Incorporation of fatty acids into phospholipids of erythrocyte membranes

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SUMMARY Incorporation of C^{14}-labeled fatty acids into phospholipids by washed ghosts, prepared from human erythrocytes, required the addition of ATP and was markedly stimulated by $MgCl_2$ and coenzyme A. Labeled palmitic acid from 1-C^{14}-palmitoyl CoA was incorporated in the absence of added cofactors. The extent of incorporation of linoleic acid was greater than that of oleic, palmitic, or stearic acids. Of the radioactive fatty acid taken up into phospholipids 80–90% was found in the lecithin fraction. Degradation of the lecithin with *Crotalus adamanteus* venom established that the labeled fatty acid was located entirely in the β-position. When whole blood was incubated with labeled fatty acid it was also found that most of the radioactivity incorporated into the ghost phospholipids was in the β-position of lecithin.

Rat erythrocyte ghosts incorporated C^{14}-linoleic acid into phospholipids at about the same rate as did human erythrocyte ghosts. Sheep erythrocyte ghosts (which contain little lecithin) incorporated very little fatty acid into phospholipids, and none into lecithin.

WHEN PRELIMINARY studies of the incorporation of C^{14}-labeled fatty acids into complex lipids by human red blood cell membranes (ghosts) were carried out using conditions similar to those suitable for demonstration of triglyceride synthesis in homogenates of liver (2–4) or of adipose tissue (5, 6), relatively little radioactivity was recovered in triglycerides, but considerably more was found in phospholipids. Uptake of fatty acids into the phospholipid fraction did not require, and was not stimulated by, the addition of α-glycerophosphate. The studies reported below were undertaken in order to define the mechanism by which the erythrocyte membranes incorporated fatty acids into phospholipid. It was found that most of the labeled fatty acid in human ghost phospholipids had been incorporated into the β-position of lecithin, presumably by reaction of fatty acyl coenzyme A derivatives with lysolecithin contained in the ghosts. Van Deenen and co-workers have recently reported similar findings (7, 8). This pathway probably accounts for a large part of the turnover or exchange of fatty acids in phospholipids of circulating erythrocytes. Whether these reactions play a role in membrane function (e.g., by determining the relative amounts of lysolecithin and lecithin in the membrane, or by effecting changes in the fatty acid composition of the membrane lecithins) remains to be determined.

METHODS

Blood from several different normal fasting donors who had been on an unrestricted diet was collected in sodium citrate or citric acid–dextrose anticoagulant mixtures.[1] The plasma and buffy coat were removed after centrifugation, and the erythrocytes were washed three times with 0.154 M sodium chloride solution. Hemolysis was carried out in 10^{-4} M ethylenediaminetetraacetic acid, adjusted to pH 7.4 with tris(hydroxymethyl)aminomethane. Ghosts, collected by centrifugation for 15 min at 15,000 × *g*, were washed with the solution employed for hemolysis to which was added 0.017 M sodium chloride. Usually 5 to 7 washes were required before the supernatant fluid was free of hemoglobin color. Hemolysis and washings were carried out at 4°. Ghosts were stored at −20° without loss of activity. Thus, a single preparation could be used for several days.

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[1] The procedure described for preparing ghosts was devised by Dr. Joseph Hoffman, who kindly provided ghosts for preliminary experiments.

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In most experiments ghosts from human erythrocytes were incubated with C^{14}-labeled fatty acid, CoA,[2] ATP, and $MgCl_2$ as indicated in Table 1. Flasks were set up in duplicate and all values reported are the means of data from duplicate flasks. At the end of the incubation period, 15 ml of a chloroform–methanol solution (2:1, v/v) were added. The contents of the incubation flask were then transferred to a separatory funnel, using 20 ml of extraction mixture to rinse the flask. After 30 or more min, 15 ml of water was added and the phases were allowed to separate overnight at room temperature. A sample of the chloroform phase was taken for radioassay and the remainder dried under nitrogen. The lipids were dissolved in 10 ml of chloroform which was then shaken with 0.15 g activated (18 hr at 85°) silicic acid. Phospholipids were adsorbed on the silicic acid and after centrifugation the supernatant solution, containing neutral lipids, was decanted. This chloroform solution plus two chloroform washes of the silicic acid were pooled and dried. The residue was dissolved in isooctane and free fatty acids were separated from glycerides by extraction of the former into alkaline ethanol (9). Phospholipids were eluted from the silicic acid with methanol. Samples were taken for radioassay and for phosphorus determination (10).

