Phospholipid Metabolism in Kidney. III. Biosynthesis of Phospholipids from Radioactive Precursors in Rabbit Renal Cortex Slices*

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ABSTRACT: The metabolism of phospholipids in renal cortex slices was studied by incubating the slices in Krebs–Ringer medium containing inorganic ^{32}P, glucose-6-^{14}C, serine-3-^{14}C, palmitic acid-1-^{14}C, or linoleic acid-1-^{14}C and determining the pattern of labeling of individual phospholipids isolated by silicic acid chromatography. Cardiolipin and sphingomyelin were metabolically inert. ^{32}P was incorporated chiefly into phosphatidylinositol and phosphatidylcholine. The glycerol of lecithin was labeled from glucose-6-^{14}C, the results suggesting that the glycerol and phosphorus of this lipid turn over as a unit. Radioactive fatty acids were rapidly incorporated into lecithin; linoleic acid esterified in the α' and β positions had the same specific activity, but palmitic acid in the β position had a much higher specific activity than that in the α' position. Serine-3-^{14}C was incorporated chiefly into the serine moiety of phosphatidylserine, but lesser amounts of radioactivity were found in the ethanolamine of phosphatidylethanolamine and the glycerol of lecithin. The results showed lecithin probably does not arise by methylation of phosphatidylethanolamine.

Cyanide (10^{-2} M) abolished ^{32}P uptake but did not affect the incorporation of serine into phosphatidylserine. Ouabain (10^{-4} M) stimulated incorporation of ^{32}P into all lipids by 50%. It is suggested that the turnover of β-esterified palmitic acid in lecithin may be involved in the oxidation of this fatty acid by renal cortex tissue.

In previous reports (Morgan *et al.*, 1963b; Tinker *et al.*, 1964) we have shown that rabbit renal cortex contains as its major phospholipid components phosphatidylcholine, phosphatidylethanolamine (plus plasmalogen), phosphatidylserine, and sphingomyelin. Lesser amounts of cardiolipin and phosphatidylinositol are also present. Studies of the incorporation of ^{32}P into these lipids *in vitro* (Tinker *et al.*, 1963) indicated that the phosphorus moieties of lecithin and phosphatidylinositol were metabolically active, while the phosphorus moieties of ethanolamine and serine phosphatides, sphingomyelin and cardiolipin, were not labeled appreciably from ^{32}P. The present report describes experiments designed to elucidate the metabolic pathways of glycerol, nitrogenous moieties, and fatty acids of the phospholipids.

Experimental Section

Palmitic acid-1-^{14}C, linoleic acid-1-^{14}C, DL-serine-3-^{14}C, and D-glucose-6-^{14}C were purchased from New England Nuclear Corp. Palmitic acid-1-^{14}C and linoleic acid-1-^{14}C were supplied with specific activities of 37.1 mcuries/mmole and 22.8 mcuries/mmole, respectively; both were diluted with the appropriate unlabeled fatty acids (obtained from Applied Science Laboratories, State College, Pa.) to a specific activity of 5.0 mcuries/mmole. Gas–liquid partition chromatography of the diluted radioactive fatty acids (see below) indicated that these preparations were radiochemically pure. DL-Serine-3-^{14}C had a specific activity of 5.23 mcuries/mmole; that of D-glucose-6-^{14}C was 4.87 mcuries/mmole. Crystalline rabbit serum albumin was purchased from Mann Biochemicals Inc., New York, N. Y., and freed from endogenous fatty acids by the method of Goodman (1957). Ouabain was a gift from the Eli Lilly Co., Indianapolis, Ind.

Techniques for preparing the tissue slices have already been described (Tinker *et al.*, 1963); 500 mg of tissue (three slices) was incubated at 37° in 20 ml of Krebs–Ringer medium III (Krebs, 1950) for 0–2 hr. Where indicated, the following substances were added to the medium in the final concentrations stated: inorganic phosphate-^{32}P, 1.0 μcuries/ml; DL-serine-3-^{14}C, 1.0 μcurie/ml or 0.5 μcurie/ml; D-glucose-6-^{14}C, 0.5 μcurie/ml; palmitic acid-1-^{14}C (albumin complex), 0.25 μcuries/ml; linoleic acid-1-^{14}C (albumin complex), 0.25 μcurie/ml; potassium cyanide, 10^{-4} to 10^{-1} M; ouabain, 10^{-4} M. All radioactive substances were present in micromolar amounts or less; when D-glucose-6-^{14}C was added to the medium, unlabeled glucose was omitted. The albumin complexes of radio-

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active fatty acids were prepared in the following manner: 10.0 μmoles (50 μcuries) of radioactive fatty acid in 0.1 ml of absolute ethanol was added to a solution of 700 mg of albumin in 10 ml of 0.9% saline at 25°. The solution cleared completely within 0.5 hr. This solution (1 ml) was then added to 19 ml of Krebs–Ringer medium. The final concentration of fatty acid was thus 0.05 μmole/ml; that of albumin was 0.35%.

Lipids were extracted from the tissue and separated by silicic acid chromatography essentially as described earlier (Morgan *et al.*, 1963b) except that with small amounts of lipids (0.2–1 mg of lipid P), a loading factor of 10 g of silicic acid/mg of lipid phosphorus was used. Sphingomyelin was purified by mild alkaline methanolysis as described below. Free fatty acids were removed from the neutral lipids fraction by the Borgström wash technique (Borgström, 1952). Lipids were degraded either by acid hydrolysis in 6 N HCl in a sealed tube at 110° or by mild alkaline methanolysis. In the latter case, the following modification of the technique of Morgan *et al.* (1963a) was used. An aliquot of lipid containing 0.5–10 μmole of ester was dissolved in 1 ml of methanol, and 5 ml of 0.5 N methanolic NaOH was added. The reaction was allowed to proceed 30 min or more at room temperature, and then the mixture was neutralized by the addition of 0.42 ml of 6 N HCl. Chloroform (12 ml) and 4.1 ml of water were added and mixed, and the phases were allowed to separate. The entire procedure was carried out in a 125-ml separatory funnel; emulsions were readily broken by cooling to 4° and warming one side of the separatory funnel with a stream of warm air. Methyl esters of the fatty acids of the lipids were recovered in the chloroform phase. Sphingomyelin was recovered intact in the chloroform phase after this treatment and was separated from fatty acid methyl esters by chromatography on a column of silicic acid–Hiflo Supercel (2:1 w/w). Preparations of phosphatidylcholine were hydrolyzed with purified phospholipase A (Saito and Hanahan, 1962), and the products (lysolecithin and fatty acids) were separated by silicic acid chromatography.

The water-soluble products obtained from certain of the above hydrolytic procedures were separated by paper chromatographic or ionophoretic techniques. Serine and ethanolamine were isolated using a two-dimensional ionophoresis–chromatography system (Walsh *et al.*, 1962); choline was isolated by descending paper chromatography using the solvent of Richmond and Hartley (1959). Substances purified by chromatographic or ionophoretic techniques were eluted from the paper with water by the micromethod described by Putnam (1957). In certain cases the aqueous hydrolysates obtained by acid hydrolysis of phosphatides were deionized with Amberlite MB-1, and the deionized hydrolysates were referred to as the "glycerol fraction" of the lipid. The efficiency of deionization was indicated by the complete removal of ^{32}P from the hydrolysates.

