravenously, into the brain (Schuberth *et al.*, 1969; Diamond, 1971; Chida & Arakawa, 1971), but only Ansell & Spanner (1971) have suggested that it is transported into the brain predominantly lipid-bound, either as phosphatidylcholine or lysophosphatidylcholine.

The present paper presents evidence that doubly labelled lysophosphatidylcholine (a mixture of 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphorylcholine and 1-acyl-*sn*-glycero-3-phosphoryl[*Me*-^{3}H]choline) injected intravenously into squirrel monkeys was taken up and rapidly metabolized by the brain.

Materials and Methods

Preparation of labelled substrates

Lysophosphatidylcholine (1-acyl-*sn*-glycero-3-phosphorylcholine), labelled with either [1-^{14}C]-palmitic acid or [*Me*-^{3}H]choline, was prepared from biosynthetically labelled phosphatidylcholine by the action of *Crotalus adamanteus* venom (Ross Allen's Reptile Institute, Silver Springs, Fla., U.S.A.) by the method of Long & Penny (1957).

[1-^{14}C]Palmitoyl-labelled phosphatidylcholine was prepared as follows. A portion (500μCi) of [1-^{14}C]-palmitic acid (sp. radioactivity 55mCi/mmol; Amersham/Searle Corp., Des Plaines, Ill., U.S.A.) and 10μmol of 2-acyl-lysophosphatidylcholine, prepared by acid hydrolysis of choline plasmalogens from rhesus-monkey heart, were evaporated to dryness and redispersed in 3ml of potassium phosphate buffer (0.2M, pH7.4) containing 10μmol of CTP, 75μmol of ATP, 2.5μmol of CoA, 210μmol of glycerol 3-phosphate, 20μmol of $MgCl_2$ and 10μmol of CDP-choline. As a source of enzyme, 2.5ml of a 45000*g*-min supernatant from a 20% (w/v) homogenate of rat liver in 0.25M-sucrose was added and the flask was incubated at 37°C for 3h. Lipids were extracted by the method of Bligh & Dyer (1959), and the phosphatidylcholine was separated by t.l.c. on silica gel H (E. Merck, Darmstadt, Germany) in the solvent system chloroform–methanol–acetic acid–water (25:15:4:2, by vol.; Skipski *et al.*, 1964). The phosphatidylcholine was detected by brief exposure of the plate to iodine vapour, eluted from the silica with 20ml of methanol, and rechromatographed in the solvent system chloroform–methanol–water (13:7:1, by vol.).

Some 17% of the added [1-^{14}C]palmitic acid was incorporated into phosphatidylcholine and 9% remained in 1-acyl-lysophosphatidylcholine. The specific radioactivity of two preparations was 0.95μCi/μmol and 2.31μCi/μmol. For the preparation of [*Me*-^{3}H]choline-labelled phosphatidylcholine, a 21-day-old rat was injected intraperitoneally with 5mCi of [*Me*-^{3}H]choline (specific radioactivity 16Ci/mmol; Amersham/Searle Corp.) in 1ml of 0.9% NaCl, and the dose was repeated 24h later. After another 24h, the animal was killed and the liver, brain and several pooled organs were extracted with chloroform–methanol (2:1, v/v; Folch *et al.*, 1957). [*Me*-^{3}H]Choline-labelled lysophosphatidylcholine derived from liver phosphatidylcholine was used in these studies. The specific radioactivity of this preparation was 5.97μCi/μmol. The samples were stored in chloroform at −12°C. At least 99% of the radioactivity of labelled lysophosphatidylcholine in both preparations co-chromatographed with authentic lysophosphatidylcholine on t.l.c. The radiochemical purity of the lysophosphatidylcholine samples was checked every 6–8 weeks and, where necessary, the lysophosphatidylcholine was repurified by t.l.c.

Preparation of lysophosphatidylcholine–albumin complex

A portion (3μmol) of lysophosphatidylcholine, containing a mixture of 1.37μCi of 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphorylcholine and 5.15μCi of 1-acyl-*sn*-glycero-3-phosphoryl[*Me*-^{3}H]choline (^{3}H:^{14}C ratio 3.75) was evaporated to dryness and dispersed in 1.0ml of 0.9% NaCl; 4.5ml of a solution containing 3μmol of purified human serum albumin (Behring Diagnostics, Woodbury, N.Y., U.S.A.) in 0.9% NaCl was added and the flask was incubated in a metabolic shaker at 37°C overnight. All these procedures were performed aseptically. These procedures did not cause any hydrolysis of the lysophosphatidylcholine.

Dietary treatment of squirrel monkeys

Sexually mature female squirrel monkeys (*Saimiri sciureus*) were maintained on either a semipurified diet, which induces hypercholesterolaemia and in which 45% of the calories were supplied as butter plus crystalline cholesterol [0.1g/418kJ (0.1g/100kcal) or 20g], or a similar control diet except that 15% of the calories came from corn oil and no cholesterol was added (Portman *et al.*, 1967; Portman & Alexander, 1969). Experiments were normally performed on pairs of animals with one control and one hypercholesterolaemic animal in each experiment. In two experiments, however, three animals were used; the additional control animal in each experiment had been maintained on Purina monkey chow.