In order to separate specific phospholipids, chromatography on silicic acid-impregnated paper (Whatman No. 1) with diisobutyl ketone–acetic acid–water, 40:30:1 (v/v/v), for 18 hr at room temperature (11) was employed. Lipids were detected by inspecting papers under ultraviolet light after they had been stained with Rhodamine 6G. Ninhydrin and phosphomolybdic acid–stannous chloride reagents were used for detection of specific phosphatides (12). For assay of radioactivity, chromatograms were divided parallel to the solvent front into 1–2 cm segments. In experiments with C^{14}-labeled palmitoyl coenzyme A, lecithin was isolated for radioassay by chromatography of the phospholipid fraction from silicic acid on alumina (13). Alumina columns were also used to prepare lecithin for enzymatic degradation. Lecithin prepared in this fashion was dissolved in 1 ml diethyl ether. A solution (0.01 ml) containing 50 μg of lyophilized *Crotalus adamanteus* venon and 0.05 μmoles of calcium chloride was added (14, 15). After incubation for 60 min at 37°, 2 ml of ethanol was added, the mixture was dried under nitrogen, and the lipids were dissolved in chloroform. Free fatty acids and lecithin or lysolecithin were separated on silicic acid columns or on silicic acid-impregnated paper.

[2] Abbreviations used are CoA, coenzyme A; ATP, adenosine triphosphate; CDP, cytidine diphosphate; CTP, cytidine triphosphate; P, phosphorus, except in Table 3.

TABLE 1 INCORPORATION OF PALMITIC ACID-1-C^{14} INTO LIPID FRACTIONS OF HUMAN ERYTHROCYTE GHOSTS*

Expt. No.	Incubation Time	C^{14} Radioactivity in: Phospholipids	Neutral Lipids
	min	*cpm*	*cpm*
1	0	400	394
	60	3552	603
2	0	552	380
	60	4232	530
3	0	532	800
	60	3955	1200
	60 (boiled)†	712	774

* The reaction mixture contained 6.25 mμmoles (ca. 100,000 cpm) of palmitate-1-C^{14} (K salt), 2 μmoles of ATP, 0.1 μmole of CoA, 3 μmoles of $MgCl_2$, 125 μmoles of buffer phosphate, and 0.4 ml of ghosts, in a total volume of 3.0 ml. Incubation was carried out at 37°.

† Ghosts heated for 10 min at 100°.

In one group of experiments, 1 ml of whole human blood (freshly drawn with sodium citrate as anticoagulant) was incubated at 37° in an atmosphere of 100% oxygen after adding 0.1 ml of a solution containing labeled fatty acid, 3 mg bovine serum albumin, and 0.2 mg glucose. At the end of the incubation period, 0.1 ml of 0.625 M sodium fluoride was added and the flasks were placed in an ice bath. The contents of the flasks were then transferred to chilled centrifuge tubes. Red cells were collected and hemolyzed, ghosts were washed, and the phospholipids isolated as described above.

C^{14} radioactivity was determined in a Tri-Carb liquid scintillation counter (Packard Instrument Co., La Grange, Ill.) with samples dissolved in, or pieces of chromatograms suspended in, 15 ml of toluene containing diphenyloxazole, 5 mg/ml.