Serine and ethanolamine were measured by the method of Spackman *et al.* (1958), using an automated apparatus (Spinco Division, Beckman Instruments Inc., Palo Alto, Calif.).[1] Choline was determined by the method of Wheeldon and Collins (1958). All other analytical techniques have been described previously (Morgan *et al.*, 1963b). Fatty acid methyl esters were separated and analyzed by gas–liquid partition chromatography, as described earlier (Morgan *et al.*, 1963b); when ^{14}C-labeled fatty acids were chromatographed, the effluent from the mass detector was combusted in a train consisting of a copper oxide furnace maintained at 800° and a magnesium perchlorate drying tube. The radioactive carbon-^{14}C dioxide was then counted in a flow-through scintillation detector, using anthracene as the fluor. The combustion furnace and detector system were products of the Packard Electronic Co., La Grange, Ill.

Radioactivity was measured by liquid scintillation counting (except in the case of fatty acid methyl esters, *vide supra*). Lipid samples were dissolved in 10 ml of toluene containing 0.5% 2,5-diphenyloxazole (PPO)[2] and 0.05% 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) (w/v). Aqueous samples (1 ml) were dissolved in 10 ml of Bray's solution (Bray, 1960). Disintegrations were detected using a Packard Tri-Carb liquid scintillation counter, Model 3002. This instrument was used to count samples doubly labeled with ^{14}C and ^{32}P: the "red" channel was set at a gain of 0.75% with an A–B window of 250–1000, while the "green" channel was set at a gain of 5.75% with an A–B window of 50–1000. ^{32}P disintegrations were counted in the "red" channel with an absolute efficiency of 70%, and in the "green" channel with an absolute efficiency of 5%; ^{14}C disintegrations were counted in the "green" channel with an absolute efficiency of 85% and were not counted in the red channel. The counts measured on the "green" channel were corrected for their ^{32}P content by employing appropriate standards.

Radioautography of thin layer chromatograms of ^{14}C-labeled lipids containing approximately 1000–2000 dpm/spot was carried out using Kodak No-Screen X-ray film, using an exposure time of four weeks.

Results

Phospholipid Composition of Renal Cortex Slices. The total lipid extract obtained from 0.5 g of tissue (equivalent to 0.5 mg of lipid phosphorus) was applied to a column of 5 g of silicic acid–2.5 g of Hiflo Supercel in chloroform. The neutral lipids were eluted with chloroform, and the column was then eluted with the three solvents shown in Figure 1. This served to separate the phospholipids into five fractions, labeled I–V, respectively. A sixth fraction, containing less than 1% of the total lipid phosphorus, was eluted from the column with methanol; it contained sphingomyelin and possibly also a trace of lysolecithin. Peaks IV (phosphatidylcholine) and V (sphingomyelin) were not well separated,

[1] Through the courtesy of Dr. K. Walsh, Department of Biochemistry, University of Washington.

[2] Abbreviations: PPO, 2,5-diphenyloxazole; POPOP: 1,4-bis-[2-(5-phenyloxazolyl)]benzene.

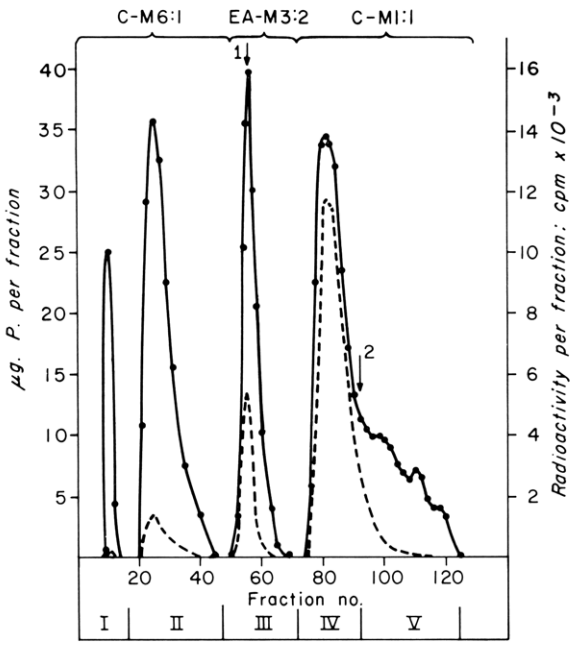

FIGURE 1: Separation of kidney cortex phospholipids on a silicic acid–Hiflo Supercel (2:1 w/w) column. Lipid phosphorus, 0.641 mg; silicic acid, 5 g. Column size 1 cm (id) × 17.2 cm; 3-ml fractions. Phospholipids had been labeled *in vitro* with ^{32}P. Solid line, lipid phosphorus; broken line, radioactivity. Roman numerals indicate combined fractions. Vertical arrow no. 1 indicates splitting of peak III into fractions IIIA and IIIB; vertical arrow no. 2 indicates cut between peaks IV and V. After tube 125, the column was eluted with five column volumes of C–M (1:9). In Figures 1, 2, and 11, the solvents used are abbreviated: C, chloroform; M, methanol; EA, ethyl acetate; W, water.

TABLE I: Lipid Phosphorus Content of Rabbit Kidney Cortex Slices.

Incubation Time (hr)[a]	mg of P/g of Tissue[b]
0.25	1.08 ± 0.190 (4)
0.50	1.05 ± 0.121 (6)
0.75	1.01 ± 0.149 (4)
1.00	0.976 ± 0.137 (6)
1.25	1.02 ± 0.067 (4)
1.50	1.02 ± 0.096 (6)
1.75	1.09 ± 0.115 (3)
2.00	1.02 ± 0.113 (6)
Grand average	1.03 ± 0.126 (39)

[a] Incubations were carried out at 37° in Krebs–Ringer medium; see text. [b] Results expressed as mean ± standard deviation; number of individual results in parentheses.

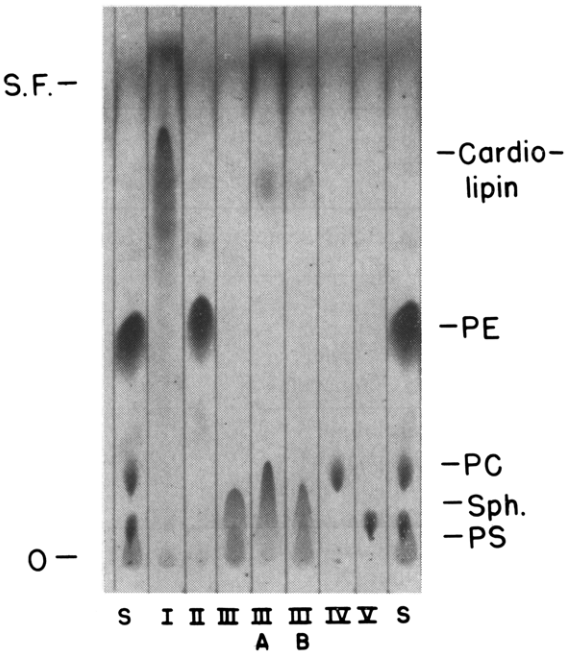

FIGURE 2: Thin layer chromatogram of phospholipid fractions of rabbit kidney cortex on silica gel G; solvent, C–M–W (95:35:4). Abbreviations: PE, phosphatidylethanolamine; PC, phosphatidylcholine; Sph, sphingomyelin; PS, phosphatidylserine; S, standard; O, origin; SF, solvent front. Roman numerals refer to peaks in Figure 1. Material at solvent front in channel IIIA is an artifact; other chromatograms did not show it.

but it was possible to obtain a pure preparation of phosphatidylcholine by pooling the tubes of peak IV which had constant ^{32}P specific activity. Sphingomyelin was freed from traces of lecithin by mild alkaline methanolysis. Figure 2 shows a thin layer chromatogram of the various fractions. In peak III (phosphatidylinositol plus phosphatidylserine) there was an asymmetric distribution of components; by pooling the first few tubes of this peak, there was obtained a fraction (IIIA) containing predominantly phosphatidylinositol, while the remaining tubes (fraction IIIB) contained predominantly phosphatidylserine together with some phosphatidylinositol. It was not possible to quantitatively separate phosphatidylinositol and phosphatidylserine.