Injection of lysophosphatidylcholine–albumin complex

Monkeys were anaesthetized with Ketamine hydrochloride (Bristol Laboratories, Syracuse, N.Y., U.S.A., 4.0mg/kg body wt.) and catheters (PV-3 tubing) were implanted in the femoral artery and vein of one leg. Biliary catheters (PE-20 tubing) were also implanted in 10 of the 13 monkeys studied. Then 2.5ml of the lysophosphatidylcholine–albumin complex (1.36μmol of lysophosphatidylcholine, containing 0.63μCi of [1-^{14}C]palmitate-labelled lysophosphatidylcholine and 2.34μCi of [*Me*-^{3}H]-choline-labelled lysophosphatidylcholine), together with a wash-in portion of 1ml of 0.9% NaCl, was injected into the femoral vein of each monkey over a 30s period. Blood samples were withdrawn at various times from the arterial catheter into heparinized syringes. Continuous fluid and electrolyte replacements were infused via this route immediately after

the withdrawal of each blood sample. At the termination of the experiments, the animals were killed under anaesthesia by exsanguination from the abdominal aorta and perfused with 300–400ml of 0.9% NaCl via the vena cava. The cranial cavity was opened and the brain rapidly removed, chilled in ice and weighed. Tissue (approx. 1g) from the brain stem and cerebral cortex (sections taken tangentially to exclude white matter) was removed for analysis and the remainder of the brain was homogenized in ice-cold water. The visual appearance of the brains after perfusion indicated that they were completely free of residual blood.

Extraction of tissues

Lipids were extracted from plasma and brain tissues by homogenization in chloroform–methanol (2:1, v/v; Folch *et al.*, 1957). The upper aqueous-methanol phase, obtained after the addition of 0.4vol. of water to the chloroform–methanol extract, was re-extracted with one-third of its volume of chloroform and the resultant aqueous-methanol phase was assayed for water-soluble radioactivity. The chloroform extracts were combined and made to a known volume, and samples were taken for the assay of lipid mass and radioactivity.

Samples of the total brain homogenate were also removed, 0.2vol. of 50% (w/v) trichloroacetic acid was added, and the samples were placed on ice for 20min. The precipitated protein was sedimented and, after the supernatant had been decanted off, was re-extracted with 4.0ml of 10% trichloroacetic acid. The two supernatants were combined and filtered through Whatman no. 541 filter paper. Trichloroacetic acid was removed from the resultant supernatant by washing with 3×0.4vol. of diethyl ether and once with 0.4vol. of light petroleum (b.p. 40–60°C)–diethyl ether (1:1, v/v).

Separation of water-soluble material

Samples of the aqueous phases from either Folch or trichloroacetic acid extractions were separated by ion-exchange chromatography on Dowex 50 (X8; H^+ form; 200 mesh; Sigma Chemical Co., St. Louis, Mo., U.S.A.). Aqueous samples from the brain stem and cerebral-cortex extracts were applied to small Dowex 50 columns (0.8cm×3.0cm) and the columns were successively washed with 20ml of water, 30ml of 0.1M-HCl and 20ml of 3M-HCl (Webster & Cooper, 1968). These three fractions contained respectively glycerophosphorylcholine, phosphorylcholine and choline. In addition, the 3M-HCl fraction contained betaine and acetylcholine. In some experiments, an improved method with larger columns (0.8cm×7cm) of Dowex 50 (Illingworth & Portman, 1972*b*) was used, which successively separated glycerophosphorylcholine, phosphorylcholine, betaine and choline into distinct fractions. In this method, the columns were washed with 25ml of water, 90ml of 0.4M-HCl and 30ml of 3M-HCl. Phosphorylcholine was normally eluted in the first 40ml of 0.4M-HCl, and betaine between 40 and 90ml of 0.4M-HCl.

Samples of aqueous extracts from the brain were also analysed by t.l.c. on silica gel G or cellulose-impregnated plates. Betaine was resolved from other labelled compounds by chromatography on 0.25mm-thick silica gel G plates in the solvent system methanol–acetone–3M-HCl (18:3:2, by vol.). R_F values in this system were betaine 0.53, choline 0.39, acetylcholine 0.39, glycerophosphorylcholine 0.30, and phosphorylcholine 0.36. Acetylcholine was separated on 0.25mm-thick cellulose plates with the solvent system 0.2M-ammonium acetate (pH4.8)–acetone (1:4, v/v; Diamond & Kennedy, 1969). Spots were located with iodine vapour.

Separation of lipids and assay of radioactivity

Individual neutral lipids and phospholipids were separated on 0.25mm-thick layers of silica gel H with the solvent systems *n*-heptane–ether–methanol–acetic acid [85:15:3:2, by vol. (Belfrage *et al.*, 1965)] and chloroform–methanol–acetic acid–water [75:55:12:6, by vol. (Illingworth & Portman, 1972*a*) or 25:15:4:2, by vol. (Skipski *et al.*, 1964)]. Because of the low concentrations of lysophosphatidylcholine in brain tissue, 50μg of unlabelled material was added to each of the samples used to separate phospholipids for radioassay.

Lipid mass was determined by a charring technique (Portman & Alexander, 1972) after t.l.c. on washed (Parker & Peterson, 1965) silica gel H plates or by scraping the plates and determining the phospholipid P (Bartlett, 1959).

After elution with methanol, phosphatidylcholine samples were further fractionated according to their degree of unsaturation by t.l.c. on $AgNO_3$-impregnated silica gel H (Arvidson, 1968). Individual bands were detected under u.v. light after the plate had been sprayed with a solution which contained 0.4g of Omnifluor (New England Nuclear Corp., Boston, Mass., U.S.A.), 100ml of toluene and 900ml of ethanol (Illingworth & Glover, 1970). Three principal components corresponding to mono-+di-, tetra- and penta-+hexa-enoic phosphatidylcholines were resolved. Each band was eluted with 20ml of chloroform–methanol–water–acetic acid (45:15:1:10, by vol.) and the eluate was washed successively with 4M-NH_3 and twice with 0.5% NaCl–methanol–chloroform (50:50:3, by vol.).