C^{14}-labeled fatty acids were purchased from Nuclear Chicago Co., Chicago, Ill. ATP and CoA from Pabst Laboratories, Milwaukee, Wis. C^{14}-palmitoyl coenzyme A was kindly provided by Dr. DeWitt S. Goodman, who prepared it. Silicic acid for columns and batch separations was purchased from Bio-Rad Laboratories, Richmond, Calif. Silicic acid-impregnated papers (11) were prepared with silicic acid (100 mesh) from the Mallinckrodt Chemical Works, St. Louis, Mo.

RESULTS

Experiments with Human Erythrocyte Ghosts

When ghosts were incubated with 1-C^{14}-palmitic acid, ATP, CoA, and $MgCl_2$, incorporation of radioactivity into phospholipids was many times greater than into neutral lipids (Table 1). Essentially no radioactivity was incorporated by boiled ghosts similarly incubated.

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TABLE 2 EFFECT OF COFACTORS ON INCORPORATION OF C^{14}-LABELED FATTY ACIDS INTO PHOSPHOLIPIDS OF MEMBRANES FROM HUMAN ERYTHROCYTES

Additions	Relative Incorporation into PL* of: Palmitate-1-C^{14}	Linoleate-1-C^{14}
Complete system†	100	100
Buffer alone	7	4
Complete system minus CoA	43	
Complete system minus $MgCl_2$	58	
Complete system minus ATP	4	3
Complete system minus ATP plus 2 μmoles CTP		5
Complete system minus ATP plus 4 μmoles CTP		13
Complete system plus 0.9 μmoles CoA		102
Complete system plus 2 μmoles ATP	96	96
Complete system plus 2 μmoles CTP	93	131
Complete system plus 2 μmoles CTP plus 1 mg CDP choline		126
Complete system plus 2 μmoles CTP plus 1 mg CDP choline plus 10 μmoles α-GP		118
Complete system plus 2 μmoles CTP plus 10 μmoles α-GP		124
Complete system plus 10 μmoles α-GP	97	

* PL, phospholipids.

† Each flask contained 0.4 ml ghosts, a tracer amount (<0.01 μmoles) palmitate-1-C^{14} or linoleate-1-C^{14} (potassium salt), 2 μmoles ATP, 3 μmoles $MgCl_2$, 0.1 μmole CoA and 125 μmole potassium phosphate buffer, pH 7.0 in a total volume of 3 ml. Incubation carried out in 25-ml Erlenmeyer flasks for 60 min at 37°. Incorporation determined as described the Methods. Value for relative incorporation based on data from 2–4 experiments (in duplicate).

Table 2 summarizes data from several experiments in which the effects of various cofactors on the incorporation of C^{14}-labeled palmitic or linoleic acid into phospholipids was examined. Omission of either coenzyme A or magnesium chloride markedly decreased but did not abolish incorporation. There was no incorporation in the absence of ATP even when an equimolar amount of CTP was substituted for it. Addition of 2 μmoles of CTP in the presence of ATP was usually associated with a small (approximately 20%) increase in incorporation of labeled fatty acid into phospholipids. The addition of α-glycerophosphate with or without CTP and CDP choline did not influence incorporation.

As shown in Fig. 1, the amount of labeled fatty acid, in this case linoleic acid-1-C^{14}, incorporated into phospholipids was a linear function of the volume of ghosts added over the range of 0.2–0.8 ml. (The dry weight, after lyophilization, of several different preparations of human ghosts was 10–11 mg/ml.)

With 0.4 ml of ghosts and addition of cofactors as described in Table 2, incorporation of palmitate-1-C^{14} into the phospholipid fraction proceeded at a constant rate for at least 60 min at 37° (Fig. 2). At 25° incorporation was considerably slower. The time course of incorporation at 50° did not differ greatly from that at 37°. The deviation from linearity at 50° is probably significant, however, since in several different experiments, the data from incubations at 37° have fitted so closely a straight line.