In Table I is shown the lipid phosphorus content of rabbit renal cortex slices, while Table II shows the distribution of phosphorus among the various fractions. The phospholipid composition of the tissue did not change during a 2-hr incubation, and it was concluded that the steady-state condition obtained with respect to the phospholipids. In Table III is shown the phospholipid composition of renal cortex, calculated from the data of Tables I and II. Thin layer chromatograms of the neutral lipid fraction revealed no striking changes in the distribution of components during in-

TABLE II: Distribution of Phosphorus in Silicic Acid Column Fractions.

Time (hr)[a]	% of Applied Phosphorus[b]					
	I	II	III	IV	V	VI
0.25	5.66 ± 0.692 (7)	27.2 ± 2.47 (7)	19.1 ± 2.41 (7)	32.6 ± 1.64 (7)	14.3 ± 1.17 (7)	1.07 ± 0.23 (3)
0.50	5.25 ± 0.368 (7)	28.9 ± 1.69 (7)	18.8 ± 0.28 (7)	32.6 ± 1.46 (7)	14.0 ± 2.68 (7)	0.72 ± 0.16 (4)
0.75	5.41 ± 0.478 (7)	29.2 ± 2.65 (7)	19.4 ± 1.76 (7)	31.1 ± 2.49 (7)	14.3 ± 2.14 (7)	0.95 ± 0.25 (4)
1.00	5.33 ± 0.913 (6)	29.2 ± 1.69 (6)	18.7 ± 1.02 (6)	31.4 ± 1.66 (6)	15.1 ± 2.27 (6)	0.54 ± 0.21 (3)
1.25	5.20 ± 0.812 (7)	29.7 ± 3.03 (7)	18.2 ± 2.33 (7)	31.5 ± 3.19 (7)	15.1 ± 3.22 (7)	0.67 ± 0.15 (4)
1.50	5.46 ± 0.615 (7)	29.7 ± 3.73 (7)	18.5 ± 1.58 (7)	30.8 ± 2.72 (7)	15.4 ± 3.07 (7)	0.41 ± 0.15 (4)
1.75	5.38 ± 0.277 (6)	28.9 ± 2.90 (6)	18.5 ± 1.19 (6)	31.3 ± 1.30 (6)	15.2 ± 2.85 (6)	1.40 ± 0.78 (3)
2.00	5.85 ± 0.771 (7)	28.3 ± 2.08 (7)	18.8 ± 0.29 (7)	31.3 ± 1.30 (7)	14.9 ± 2.43 (7)	1.67 ± 1.38 (4)
Grand average	5.44 ± 0.649 (54)	28.9 ± 2.62 (54)	18.8 ± 1.64 (54)	31.6 ± 2.10 (54)	14.8 ± 2.55 (54)	0.92 ± 0.25 (29)

[a] Time of incubation at 37°. [b] Results quoted as mean ± standard deviation (number of individual results in parentheses). Roman numerals refer to fractions shown in Figure 1.

TABLE III: Phospholipid Composition of Kidney Cortex.

Fraction	Chief Components	mg of P/g of Tissue	μmoles of P/g of Tissue
I	Cardiolipin	0.056	1.82
II	Phosphatidyl-ethanolamine	0.297	9.58
III	Phosphatidylserine, phosphatidyl-inositol	0.193	6.23
IV	Phosphatidylcholine	0.325	10.48
V	Sphingomyelin	0.152	4.90
VI	Sphingomyelin, lysolecithin (?)	0.007	0.23
Total		1.03	33.23

cubation. The distribution of components was qualitatively similar to that described by Morgan *et al.* (1963b). Fraction I contained, as its major component, cardiolipin; however, there was also present a small amount of glycolipid, visible in Figure 2 as material moving with the solvent front. Fraction I had a molar N/P ratio of approximately 0.3, and after acid hydrolysis 80% of the nitrogen remained ether soluble. Paper chromatography of the aqueous hydrolysate (Morgan *et al.*, 1963b) revealed the presence of considerable amounts of hexose, as well as glycerol. It was concluded that fraction I probably contains glycosphingolipids, but the chemical nature of these components was not further examined.

^{32}P Incorporation. Figure 3 shows the time-course curves for uptake of ^{32}P into the various phospholipids; these curves are similar to those previously reported (Tinker *et al.*, 1963), and are included for comparison with the following figures. It is of interest that there is a rapid uptake of ^{32}P into fraction VI, as well as into phosphatidylinositol and lecithin; this is presumed to represent labeling of lysolecithin. The labeling of "phosphatidylserine" probably represents contamination with phosphatidylinositol. A definite lag period in the uptake of ^{32}P into phosphatidylcholine is to be noted.

Glucose-6-^{14}C Incorporation. It was important to know whether the ^{32}P incorporation described above, especially that into the lecithin, represented true biosynthesis, or whether a phosphorus-exchange reaction was operative. Accordingly, slices of kidney cortex were incubated with trace amounts of glucose-6-^{14}C, and the labeling of the phospholipids followed as a function of time. Figure 4 summarizes the results of one such experiment: there was a continuous increase in the specific activity of all lipid fractions except the "lysolecithin" fraction, wherein the specific activity rose for 1 hr, then declined. Radioautography of a thin layer chromatogram of the various fractions showed that the radio-

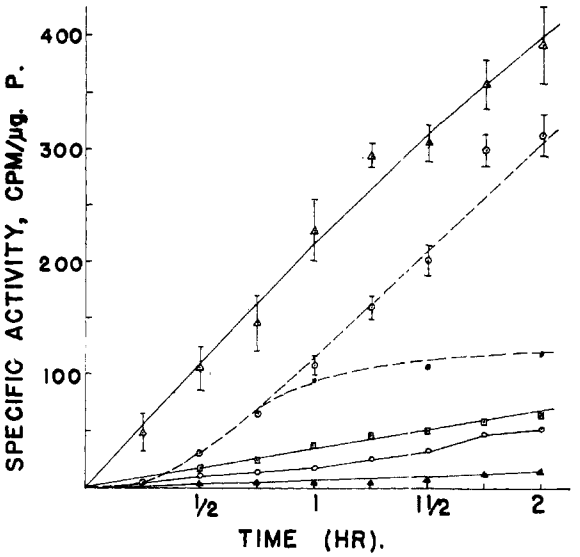

FIGURE 3: Time-course uptake curves for phospholipids of rabbit kidney cortex slices incubated with ^{32}P. For each point, 500 mg of tissue was incubated at 37° in 20 ml of Krebs–Ringer medium containing 20 μcuries of inorganic phosphate-^{32}P. △, phosphatidylinositol; ⊙, phosphatidylcholine; ●, lysolecithin fraction; ⊡, phosphatidylserine; ○, phosphatidylethanolamine; ▲, cardiolipin. Sphingomyelin was negligibly labeled. Short vertical lines through PI and PC points represent average deviation from mean in five experiments.