Radioactivity was determined by previously described methods (Portman & Alexander, 1970) except that 10% BBS3 (Beckman Instruments Co., Palo Alto, Calif., U.S.A.) was added to the scintillation fluid as a solubilizer. Radioactive lipids were

counted in the presence of the silica gel after the addition of 0.5ml of water to each vial. Under these conditions, all lipids were fully solubilized. The presence of silica gel in the vials did not alter the ^{3}H:^{14}C ratios. Water-soluble compounds, eluted from ion-exchange columns, were concentrated by freeze-drying and evaporated to dryness under N_2. The samples were redissolved in 1ml of water, and radioactivity was assayed after the addition of 10ml of Aquasol (New England Nuclear Corp.). This procedure was also used to assay radioactivity in samples of betaine or acetylcholine separated by t.l.c.

Results

Uptake of lysophosphatidylcholine by brain

The uptake of lysophosphatidylcholine by the brain *in vivo* was studied in 13 adult squirrel monkeys (body wt. 600–1020g; mean 760g) maintained on either control diets or one inducing hyperlipaemia. As Fig. 1 illustrates, the injected doubly labelled lysophosphatidylcholine was cleared very rapidly

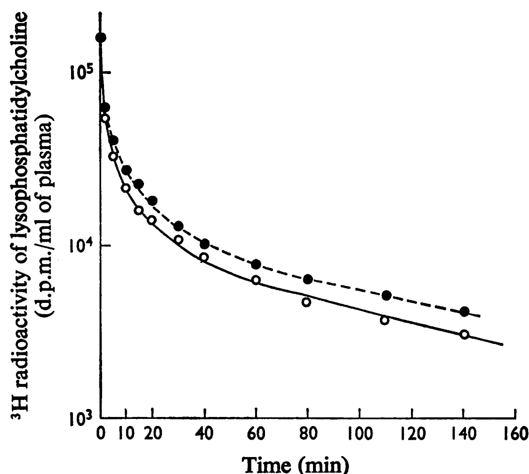

Fig. 1. *Disappearance of intravenously injected [Me-^{3}H]choline-labelled lysophosphatidylcholine from the plasma of squirrel monkeys as a function of time*

Each monkey received 1.36μmol of albumin-bound lysophosphatidylcholine and 2.34μCi of ^{3}H at the starting time. Lysophosphatidylcholine radioactivity was determined as indicated in the Materials and Methods section. Each line is a composite of the individual decay curves for seven control (○) and six hyperlipaemic (●) monkeys.

from the plasma of both groups of animals. The slower disappearance of labelled lysophosphatidylcholine in the plasma of the hyperlipaemic group of animals was due to the higher concentrations of lysophosphatidylcholine in their plasma and total body pool (Portman *et al.*, 1970). Although hyperlipaemia did influence the incorporation of lysophosphatidylcholine into phosphatidylcholine in both the liver and the bile, no consistent changes in the uptake of this compound by the brain were observed. The results from both groups of animals for the brain were therefore combined.

The incorporation of [*Me*-^{3}H]choline-labelled lysophosphatidylcholine into the lipid- and water-soluble fractions of squirrel-monkey cerebral cortex and brain stem are shown in Figs. 2 and 3 respectively. In both areas of the brain, radioactivity was about twice as great in the lipid extract as in the aqueous phase. The overall concentration of radioactivity in cortical grey matter was, however, some 15–20% greater than that in white matter from the brain stem, although the time-course of incorporation by both samples was similar. The minute quantities of ^{14}C radioactivity in the aqueous phases (^{3}H:^{14}C ratios >20) indicated that the ^{3}H radioactivity in this fraction was not due to unextracted lysophosphatidylcholine (which had a ^{3}H:^{14}C ratio of 3.75). The ^{3}H:^{14}C ratios for the combined lipid plus aqueous fractions (Figs. 2 and 3) did not change appreciably up to 3h, an indication that the newly formed phosphatidylcholine (^{3}H:^{14}C ratios >3.0; see Table 3) or the products of lysophosphatidylcholine catabolism were not being rapidly lost from the brain. Values for the total uptake of labelled lysophosphatidylcholine by the brain (mean weight 22.9 ± 0.4g), expressed as a percentage of the injected dose, were 0.45% after 10min (no. of animals = 3), 1.04% after 22min (no. of animals = 2) and 0.99% after 140–180min (no. of animals = 6).

Distribution of radioactivity in lipid classes

The phospholipid composition of squirrel-monkey brain (Table 1) resembles that of other mammalian species (Dickerson, 1968). In our study, white matter was characterized by a higher concentration of total phospholipid and was relatively richer in sphingomyelin, phosphatidylserine and phosphatidylethanolamine, but poorer in phosphatidylcholine, than corresponding samples of cortical grey matter. The lysophosphatidylcholine content of both samples was very low.