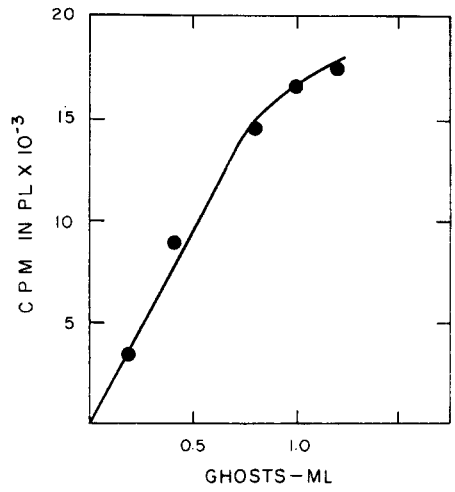

FIG. 1. Incorporation of linoleic acid-1-C^{14} into ghost phospholipids as a function of volume of ghosts added. Incubation conditions as described in the legend for Table 2.

Incorporation of C^{14}-labeled palmitate or linoleate into the phospholipid fraction was markedly inhibited by cupric chloride: 88% by 3×10^{-5} M, 90% by 2×10^{-3} M. In the same concentrations calcium chloride was without effect, as shown in Table 3. Sodium fluoride did not inhibit, even at very high concentration. A small percentage inhibition was consistently observed with mercaptoethanol. On the other hand, cysteine was

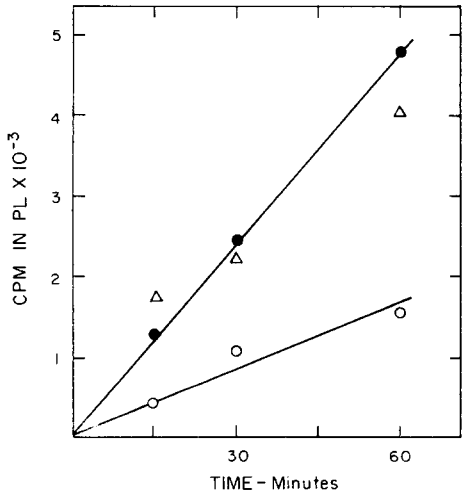

FIG. 2. Incorporation of palmitic acid-1-C^{14} into ghost phospholipids as a function of incubation time at three different temperatures. Conditions as described in the legend for Table 2. ○ = 25°, ● = 37°, △ = 50°.

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without effect in some studies and in others seemed to be slightly inhibitory. Iodoacetate, *p*-chloromercuribenzoate or N-ethylmaleimide at 10^{-4} M had little or no effect. Phloridizin, 30 or 300 μg/ml, did not influence incorporation of palmitate-C^{14} into phospholipids. In one experiment chlorpromazine, 10^{-3} M, inhibited incorporation by 25%.

Linoleic acid was incorporated much more rapidly than was palmitic acid into ghost phospholipids when each was added in the same tracer amount (6.25 mμmoles). In several instances more than 50% of the added linoleate-C^{14} was recovered in phospholipids after a 1 hr incubation. Under similar conditions, about 5–6% of the labeled palmitate was incorporated. The fraction of oleic acid incorporated was slightly greater but utilization of these two acids was never compared directly in the same experiment. In a single experiment stearic acid was incorporated less well than palmitic acid. After incubation with either labeled linoleate or palmitate, 80–90% of the total phospholipid radioactivity was recovered with lecithin after chromatography (Table 4). The fractions of the total radioactivity contained in phosphatidylethanolamine (approx 4%), sphingomyelin (approx 3%), or phosphatidylserine (approx 1%) were independent of the labeled fatty acid used. The distribution of radioactivity among the phospholipid classes was not altered when CTP and/or α-glycerophosphate was added. No phosphatidic acid spot was seen on chromatograms stained with Rhodamine 6G, nor was any radioactivity detected in the area where phosphatidic acid is usually found. Lysolecithin was not detected on these chromatograms.