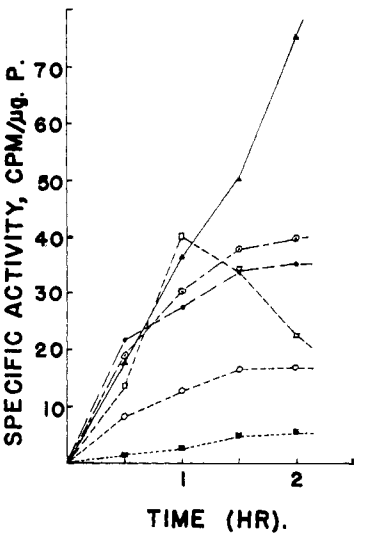

FIGURE 4: Time-course uptake curves for phospholipids of rabbit kidney cortex slices incubated with glucose-6-^{14}C. For each point, duplicate aliquots of 500 mg of tissue was incubated at 37° in 20 ml of Krebs–Ringer medium containing 10 μ curies of glucose-6-^{14}C. ▲, phosphatidylcholine; ⊙, fraction III; ●, fraction I; □, lysolecithin (fraction VI); ○, phosphatidylethanolamine; ■, sphingomyelin. Radioactivity in fraction I was found to be in glycolipids.

activity was in each case associated with the major phosphorus-containing spot, except in fraction I (Figure 1). In the latter fraction, the radioactivity was confined to the glycolipid area; cardiolipin was not labeled. It is to be noted that the time course for uptake of ^{14}C into lecithin was quite similar to the time course for ^{32}P uptake; in experiments in which slices were incubated in a medium containing both ^{32}P and glucose-6-^{14}C, the time courses for labeling of lecithin with ^{32}P and ^{14}C were exactly parallel.

The position of the ^{14}C label in the lecithin molecule was located by subjecting the doubly labeled lecithin preparations to acid hydrolysis. More than 95% of the ^{32}P and ^{14}C became water soluble after this treatment. After deionizing the aqueous hydrolysate (which removed all the ^{32}P but no ^{14}C), the deionized hydrolysate was analyzed for glycerol and counted. The results are shown in Table IV, which indicates that the molar specific activity of the glycerol was identical (within the limits of error) with that of the intact lecithin from which it was derived.

Serine-^{14}C Incorporation. In an attempt to label the nitrogenous moieties of the phosphatides, slices of renal cortex were incubated in a medium containing DL-serine-3-^{14}C. Figure 5 summarizes the results of an experiment in which 500 mg of tissue was incubated in 20 ml of medium containing 1.0 μcurie (0.19 μmole) of serine-^{14}C/ml. There was an extremely rapid labeling of phosphatidylserine, and a much slower labeling of other fractions. Radioactivity in fraction I was confined to the glycolipids.

TABLE IV: Specific Activities of Lecithin and Lecithin Glycerol[a] Preparations Labeled from Glucose-6-^{14}C and ^{32}P.

Sample	Time (hr)[b]	^{14}C (counts/min per μmole)	^{32}P (counts/min per μmole)
Lecithin 1	0.5	285.0	272.0
Lecithin 2	1.0	737.4	1515.0
Lecithin 3	1.5	1236.8	3910.0
Lecithin 4	2.0	1460.9	5550.0
Glycerol 1	0.5	240.7	0
Glycerol 2	1.0	783.9	0
Glycerol 3	1.5	1286.9	0
Glycerol 4	2.0	1569.9	0

[a] Each glycerol preparation was obtained from the lecithin preparation of the same number by acid hydrolysis as described in the text. [b] Time of incubation at 37°.

In order to determine the location of the label within the phosphatide molecules, the radioactive lipid preparations, obtained from slices of renal cortex incubated

TABLE V: Distribution of Label in Purified Phospholipids Labeled From Serine-^{14}C.

Fraction	Major Phospholipid	Components	Specific Activity (counts/min per μmole)	Ratio[a]
II	Phosphatidylethanolamine	Ethanolamine	53.6	5.76
		Glycerol	9.3	
IIIA	Phosphatidylserine + phosphatidylinositol	Serine	1256	72.7
		Glycerol[b]	17.3	
IIIB[c]	Phosphatidylserine	Serine	1570	Infinity
		Glycerol	0	
IV	Phosphatidylcholine	Choline	2.3	0.018
		Glycerol	125.1	

[a] Ratio of specific activity of nitrogenous moiety to that of glycerol. [b] Also contains inositol. [c] The third peak from the silicic acid column was divided in a different manner from that shown in Figure 1; all of the phosphatidylinositol was obtained in fraction IIIA.

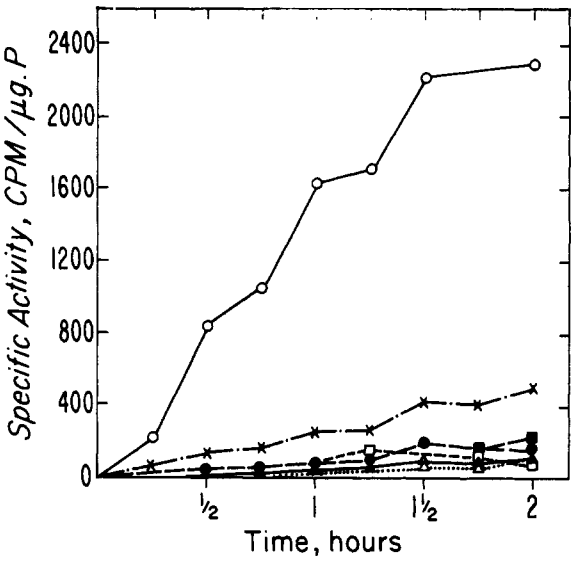

FIGURE 5: Time-course uptake curves for the incorporation of the radioactivity of DL-serine-3-^{14}C into the phospholipids of rabbit kidney cortex slices. For each point, 500 mg of tissue was incubated in 20 ml of Krebs–Ringer medium containing 20 μcuries of serine-^{14}C. O—O, phosphatidylserine; ×—·—×, total phospholipids; ■—■, sphingomyelin; □---□, lecithin; ▲—▲, phosphatidylethanolamine; △—△, phosphatidylinositol; ●---●, fraction I. The radioactivity in fraction I was found to be in glycolipids.

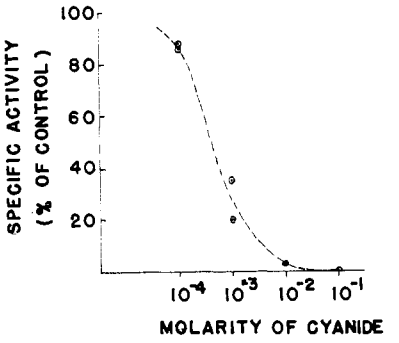

FIGURE 6: Effect of potassium cyanide on uptake of ^{32}P into total phospholipids of rabbit kidney cortex slices. For each point, 0.5 g of tissue was incubated 1 hr in 20 ml of Krebs–Ringer medium containing 20 μcuries of ^{32}P and the molarity of cyanide shown. (Duplicates at 10^{-2} M and 10^{-1} M cyanide were identical.) Specific activity of phospholipids is expressed as per cent of that found in control slices from the same rabbit, incubated 1 hr in the absence of cyanide.

1 hr in the presence of serine-^{14}C, were subjected to acid hydrolysis. After extraction of the hydrolysates with petroleum ether (bp 40–60°), the radioactivity was quantitatively recovered in the aqueous phase. Aliquots of the aqueous hydrolysates were deionized, and the deionized hydrolysates were labeled "glycerol fraction." Serine, ethanolamine, and choline were isolated from the appropriate nondeionized hydrolysates by paper ionophoresis and chromatography. The specific activities of various portions of the phospholipid molecules are shown in Table V.

The specific activity of the serine in fractions IIIA and IIIB is essentially the same; the lower ratio of specific activity of serine to "glycerol" in fraction IIIA as compared to IIIb presumably represents a small amount of labeling of the glycerol and/or inositol of phosphatidylinositol. The data show that serine-3-^{14}C is incorporated chiefly into the nitrogenous moieties of phosphatidylserine and phosphatidylethanolamine, but predominantly into the glycerol of phosphatidylcholine.