Table 2 shows the distribution of ^{14}C radioactivity derived from [1-^{14}C]palmitoyl-labelled lysophosphatidylcholine within the different lipid classes of brain. At all time-periods studied, most of the radioactivity was present in phosphatidylcholine, with comparatively little in lysophosphatidylcholine. Free

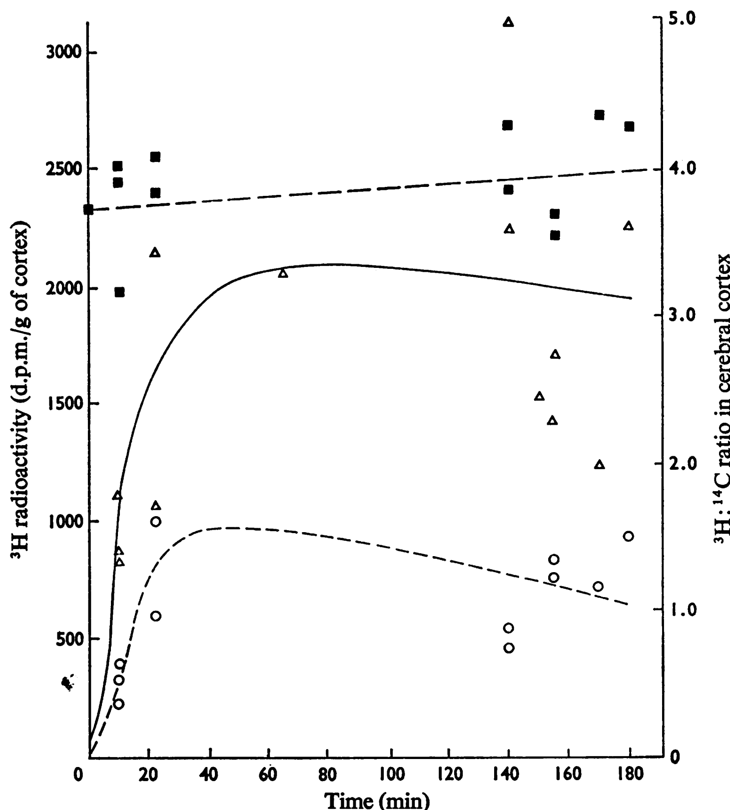

Fig. 2. *Incorporation of ^{3}H radioactivity into the total lipid and water-soluble fractions from squirrel-monkey cerebral cortex and the ^{3}H:^{14}C ratios of combined lipid and water-soluble fractions as a function of time after the intravenous injection of [Me-^{3}H]choline- and [1-^{14}C]palmitoyl-labelled lysophosphatidylcholine*

The ^{3}H:^{14}C ratio of the injected lysophosphatidylcholine was 3.75. ■, ^{3}H:^{14}C ratio in cortex; △, lipid-bound ^{3}H radioactivity; o, water-soluble ^{3}H radioactivity. The technique for isolation and assay of samples is described in the Materials and Methods section.

fatty acids, and to a lesser extent triglycerides and phosphatidylethanolamine, were also labelled, an indication of hydrolysis and further metabolism of the palmitate moiety from [1-^{14}C]palmitoyl-labelled lysophosphatidylcholine. No significant radioactivity was present in other lipid fractions. The fact that virtually no radioactivity was detected in either the phosphatidylcholine or free fatty acid fractions of plasma during the first 20–30min after injection of doubly labelled lysophosphatidylcholine precludes the possibility that the rapid labelling of these compounds in the brain was due to either uptake from (Dhopeshwarkar & Mead, 1969; Hoelzl & Franck, 1969) or possible exchange with (Illingworth & Glover, 1971) their counterparts in plasma. The very low ^{3}H radioactivity (<0.1% of total plasma ^{3}H content) in the aqueous phase of chloroform–methanol extracts from plasma indicated that the water-soluble ^{3}H radioactivity found in brain must also have resulted from the uptake and subsequent metabolism of lysophosphatidylcholine within the brain.

Fatty acid specificity of lysophosphatidylcholine acylation

To ascertain whether or not lysophosphatidylcholine was being selectively acylated with fatty acids of a particular unsaturation, we separated phosphatidylcholine samples from both grey and white matter into three fractions by t.l.c. on $AgNO_3$-impregnated silica gel H. As Table 3 illustrates, the relative specific radioactivities of tetraenoic phosphatidylcholine fractions were some two to three times as high as those of the mono-+di-ene or penta-+hexa-ene

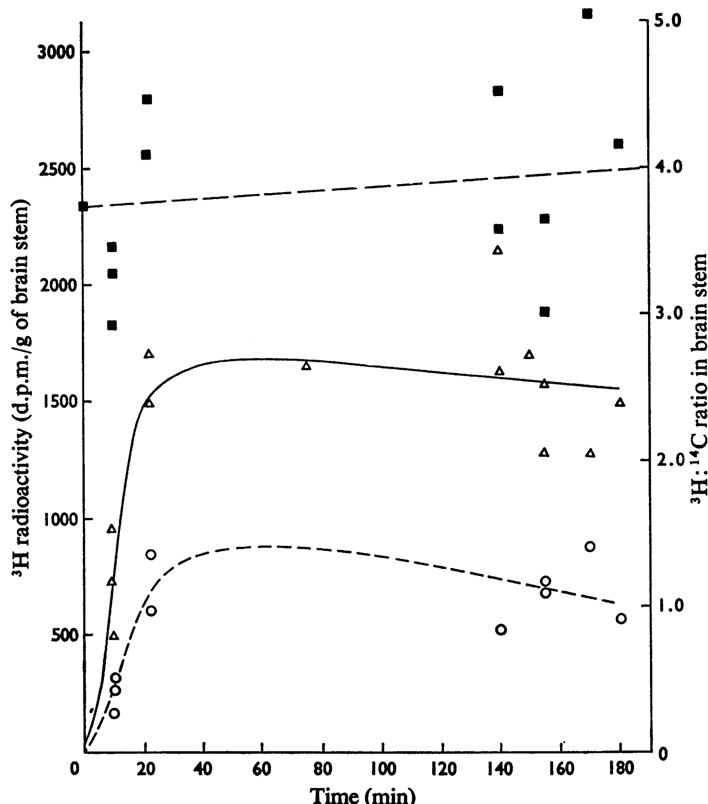

Fig. 3. *Incorporation of* ^{3}H *radioactivity into the total lipid and water-soluble fractions from squirrel-monkey brain stem and the* ^{3}H:^{14}C *ratios of combined lipid and water-soluble fractions as a function of time after the intravenous injection of* [*Me*-^{3}H]*choline- and* [1-^{14}C]*palmitoyl-labelled lysophosphatidylcholine*