Effects of Cyanide and Ouabain. Since the preceding

data indicated a rapid replacement of the serine moiety of phosphatidylserine but only a slow replacement of the phosphorus moiety, it was considered probable that the incorporation of serine-^{14}C into phosphatidylserine represented exchange of the serine moiety independently of the phosphorus moiety. In order to investigate this hypothesis, the effects of cyanide upon incorporation of ^{32}P and serine-^{14}C into the lipids were tested. Figure 6 shows the effects of various concentrations of cyanide upon the incorporation of ^{32}P into the total phospholipids of kidney cortex slices. In subsequent experiments, 10^{-2} M cyanide was employed, a concentration at which ^{32}P incorporation into the phospholipids was almost completely abolished. This concentration of cyanide produced only a slight decrease in the total lipid phosphorus after a 1-hr incubation.

Burg and Orloff (1963) have shown that 3×10^{-4} M ouabain inhibits 40% of the active sodium–potassium transport in rabbit renal cortex slices. In order to investigate the possibility that phospholipid metabolism might be affected by changes in intracellular electrolyte concentration, or by the active transport of sodium and potassium, the effects of ouabain upon the incorporation of ^{32}P and serine-^{14}C into the phospholipids were studied. A medium containing 10^{-4} M ouabain was chosen as one which would severely inhibit active cation transport and would be expected to effect a decrease in intracellular potassium concentration.

Table VI shows the effects of cyanide and ouabain upon the incorporation of ^{32}P and serine-3-^{14}C into the total lipids of renal cortex slices (the figures for ^{14}C uptake include incorporation into the neutral lipids). Cyanide produced a striking inhibition of ^{32}P uptake, but 50% of the ^{14}C incorporation was unaffected; ouabain caused a significant stimulation of ^{32}P incorporation, and had a highly variable effect upon serine-3-^{14}C incorporation. The distribution of radioactivity (^{14}C) in various lipid fractions of control and cyanide-treated slices is shown in Table VII; it can be seen that the effect of cyanide was to decrease the incorporation of serine-^{14}C into components other than phosphatidylserine. As is evident, the labeling of phosphatidylserine was unaffected. The stimulation of ^{32}P incorporation in the presence of ouabain was not confined to any specific phospholipid, as can be seen in Table VIII.

TABLE VI: Effects of 10^{-2} M Cyanide and 10^{-4} M Ouabain upon Incorporation of Radioactivity of ^{32}P and Serine-3-^{14}C into Total Lipids of Rabbit Kidney Cortex Slices.[a]

Treatment	Specific Activity (counts/min per μg of P)	Ratio[b]	Significance (P)[c]
	P^{32} Incorporation		
Control	153.0 ± 19.2 (8)	1.00	
Cyanide	4.0 ± 0.8 (6)	0.03	<0.001
Ouabain	234.5 ± 49.6 (6)	1.53	<0.01
	Serine-3-^{14}C Incorporation		
Control	71.3 ± 9.43 (8)	1.00	
Cyanide	39.9 ± 7.27 (6)	0.55	<0.01
Ouabain	104.9 ± 22.8 (6)	1.47	>0.05

[a] Results are expressed as specific activity of total lipids ± standard deviation (number of individual experiments in parentheses). [b] "Ratio" indicates ratio of experimental specific activity to control. [c] Statistical significance was assessed by Student's t test.

TABLE VII: Distribution of Radioactivity from Serine-3-^{14}C in Various Fractions of Rabbit Renal Cortex Lipids.

Fraction	% of Total Incorporated Radioactivity: Control	% of Total Incorporated Radioactivity: 10^{-2} M Cyanide
Neutral lipids	16.1	0.7
Phosphatidylserine[a]	58.6	85.0
All other phospholipids	25.3	14.3
	100.0	100.0

[a] Specific activity of phosphatidylserine: control, 137.1 counts/min per μg of P; cyanide, 132.6 counts/min per μg of P.

Incorporation of Fatty Acids. Linoleic acid and palmitic acid were selected as a typical unsaturated and saturated acid, respectively, for use in a study of the metabolism of the fatty acyl moieties of the phosphatides. Tissue slices (500 mg) were incubated in 20 ml of

TABLE VIII: Effects of Ouabain on Specific Activities of Phospholipid Fractions from Rabbit Renal Cortex Slices Incubated with ^{32}P.

Fraction	Specific Activity (counts/min per μg): Control	Ouabain	Ratio
Total phospholipids	140.0	215.2	1.54
Cardiolipin	19.8	40.7	2.05
Phosphatidylethanolamine	19.8	44.7	2.25
Phosphatidylinositol + phosphatidylserine	196.2	556.8	2.83
Phosphatidylcholine	300.9	595.0	1.92
Sphingomyelin	8.9	11.3	1.27

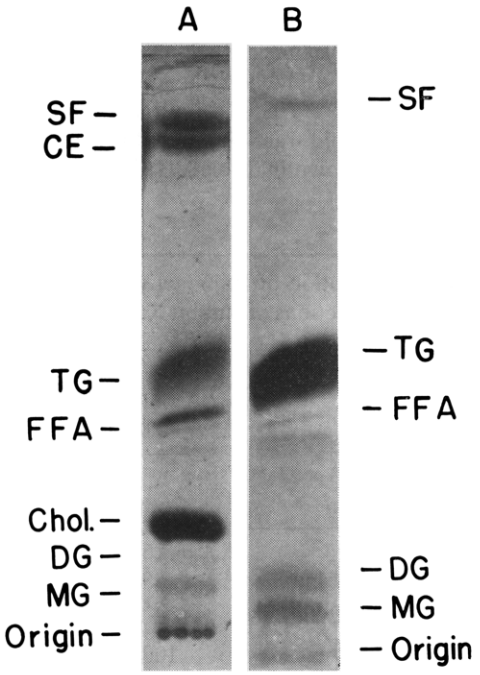

FIGURE 7: Thin layer chromatogram of rabbit kidney cortex neutral lipids on silica gel G; solvent, petroleum ether–diethyl ether–acetic acid (90:10:1, v/v). A, charring pattern of chromatogram; B, radioactivity (linoleate-1-^{14}C) as determined by radioautography. Abbreviations: SF, solvent front; CE, cholesterol esters; TG, triglycerides; FFA, free fatty acids; Chol., cholesterol; DG, diglyceride; MG, monoglyceride.

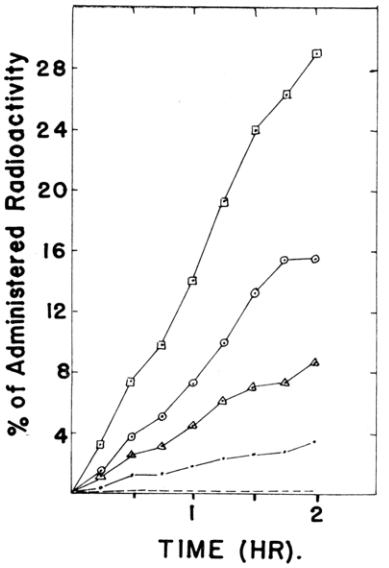

FIGURE 8: Uptake of linoleic acid-1-^{14}C into various fractions of rabbit kidney cortex lipids. For each point, 0.5 g of tissue was incubated in 20 ml of Krebs–Ringer medium containing 5 μcuries of linoleic acid-1-^{14}C (albumin complex). ⊡, total lipids; ⊙, esterified neutral lipid; ◬, lecithin; ●, other phosphatides; – – –, free fatty acids.

media containing 5 μcuries of the appropriate 1-^{14}C-labeled fatty acid as the albumin complex. This amount of radioactivity was equivalent to 1 μmole of fatty acid. A rapid uptake and esterification of both linoleic acid-^{14}C and palmitic acid-^{14}C was observed; after 2 hr, 29.1% of the administered linoleic acid-^{14}C and 12.2% of the administered palmitic acid-^{14}C, respectively, were recovered in the total lipids of the tissue slices. After separation of the lipid mixtures into various fractions, slightly over half of the incorporated counts were found in the neutral lipid fraction. Radioautography of thin layer chromatograms of the neutral lipid fractions was employed to qualitatively determine the distribution of the label. Figure 7 shows a radioautogram of the neutral lipids of tissue incubated 2 hr in the presence of linoleic acid-1-^{14}C; only a small amount of the label was found in nonesterified fatty acids, the bulk of the radioactivity being in triglycerides. In order to quantitate the radioactivity in nonesterified fatty acids, these acids were recovered from the neutral lipid mixture by the Borgström wash technique (Borgström, 1952). Figure 8 shows the distribution of administered linoleic acid-1-^{14}C in various lipid fractions. In the case of the fractions containing esterified fatty acids, aliquots were degraded by mild alkaline hydrolysis; the label was quantitatively recovered as fatty acid methyl esters. The latter were separated by gas–liquid partition chromatography, and the label was found to coincide with the methyl linoleate peak. Essentially the same results were obtained using palmitic acid-1-^{14}C, except that the total recovery of administered label was smaller. A rapid uptake and esterification of each fatty acid, chiefly into triglycerides and lecithin, was observed.

In Figures 9 and 10 are shown the specific activities of linoleic and palmitic acid in individual phospholipid fractions, calculated from the fatty acid composition of the various fractions. There was much less variation in the specific activity of palmitic acid among the phospholipids than was the case with linoleic acid; evidently palmitic acid is replaced at approximately equal rates in various phospholipids, while linoleic acid is replaced much faster in phosphatidylcholine than in other phosphatides. It is interesting that there is virtually no labeling of the linoleic acid of the cardiolipin fraction, although this acid comprises 60% of the fatty acids of this fraction (Morgan *et al.*, 1963b). Together with the low incorporation of ^{32}P and glucose-6-^{14}C into cardiolipin, these data suggest that the cardiolipin component is metabolically inert. The high specific activity of palmitic acid in fraction I was quite surprising; it is considered that this incorporation was into the glycolipids rather than the cardiolipin, but this could not be checked by radioautography due to the very low absolute levels of radioactivity.

In view of the positional specificity in the fatty acids esterified in renal cortex lecithin, as reported by Morgan

TABLE IX: Fatty Acid Composition of Lecithins from Rabbit Kidney Cortex Slices.[a]

Incubation Time (hr)	Chain Length: No. of Double Bonds									
	14:0	15:0	16:0	16:1	18:0	18:1	18:2	20:4	Other	Total Unsat.
					Total Fatty Acids					
Fresh control	2.2	1.7	21.7	5.3	13.5	13.7	28.0	11.3	2.6	60.2
0.5	2.6	1.8	21.6	5.3	13.3	13.9	28.3	11.0	2.6	59.4
1	2.7	1.7	22.2	6.3	13.1	13.3	28.2	11.6	1.0	60.1
1.5	2.2	2.1	19.7	5.7	14.5	14.0	25.7	11.7	3.3	61.2
2	2.2	1.3	19.1		14.8	14.2	26.9	12.2	3.6	60.2
					β Fatty Acids					
Fresh control	1.7	0.9	13.8	4.2	2.4	16.6	41.7	15.8	3.4	81.2
0	2.0	1.0	13.9	5.2	2.2	15.3	39.6	17.4	3.4	80.8
0.5	1.7	0.8	13.4	3.4	1.3	15.0	44.9	19.5	—	82.8
1	1.5	0.6	12.7	3.7	2.1	12.1	37.7	29.6	—	83.1
2	1.9	1.0	13.6	4.7	1.5	13.0	42.0	21.6	0.7	82.0

[a] All figures are given in mole per cent.

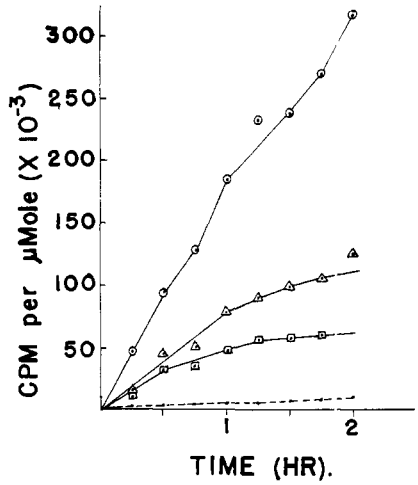

FIGURE 9: Specific activities of linoleic acid-1-^{14}C esterified in various phospholipid fractions of rabbit kidney cortex. For each point, 0.5 g of tissue was incubated with 20 ml of Krebs–Ringer medium containing 5 μcuries of linoleic acid-1-^{14}C (albumin complex). ⊙, phosphatidylcholine; △, phosphatidylethanolamine; ⊡, phosphatidylserine plus phosphatidylinositol; – – –, cardiolipin. Only a negligible amount of label was found in sphingomyelin and lysolecithin.

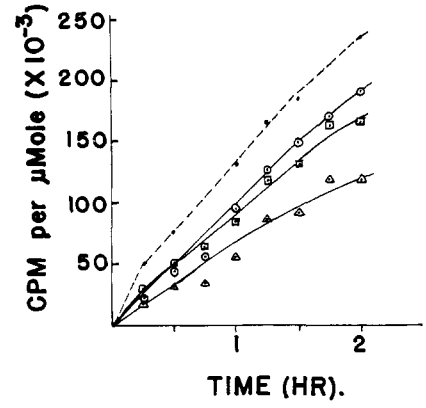

FIGURE 10: Specific activities of palmitic acid-1-^{14}C esterified in various phospholipid fractions of rabbit kidney cortex. For each point, 0.5 g of tissue was incubated with 20 ml of Krebs–Ringer medium containing 5 μcuries of palmitic acid-1-^{14}C (albumin complex). – – –, cardiolipin; ⊙, phosphatidylcholine; ⊡, phosphatidylserine (plus phosphatidylinositol); △, phosphatidylethanolamine. Only a negligible amount of label was found in sphingomyelin and lysolecithin.

et al. (1963b), it was of interest to determine whether there was any metabolic difference between the α' and β positions of phosphatidylcholine. Accordingly, the lecithins labeled with ^{14}C in the fatty acids (linoleic or palmitic) were degraded with phospholipase A as described earlier (Morgan *et al.*, 1963b) and the reaction products separated by chromatography on silicic acid columns. Fatty acids released from the β position were eluted from the columns with chloroform, and the polar products were eluted with methanol. The lipid phosphorus of the starting material was quantitatively recovered in the methanol eluate, which contained only lysolecithin, as can be seen in Figure 11; the original lecithin was totally hydrolyzed.

In Table IX is shown the distribution of individual fatty acids among the total and β-esterified fatty acids of renal cortex lecithin, as determined by gas–liquid partition chromatography of their methyl esters. Except for a slight and possibly insignificant increase in β-

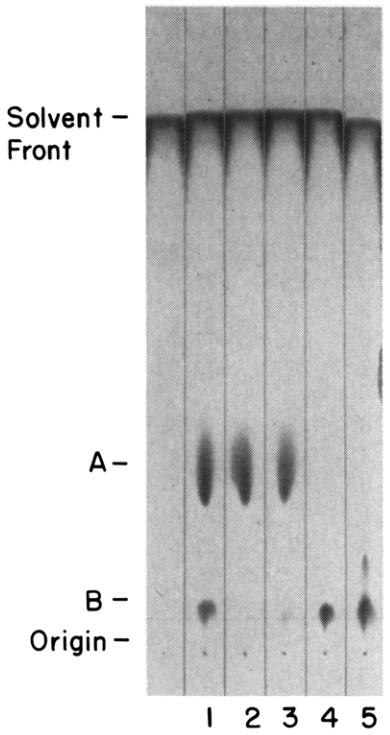

FIGURE 11: Thin layer chromatogram of products of hydrolysis of renal cortex lecithin with phospholipase A on silica gel G. Solvent, C–M–W (95:35:4, v/v); 1, standard mixture of lecithin (A) and lysolecithin (B); 2 and 3, preparations of renal cortex lecithin; 4 and 5, same preparations after hydrolysis with phospholipase A and separation of fatty acids. It is not known what caused the streaking of the lysolecithin spot in channel 5.