The ^{3}H:^{14}C ratio of the injected lysophosphatidylcholine was 3.75. ■, ^{3}H:^{14}C ratio in brain stem; △, lipid-bound ^{3}H radioactivity; o, water-soluble ^{3}H radioactivity. The technique for isolation and assay of samples is described in the Materials and Methods section.

fraction, and thereby indicate a selective acylation of lysophosphatidylcholine with tetraenoic fatty acids. The ^{3}H:^{14}C ratios of the tetra- and penta-+hexa-enoic fractions were generally higher than those of the mono-+di-enoic phosphatidylcholines and more closely resembled that of the originally injected lysophosphatidylcholine (^{3}H:^{14}C ratio 3.75). The slightly lower ratios of the latter fraction probably reflect the incorporation of [1-^{14}C]palmitic acid, produced from the hydrolysis of [1-^{14}C]palmitoyl-labelled lysophosphatidylcholine, into phosphatidylcholine via the diglyceride–CDP-choline pathway. This theory is consistent with the preferential biosynthesis of mono- and di-enoic phosphatidylcholines that occurs via this pathway (Balint *et al.*, 1967; Rytter *et al.*, 1968; Kanoh, 1969; Åkesson *et al.*, 1970). Nevertheless, the overall similarity between the ^{3}H:^{14}C ratio of the injected lysophosphatidylcholine and that of brain phosphatidylcholine clearly demonstrates that the major pathway of phosphatidylcholine formation in these experiments is by a direct acylation of lysophosphatidylcholine (Lands, 1960).

Distribution of water-soluble radioactivity

Samples of the aqueous phase from chloroform–methanol extracts of brain were separated into three fractions by chromatography on Dowex 50 (Webster & Cooper, 1968). As Table 4 illustrates, glycerophorylcholine, produced from the deacylation of lysophosphatidylcholine, was rapidly metabolized by both grey and white matter and after 3h accounted for less than 13% of the total radioactivity in the aqueous phase. Labelling of the 3M-HCl

Table 1. *Phospholipid composition of different parts of the squirrel-monkey brain*

Lipid fractions from each of 13 monkey brains were separated by t.l.c. (Skipski *et al.*, 1964), and lipid mass was determined by a charring technique (Portman & Alexander, 1972).

	Whole brain	Cerebral cortex	Brain stem
Total phospholipid (mg/g wet wt.)	61.5	43.2	63.5
Percentage distribution of P			
Origin	0.4	1.0	0.2
Lysophosphatidylcholine	0.4	0.5	0.4
Sphingomyelin	11.0	6.1	13.6
Phosphatidylcholine	32.2	35.5	26.0
Phosphatidylinositol	2.6	4.8	2.3
Phosphatidylserine	15.9	17.2	18.8
Phosphatidylethanolamine	34.1	32.1	36.6
Phosphatidic acid+cardiolipin	2.5	2.9	2.4

Table 2. *Percentage distribution of* ^{14}C *radioactivity derived from intravenously injected* [1-^{14}*C*]*palmitoyl-labelled lysophosphatidylcholine in brain lipids of squirrel monkeys*

The lysophosphatidylcholine was bound to albumin. At various intervals after injection, the monkeys were killed and the brains were thoroughly perfused with 0.9% NaCl. Brain lipids were separated into different subfractions by t.l.c. (Skipski *et al.*, 1964; Belfrage *et al.*, 1965). N.D., not determined.

			Distribution of ^{14}C radioactivity (%)				
Time (min)	No. of animals	Region of brain	Phosphatidylcholine	Lysophosphatidylcholine	Phosphatidylethanolamine	Free fatty acid	Triglyceride
10	3	Cortex	72.2	8.0	—	14.5	5.3
		Stem	67.5	10.6	—	16.1	5.8
22	2	Cortex	71.4	5.0	3.3	12.2	8.3
		Stem	56.3	7.7	3.5	23.0	9.5
75	1	Cortex	68.8	9.1	N.D.	— 22.1	—
		Stem	67.1	8.1	N.D.	— 24.8	—
140–180	7	Cortex	74.3	1.6	5.6	15.5	3.0
		Stem	70.0	2.6	6.0	18.4	3.0

fraction (containing choline, betaine and acetylcholine) was rapid but declined somewhat in the later samples. In contrast, the amount of radioactive phosphorylcholine showed a marked increase with time and accounted for some 30% of the water-soluble ^{3}H radioactivity 140–180 min after the injection of doubly labelled lysophosphatidylcholine.

The distribution of ^{3}H radioactivity from [*Me*-^{3}H]-choline-labelled lysophosphatidylcholine in acetylcholine, betaine and choline (the principal constituents of the 3M-HCl fraction) from aqueous extracts of whole brain homogenates is shown in Table 5. These values are derived from experiments in which acetylcholine, present in total aqueous extracts, was separated by t.l.c. on plates coated with cellulose (see the Materials and Methods section) and in which betaine was separated either by t.l.c. on 0.25mm-thick silica gel G plates or by ion-exchange chromatography on columns (0.8cm × 7.0cm) of Dowex 50 (see the Materials and Methods section). We did not take any special precautions to prevent the hydrolysis of acetylcholine between the times brain samples were taken and were homogenized except for rapid transfer to iced containers. Therefore the radioactivity in acetylcholine was probably underestimated. The results demonstrate a progressive increase in the labelling of betaine, together with a decrease in the radioactivity of choline, between 20min and 3h. Values for choline in Table 5 may, in fact, be too high, since this fraction probably also contains betaine aldehyde, an intermediate in the oxidation of choline to betaine (Speed & Richardson, 1968). The extent of labelling of this compound was not studied. Nevertheless, with these limitations in mind, it is