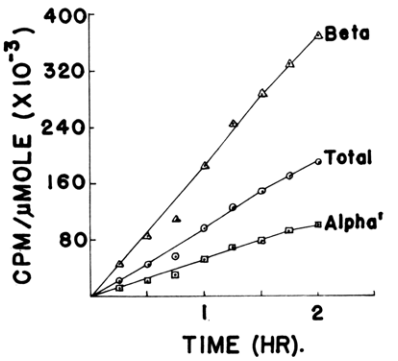

FIGURE 12: Specific activities of β-esterified, α'-esterified, and total palmitate in phosphatidylcholine labeled *in vitro* with palmitic acid-1-^{14}C.

TABLE X: Specific Activities of Linoleic Acid Esterified in Lecithin of Rabbit Kidney Cortex Slices Incubated with Linoleic Acid-1-^{14}C.

Incubation Time (hr)	Specific Activity (counts/min per μmole)		
	Total	α' Esterified	β Esterified
0.25	46,870	45,941	47,176
0.50	92,891	91,058	93,497
0.75	128,320	125,792	129,159
1.00	188,091	184,384	189,325
1.25	237,340	232,666	238,895
1.50	242,753	237,965	244,341
1.75	269,907	264,586	271,674
2.00	318,720	312,445	320,806

esterified arachidonic acid, there was no effect of incubation upon the positional specificity of the fatty acids esterified in renal cortex lecithin. The β position was predominantly (80%) esterified with unsaturated fatty acids, and of the remaining 20% saturated acids, palmitic acid was the predominant species.

Lecithin preparations labeled with linoleic acid-1-^{14}C or palmitic acid-1-^{14}C were degraded with phospholipase A, the α' and β fatty acids were isolated, and the specific activities of α'- and β-esterified linoleic acid or palmitic acid were determined. The results are presented in Table X and Figure 12. In Table X, it can be seen that the specific activities of α'- and β-esterified linoleic acid of lecithin were about the same when slices were incubated in the presence of linoleic acid-1-^{14}C; however, there was a tendency for β-esterified linoleic acid to have a higher specific activity. The results for lecithins of tissue incubated with palmitic acid-1-^{14}C are shown in Figure 12; the specific activity of β-esterified palmitic acid rose much faster than that of α'-esterified palmitate, indicating that the palmitic acid in the β position is "turned over" much faster than that in the α' position.

Discussion

The present data indicate that, with the exception of cardiolipin and sphingomyelin, all the phospholipids of rabbit renal cortex slices are metabolically active; the former two lipids seem to be metabolically inert and probably play a structural role in this tissue. The general conclusion that can be drawn is that the various phospholipid fractions exhibit marked differences in metabolic rates. Thus the serine moiety of phosphatidylserine exhibits quite a rapid turnover, although there is not an appreciable phosphorus turnover in this lipid. Moreover, the effects of cyanide upon serine-^{14}C incorporation indicated that the exchange of the serine moiety of phosphatidylserine is not dependent on a supply of metabolic energy. Evidently the serine moiety may be exchanged independently of the rest of the molecule. The phosphorus of phosphatidylethanolamine is also quite stable metabolically; nevertheless, there is a small but significant labeling of the ethanolamine moiety when serine-3-^{14}C is administered as a precursor. This may be due to the direct decarboxylation

of phosphatidylserine, as described by Borkenhagen *et al.* (1962). An appreciable labeling of phosphatidylethanolamine from radioactive palmitic acid and linoleic acid was also observed; since this is not accompanied by exchange of phosphorus, it probably does not represent *de novo* synthesis, but rather a fatty acid exchange mediated by a phospholipase and the acyl transference enzyme of Merkl and Lands (1963).

The phosphorus moiety of phosphatidylinositol has been shown to become labeled quite rapidly from inorganic ^{32}P, but the present data do not indicate whether this represents *de novo* synthesis or merely an exchange of part of the molecule. Hauser (1963) has shown that kidney tissue carries out a fairly rapid synthesis of lipid inositol from glucose-^{14}C, but his data cannot be directly compared to the present data on phosphorus turnover in this lipid. It is likely, however, that both the inositol and phosphorus are in a state of metabolic flux. A significant incorporation of both palmitic acid and linoleic acid into the phosphatidylinositol–phosphatidylserine fraction of renal cortex lipids was observed in the present study, and, in experiments in which this fraction was split into inositol- and serine-containing portions, both these lipids were labeled to approximately the same degree; thus there is some evidence that *de novo* synthesis of phosphatidylinositol may occur, although data on turnover of the glycerol moiety are lacking.

In the phosphatidylcholine fraction, the fatty acids, glycerol, and phosphorus moieties have all been shown to be metabolically active, and it is concluded that considerable catabolism and *de novo* synthesis occur. There are two known metabolic pathways by which lecithin may be synthesized *de novo* in mammalian tissues. One of these is the classic reaction described by Kennedy and Weiss (1956), involving the reaction of D-1,2-diglyceride with cytidine diphosphate choline (CDP-choline); the other pathway involves N methylation of phosphatidylethanolamine (Bremer *et al.*, 1960; Gibson *et al.*, 1961; Bremer and Greenberg, 1961; Artom, 1964). The methyl donor may be *S*-adenosylmethionine. This pathway can be tentatively ruled out as an important route of lecithin biosynthesis in renal cortex on the basis of the present data on serine-3-^{14}C uptake. Conversion of serine-3-^{14}C to lipid serine, ethanolamine, and glycerol was observed; had phosphatidylethanolamine been a precursor of phosphatidylcholine, the ratio of specific activities of choline:glycerol in the latter lipid would have been expected to be the same as the ratio of specific activities of ethanolamine:glycerol in the former lipid. Since this was not the case, the methylation pathway is probably only of minor importance as a route of lecithin biosynthesis. The very low incorporation of ^{32}P into phosphatidylethanolamine as compared to phosphatidylcholine would tend to support this conclusion. The present data for renal cortex slices are similar to the data of Ansell and Spanner (1962) for rat brain *in vivo;* in brain tissue, the pattern of labeling of phosphatidylethanolamine and phosphatidylcholine from serine-3-^{14}C was similar to that reported here for renal cortex slices.

One facet of phosphatidylcholine metabolism remains to be elucidated, namely, whether a metabolic inhomogeneity exists in the lecithin pool. A recent paper by Wurster and Copenhaver (1965) indicates that lecithins of rabbit renal cortex esterified with two saturated fatty acids may turn over much faster than other species. These authors incubated rabbit renal cortex slices for 1 hr in a ^{32}P-containing medium and isolated the labeled lecithin. The latter was subjected to reductive ozonolysis, and the oxidized "cores" were separated by thin layer chromatography. In this manner, ^{32}P-labeled "cores" corresponding to the following four types of lecithin were obtained: (i) disaturated, (ii) α'-saturated, β-unsaturated, (iii) α'-unsaturated, β-saturated, (iv) diunsaturated. The disaturated lecithins, which passed through the ozonolysis unchanged, had a specific activity twice that of the other types. However, from the distribution of phosphorus in the four "cores," it was calculated that the original lecithin must have been predominantly unsaturated in the α' position, whereas the present data, using phospholipase A to release the β-esterified acids, clearly show that it is the β position which is unsaturated. Considerable doubt is cast on one or other of the experimental methods by this discrepancy. It seems conclusively established (Tattrie, 1959; DeHaas and van Deenen, 1961; Hanahan *et al.*, 1961; Long *et al.*, 1963) that phospholipase A specifically releases the β fatty acids of L-α-lecithins. Moore and Williams (1964) showed that there are differences in the rates at which various lecithins are attacked by phospholipase A; however, in the present study 100% hydrolysis of lecithin to lysolecithin was achieved, and errors due to differential hydrolysis of the β fatty acids would not enter into the results. Thus the results obtained with phospholipase A would seem to give a true idea of the positional specificity of lecithin. Nevertheless, the methods used by Wurster and Copenhaver also seem valid, and the discrepancy in the results remains unexplained.

In addition to the observations on the turnover of the glycerol and phosphorus moieties of the lecithin, evidence has been obtained in the present study for a turnover of β-esterified palmitic acid independently of the rest of the molecule. If lecithin is biosynthesized by the Kennedy–Weiss (1956) pathway, the fatty acids would be esterified to the glycerol at the level of phosphatidic acid, which is dephosphorylated to give D-1,2-diglyceride. Thus if a ^{14}C-labeled fatty acid is administered, for example, linoleic acid, then linoleic acid at both the α' and β positions of lecithin would have the same specific activity. The latter result would be irrespective of the amount actually esterified in each position, since fatty acids in both α' and β positions arise from the same pool of free fatty acids. Only if the fatty acids at one position turn over faster than, or independently of, those in the other position would there be a difference in specific activities. The present data indicate that β-esterified palmitic acid but not linoleic acid turns over faster than the α'-esterified acid. The enzymatic basis for this exchange would seem to be the combined action of a phospholipase A and the

acylating enzyme of Lands (1960); the former enzyme is known to be present in renal tissue (Contardi and Ercoli, 1933; Robertson and Lands, 1962; Gallai-Hatchard and Thompson, 1965). It is presumed that transacylase activity does exist in renal cortex; if so, it must have a specificity for esterification of the β position of lysolecithin with palmitic acid in order to account for the specific exchange of β-esterified palmitic acid in lecithin. This would be in striking contrast to the specificity of the liver enzyme.

It seems improbable that phospholipid turnover has any direct relation to active cation transport in this tissue, as postulated by Wurster and Copenhaver (1965). Thus Davies and Galston (1951) found that the turnover time for potassium (^{42}K) in rabbit renal cortex slices was 7 min. Since the intracellular potassium content of such slices is about 50 μequiv/g (Tinker *et al.*, 1963), this gives a turnover rate of 430 μmoles/g of tissue per hr; this rate would seem to be much higher than the rates of synthesis of radioactive phospholipids observed in the present study. Further, in the present studies, under conditions in which cation transport would be strongly inhibited, *i.e.*, in the presence of 10^{-4} M ouabain, the turnover of lipid phosphorus was increased in a nonspecific manner. This conclusion agrees with those of Nelson and Cornatzer (1964), who could find no evidence for the participation of phospholipid turnover in sodium transport by the kidney *in vivo*.

It is possible that the lecithin of rabbit renal cortex may participate in fatty acid oxidation by this tissue. Gold and Spitzer (1964) found that, *in vivo*, dog kidney preferentially took up and oxidized plasma free palmitic acid; oleic, linoleic, and stearic acid were not oxidized. On the basis of Gold and Spitzer's data on palmitic acid uptake, and Smith's data (1951) on renal plasma flow in dogs, it is calculated that the turnover rate for renal palmitic acid in fasted dogs is 1.86 μmoles/g of tissue/hr, assuming complete oxidation of the palmitic acid taken up. This is probably in the range of the turnover rate for β-esterified palmitic acid in rabbit renal cortex lecithin. It is proposed that the hydrolysis of lecithin by mitochondrial phospholipase A (Scherphof and van Deenen, 1965; Rossi *et al.*, 1965) and reacylation of the resulting lysolecithin with palmitic acid by extramitochondrial transacylases (Lands, 1960) provide a cyclic "carrier" mechanism which facilitates the diffusion of palmitic acid to the site of oxidation. This proposal would relate the rapid exchange of β-esterified palmitic acid of lecithin to the high rate of palmitic acid oxidation by renal cortex; linoleate, which is apparently not oxidized preferentially by this tissue, would not participate in the cycle, and hence a rapid exchange of β-esterified linoleic acid does not take place. This hypothesis has an interesting precedent in that Fritz and Yue (1963) found that palmitoylcarnitine rather than palmitoyl coenzyme A could more readily contribute its acyl group to the fatty acid oxidase system of heart muscle mitochondria *in vitro*. These authors suggested the same function for carnitine that is here proposed for lysolecithin, namely, that it serves as a carrier in the transport of fatty acids into the mitochondria. It is of interest that, like lecithin, carnitine also has a quaternary ammonium group; this similarity may be more than coincidental.

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The Glycolipids of Dog Intestine*

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ABSTRACT: Preparative silicic acid chromatography of whole lipid extracts of dog intestine gave three fractions which contained glycolipid. The largest fraction contained all of the ceramide oligoglycosides and these were separated from phospholipid and other impurities by solvent fractionation, Florisil chromatography, and dialysis. The ceramide oligoglycosides were then separated into ganglioside and ceramide oligohexoside fractions by DEAE-cellulose chromatography. The latter was partially resolved by cold methanol fractionation followed by silicic acid chromatography. The major component, a ceramide pentaglycoside, was recovered in pure form on the basis of column and thin layer chromatography and on analysis. This is a novel substance giving analytical data and hydrolytic cleavage products consistent with an *N*-acylgalactosaminyl-*N*-acylgalactosaminylgalactosylgalactosylglucosylceramide structure. It contains 30% of the total intestine lipid sugar and most of the galactosamine. The ganglioside fraction was partially resolved on a silicic acid column, yielding a chromatographically homogeneous ganglioside as the major component. Analyses of this substance were consistent with a sialyldigalactosylglucosylceramide structure and with a minor glucosamine-containing impurity.

The sequence of sugars and nature of the long-chain base are not known. Both the ganglioside and the pentaglycoside contain predominantly long-chain fatty acids with average molecular weights of 338 and 321, respectively.

Studies on the chemistry and metabolism of the glycolipids of vertebrate tissues have been largely confined to those of nervous tissues, erythrocyte stroma, spleen, and kidney. All glycolipids isolated from these sources appear to be glycosides of a ceramide with glucose, galactose, galactose sulfate ester, lactose, or oligosaccharides which may contain in addition *N*-acylhexosamine or sialic acid or both. The chemistry and metabolism of these substances have been reviewed (Law, 1960; Svennerholm, 1964; Carter *et al.*, 1965). In this laboratory studies have been carried out on the composition of glycolipids of intestine and liver. These tissues contain some unique ceramide glycosides as well as other lipids containing fatty acyl primary amines. This paper describes the preparation of the ceramide oligoglycoside[1,2] fraction of dog intestine and the isolation and composition of the major component. This substance is a ceramide pentaglycoside which contains two *N*-acylgalactosamines, two galactoses, and one glucose in that order on the ceramide. A description of the isolation and composition of another major glycolipid component, a ganglioside, is also included in this paper.

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[1] In this paper the following terminology is used: "ceramide oligoglycoside" is a ceramide glycoside containing more than three sugar residues. "Ganglioside" is a glycolipid which contains sialic acid. "Ceramide oligohexoside" is a ceramide oligoglycoside which does not contain sialic acid. The composition of all solvent mixtures is expressed on a volume per volume basis.

[2] The following abbreviations are used in this paper: tlc, thin layer chromatography; CPG, ceramide pentaglycoside.