Table 3. *Relative specific radioactivities of phosphatidylcholine subclasses from the brain of squirrel monkeys which had been injected with a mixture of [1-^{14}C]palmitoyl-labelled and [Me-^{3}H]choline-labelled lysophosphatidylcholine bound to albumin*

The ^{3}H:^{14}C ratio of the injected dose was 3.75. The relative specific radioactivity refers to the specific radioactivity of the phosphatidylcholine subfraction relative to the total phosphatidylcholine specific radioactivity taken as unity. Each value is the mean from two or more animals (indicated in parentheses) and is derived from the average of the relative specific radioactivities of ^{3}H and ^{14}C in each fraction.

		Relative specific radioactivity and ^{3}H:^{14}C ratios of phosphatidylcholine fractions							
		Mono-+di-ene		Tetraene		Penta-+hexa-ene		Total phosphatidylcholine	
Time (min)	Sample	Relative sp. radioactivity	^{3}H:^{14}C ratio	Relative sp. radioactivity	^{3}H:^{14}C ratio	Relative sp. radioactivity	^{3}H:^{14}C ratio	Relative sp. radioactivity	^{3}H:^{14}C ratio
10	Cortex (2)	0.78	3.19	1.86	3.15	0.69	4.13	1.0	3.30 (3)
	Stem (2)	0.74	2.79	2.60	3.80	0.68	4.10	1.0	3.10 (3)
22	Cortex (2)	0.71	2.74	2.01	3.27	0.93	3.60	1.0	3.06 (2)
	Stem (2)	0.72	3.39	2.51	3.34	0.73	3.27	1.0	3.60 (2)
140–180	Cortex (3)	0.87	3.11	1.39	3.70	1.03	4.09	1.0	3.46 (7)
	Stem (3)	0.79	3.33	2.11	3.93	0.82	3.89	1.0	3.57 (7)

evident that at all times acetylcholine and choline were the principal sources of the radioactivity in the 3M-HCl fractions from Dowex 50 columns eluted by the method of Webster & Cooper (1968).

Discussion

The results of the present study clearly demonstrate that lysophosphatidylcholine in plasma is taken up and metabolized by the brain, where it serves as a precursor of both phosphatidylcholine and choline. By using an isotopic mixture of lysophosphatidylcholine, in which both the choline and fatty acid moieties were labelled, we simultaneously determined the fate of both of these constituents of the lysophosphatidylcholine molecule and their relative rates of incorporation into phosphatidylcholine.

The similarity between the ^{3}H:^{14}C ratios of injected lysophosphatidylcholine and of brain phosphatidylcholine clearly demonstrates that the principal pathway of synthesis of the latter from the former proceeds via a direct acylation (Lands, 1960; Webster, 1965) and not by synthesis *de novo* after initial hydrolysis or by a condensation of two lysophosphatidylcholine molecules to form one molecule of phosphatidylcholine and one molecule of glycerophosphorylcholine (Erbland & Marinetti, 1965). Further, since the incorporation of [*Me*-^{3}H]choline-labelled lysophosphatidylcholine into lipid-soluble material, of which over 85% was present in phosphatidylcholine, exceeded that in the water-soluble phase by about twofold, we conclude that under these conditions *in vivo* the relative rates of acylation versus hydrolysis of the lysophosphatidylcholine taken up by the brain are 2:1. These findings contrast sharply with those studies *in vitro* on homogenates of both human (Webster, 1965; Illingworth & Glover, 1969) and rhesus-monkey brain (D. R. Illingworth & O. W. Portman, unpublished work) where the rates of hydrolysis of lysophosphatidylcholine under optimum conditions were severalfold greater than the corresponding optimum rates of acylation. The obvious differences between the metabolism of lysophosphatidylcholine *in vivo* and *in vitro* may be due to differences in the subcellular localization or latency of the enzymes responsible. Thus enzymes for the acylation of lysophosphatidylcholine to phosphatidylcholine are present in the plasma membrane, mitochondria and microsomal fraction (Stahl & Trams, 1968; Stein *et al.*, 1968; Sarzala *et al.*, 1970; Wright & Green, 1971), whereas lysophospholipase (lysophosphatidylcholine acylhydrolase, EC 3.1.1.5) appears to be predominantly microsomal (Leibovitz & Gatt, 1968; Hortnagl *et al.*, 1969).

In addition to demonstrating direct acylation of lysophosphatidylcholine to phosphatidylcholine, the

Table 4. *Distribution of ^{3}H radioactivity in water-soluble material in the brains of squirrel monkeys at various intervals after the intravenous injection of [Me-^{3}H]choline-labelled lysophosphatidylcholine bound to albumin*

The water-soluble fraction was isolated by the method of Folch *et al.* (1957) and was subfractionated on Dowex 50 by the method of Webster & Cooper (1968). The numbers of animals are given in parentheses.

Time (min)	Sample	Percentage of ^{3}H radioactivity in fraction: Water (glycerophosphorylcholine)	0.1 M-HCl (phosphorylcholine)	3 M-HCl (choline + betaine + acetylcholine)
10 (3)	Cortex	20.6	10.2	69.2
	Stem	24.2	9.6	66.2
22 (2)	Cortex	29.0	12.4	58.6
	Stem	25.9	13.6	60.5
140–180 (5)	Cortex	12.6	30.6	56.8
	Stem	11.6	33.4	55.0

Table 5. *Distribution of ^{3}H radioactivity in the principal constituents of the 3 M-HCl fraction from the Dowex 50 column (Webster & Cooper, 1968) described in Table 4 and in the text*

Acetylcholine was separated by t.l.c. on cellulose-impregnated plates (see the Materials and Methods section) and betaine was separated either by t.l.c. on silica gel G plates or on columns (0.8 cm × 7.0 cm) of Dowex 50 (see the Materials and Methods section). The numbers of animals are given in parentheses.

Time (min)	Distribution (%): Acetylcholine	Betaine	Choline
10 (3)	33.8	8.6	57.6
22 (2)	46.5	11.9	41.6
140–180 (2)	43.2	21.4	35.4

relative constancy of the $^{3}H:^{14}C$ ratios of brain phosphatidylcholine also argues against a significant incorporation of choline via base-exchange reactions (Dils & Hübscher, 1961; Treble *et al.*, 1970). Recent studies on extraneural tissues (Bjerve, 1971; Plageman, 1971) as well as in brain (Porcellati *et al.*, 1971) also support this conclusion.

Our findings that the relative specific radioactivities of tetraenoic phosphatidylcholine species from brain were two to three times as great as those of other fractions are consistent with current views on phosphatidylcholine biosynthesis. Thus the mono- and di-enoic species are formed predominantly by synthesis *de novo* via the diglyceride–CDP-choline pathway, whereas tetraenoic phosphatidylcholines appear to be formed selectively by acylation of lysophosphatidylcholine (Balint *et al.*, 1967; Kanoh, 1969; Holub *et al.*, 1971). Although Webster (1965) demonstrated a lower acylation of lysophosphatidylcholine *in vitro* with arachidonate than with oleate or linoleate by human brain homogenates, it is not clear whether these results are due to physical differences between the fatty acids or whether they reflect true differences in acyltransferase specificity. Since in the present experiments we did not determine the composition of the free fatty acid pool in the brain, we cannot say definitely whether the higher specific radioactivities of the tetraenoic phosphatidylcholines are due to acyltransferase specificity or whether they simply reflect a larger pool of tetraenoic than of mono- and di-enoic acids in squirrel-monkey brain. Studies on the composition of the free fatty acid pool in other species (Bazan, 1970; Lunt & Rowe, 1971), however, support the first hypothesis.

The appearance of ^{3}H radioactivity in the water-soluble phase of chloroform–methanol or trichloroacetic acid extracts of brain, as well as of [1-^{14}C]-palmitate in the lipid extract, clearly demonstrates, in agreement with previous studies *in vitro* (Leibovitz & Gatt, 1968; Illingworth & Glover, 1969), that lysophospholipase is present in brain and, further, that it is involved in the metabolism of lysophosphatidylcholine *in vivo*. Glycerophosphoryl[*Me*-^{3}H]choline, produced from the deacylation of [*Me*-^{3}H]choline-labelled lysophosphatidylcholine, did not accumulate to any appreciable extent in brain, and this confirms the presence of the enzyme glycerophosphorylcholine diesterase (EC 3.1.4.2) in this tissue (Webster *et al.*, 1957; Baldwin & Cornatzer, 1968). Further metabolism of the labelled choline, produced by glycerophosphorylcholine diesterase activity, appeared to follow the known pathways of choline metabolism in the brain, with radioactivity appearing in acetylcholine, phosphorylcholine and betaine (Diamond &

Kennedy, 1969; Ansell & Spanner, 1968; Schuberth *et al.*, 1969; Diamond, 1971). About 30% of the ^3H taken up by the brain as lysophosphatidylcholine appeared in choline or its derivatives.

In conclusion, the rapid turnover of lysophosphatidylcholine in plasma, together with the finding that 1% of the injected doubly labelled lysophosphatidylcholine was taken up by the brain, suggests that plasma lysophosphatidylcholine serves as an important precursor for both phosphatidylcholine and choline in the brain. The absence of radioactivity in the phosphatidylcholine, free fatty acid and water-soluble fractions from plasma within 30min after the injection of doubly labelled lysophosphatidylcholine clearly proves that the labelling of these compounds in the brain cannot be due to the entry of their labelled counterparts from plasma. These findings are consistent with the theory that the entry of compounds from plasma into the brain depends on their lipid solubility (Davson, 1971). Although the presence of lysophosphatidylcholine in human cerebrospinal fluid (Illingworth & Glover, 1971) indicates that this compound can also pass the blood–cerebrospinal barrier, it may be formed directly in the cerebrospinal fluid by the action of phosphatidylcholine–cholesterol acyltransferase (Illingworth & Glover, 1970).

This work was supported by a Grant-in-Aid from the National Institutes of Health (HE 09744), Bethesda, Md., U.S.A. This is Publication no. 598 from the Oregon Regional Primate Research Center, supported in part by Grant no. RR 00163 from the National Institutes of Health.

References

Åkesson, B., Elovson, J. & Arvidson, G. (1970) *Biochim. Biophys. Acta* **218**, 44–56
Ansell, G. B. & Spanner, S. (1967) *J. Neurochem.* **14**, 873–885
Ansell, G. B. & Spanner, S. (1968) *Biochem. J.* **110**, 201–206
Ansell, G. B. & Spanner, S. (1971) *Biochem. J.* **122**, 741–750
Arvidson, G. A. E. (1968) *Eur. J. Biochem.* **4**, 478–486
Baldwin, J. J. & Cornatzer, W. E. (1968) *Biochim. Biophys. Acta* **164**, 195–204
Balint, J. A., Beeler, D. A., Treble, D. H. & Spitzer, H. L. (1967) *J. Lipid Res.* **8**, 486–493
Bartlett, G. R. (1959) *J. Biol. Chem.* **234**, 466–468
Bazan, N. G., Jr. (1970) *Biochim. Biophys. Acta* **218**, 1–10
Belfrage, P., Elovson, J. & Olivecrona, T. (1965) *Biochim. Biophys. Acta* **106**, 45–55
Bjerve, K. S. (1971) *FEBS Lett.* **17**, 14–16
Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–917
Bremer, J. & Greenberg, D. M. (1961) *Biochim. Biophys. Acta* **46**, 205–216
Chida, N. & Arakawa, T. (1971) *Tohoku J. Exp. Med.* **104**, 359–371
Davson, H. (1971) *Advan. Exp. Med. Biol.* **13**, 381–396
Dhopeshwarkar, G. A. & Mead, J. F. (1969) *Biochim. Biophys. Acta* **187**, 461–467
Diamond, I. (1971) *Arch. Neurol. (Chicago)* **24**, 333–339
Diamond, I. & Kennedy, E. P. (1969) *J. Biol. Chem.* **244**, 3258–3263
Dickerson, J. W. T. (1968) in *Applied Neurochemistry* (Davison, A. W. & Dobbing J., eds.), pp. 48–115, Blackwell Scientific Publications, Oxford and Edinburgh
Dils, R. R. & Hübscher, G. (1961) *Biochim. Biophys. Acta* **46**, 505–513
Erbland, J. F. & Marinetti, G. V. (1965) *Biochim. Biophys. Acta* **106**, 128–138
Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497–509
Glomset, J. A. (1968) *J. Lipid Res.* **9**, 155–167
Hoelzl, J. & Franck, H. P. (1969) *Proc. Int. Meet. Int. Soc. Neurochem. 2nd* 219–220
Holub, B. J., Breckenridge, W. C. & Kuksis, A. (1971) *Lipids* **6**, 307–313
Hortnagl, H., Winkler, H. & Hortnagl, H. (1969) *Eur. J. Biochem.* **10**, 243–248
Illingworth, D. R. & Glover, J. (1969) *Biochem. J.* **115**, 16P
Illingworth, D. R. & Glover, J. (1970) *Biochim Biophys. Acta* **220**, 610–613
Illingworth, D. R. & Glover, J. (1971) *J. Neurochem.* **18**, 769–776
Illingworth, D. R. & Portman, O. W. (1972*a*) *J. Lipid Res.* **13**, 220–227
Illingworth, D. R. & Portman, O. W. (1972*b*) *J. Chromatogr.* in the press
Kanoh, H. (1969) *Biochim. Biophys. Acta* **176**, 756–763
Lands, W. E. M. (1960) *J. Biol. Chem.* **235**, 2233–2237
Leibovitz, Z. & Gatt, S. (1968) *Biochim. Biophys. Acta* **164**, 439–441
Long, C. & Penny, I. F. (1957) *Biochem. J.* **65**, 382–389
Lunt, C. G. & Rowe, C. E. (1971) *Brain Res.* **35**, 215–220
Nelson, G. J. (1967) *Lipids* **2**, 323–328
Parker, F. & Peterson, N. F. (1965) *J. Lipid Res.* **6**, 455–460
Plageman, P. G. W. (1971) *J. Lipid Res.* **12**, 715–724
Porcellati, G., Arienti, G., Pirotta, M. & Giorgini, D. (1971) *J. Neurochem.* **18**, 1395–1417
Portman, O. W. & Alexander, M. (1969) *J. Lipid Res.* **10**, 158–165
Portman, O. W. & Alexander, M. (1970) *J. Lipid Res.* **11**, 23–30
Portman, O. W. & Alexander, M. (1972) *Biochim. Biophys. Acta* **260**, 460–474
Portman, O. W., Alexander, M. & Maruffo, C. A. (1967) *J. Nutr.* **91**, 35–46
Portman, O. W., Soltys, P., Alexander, M. & Osuga, T. (1970) *J. Lipid Res.* **11**, 596–604
Rytter, D., Miller, J. E. & Cornatzer, W. E. (1968) *Biochim. Biophys. Acta* **152**, 418–421
Sarzala, M. G., van Golde, L. M. G., de Kruyff, B. & van Deenen, L. L. M. (1970) *Biochim. Biophys. Acta* **202**, 106–119
Schuberth, J., Sparf, B. & Sundwell, A. (1969) *J. Neurochem.* **16**, 695–700
Skipski, V. P., Peterson, R. F. & Barclay, M. (1964) *Biochem. J.* **90**, 374–378
Speed, D. & Richardson, M. (1968) *J. Chromatogr.* **35**, 497–505

Stahl, W. L. & Trams, E. G. (1968) *Biochim. Biophys. Acta* **163**, 459–471
Stein, Y. & Stein, O. (1966) *Biochim. Biophys. Acta* **116**, 95–107
Stein, Y., Widnell, C. & Stein, O. (1968) *J. Cell Biol.* **39**, 185–192
Switzer, S. & Eder, H. A. (1965) *J. Lipid Res.* **6**, 506–511
Treble, D. H., Frumkin, S., Balint, J. A. & Beeler, D. A. (1970) *Biochim. Biophys. Acta* **202**, 163–171
Webster, G. R. (1965) *Biochim. Biophys. Acta* **98**, 512–519
Webster, G. R. & Cooper, M. (1968) *J. Neurochem.* **15**, 795–802
Webster, G. R., Marples, E. A. & Thompson, R. H. S. (1957) *Biochem. J.* **65**, 374–377
Wright, J. D. & Green, C. (1971) *Biochem. J.* **123**, 837–844