Lecithin, purified from ghosts that had been incubated with radioactive palmitate or linoleic acid, was hydrolyzed enzymatically with phospholipase A from snake venom. As shown in Table 5, virtually all of the radioactivity present in the purified lecithin was recovered in the free fatty acid fraction after incubation with snake venom, indicating that the labeled fatty acid, linoleate or palmitate, had been incorporated entirely into the β-position of lecithin (16–18).

This finding suggested that incorporation resulted from esterification of lysolecithin with the labeled fatty acid, the latter presumably in the form of the fatty acyl coenzyme A derivative. As shown in Table 6, incorporation of radioactive palmitate from 1-C^{14}-palmitoyl CoA into phospholipids proceeded in the absence of added cofactors whereas there was essentially no incorporation of the free fatty acid in the absence of ATP and CoA.

Addition of lysolecithin (prepared from rat liver lecithin with snake venom) to the incubation medium

TABLE 3 INHIBITION OF INCORPORATION OF LABELED FATTY ACIDS INTO GHOST PHOSPHOLIPIDS*

Additions		Relative Incorporation	C^{14}-Fatty Acid Incorporated†
	μmoles/3 ml		
None		100	P, L
Cupric Chloride	0.1	12	P, L
	5	4	P, L
Calcium Chloride	0.1	109	P, L
	5	108	P, L
Sodium Fluoride	0.1	112	P
	5	107	P
	42	105	P
Mercaptoethanol	0.1	75	L
	5	72	L
Cysteine	0.8	107	P
	0.8	74	P

* Incubation conditions as described in Table 2.
† P = palmitic acid-1-C^{14}, L = linoleic acid-1-C^{14}.

TABLE 4 INCORPORATION OF C^{14}-LABELED PALMITATE AND LINOLEATE INTO SPECIFIC PHOSPHOLIPIDS BY GHOSTS*

Phospholipid†	C^{14} Radioactivity from: Linoleic Acid	Palmitic Acid
	cpm	*cpm*
Sphingomyelin	325	59
Lecithin	10,697	1239
Phosphatidylserine	190	51
Phosphatidylethanolamine	467	157

* Samples of a single preparation of ghosts were incubated with 6.25 mμmoles (1.6×10^4 cpm/mμmole) of either palmitate-1-C^{14} or linoleate-1-C^{14} as described in Table 2. Phospholipids were separated on silicic acid-impregnated paper as described in Methods.

† It is recognized that these fractions may contain phosphatidal, as well as phosphatidyl, compounds. Our finding that radioactive fatty acid in the lecithin fraction was rapidly and completely released by treatment with *Crotalus adamanteus* venom suggests that in this fraction, at least, incorporation was into the phosphatidyl compounds. (Gottfried and Rapport (26) observed that phosphatidyl choline was hydrolyzed considerably more rapidly than was phosphatidal choline by *Crotalus atrox* venom.)

TABLE 5 DEGRADATION WITH SNAKE VENOM OF LECITHINS ISOLATED FROM GHOSTS*

Expt. No.	C^{14}-Fatty Acid	Incubation with Venom	C^{14} Radioactivity in: Phospholipids	Free Fatty Acids
		min	*cpm*	*cpm*
1	Linoleic	0	1573	69
		60	96	1533
2	Linoleic	0	3393	86
		60	129	2227
3	Palmitic	0	1076	96
		60	132	1024

* Ghosts were incubated with labeled fatty acid under conditions described in Table 1. In each experiment a sample of lecithin isolated from the ghosts was purified by chromatography on alumina. C^{14} radioactivity was determined in phospholipid and in free fatty acid fractions both before and after 60 min incubation with *Crotalus adamanteus* venom. Procedures are described in detail in the text.

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TABLE 6 INCORPORATION OF 1-C^{14}-PALMITOYL CoA INTO PHOSPHOLIPIDS BY GHOSTS*

Radioactive Substrate	Other Additions	Palmitate-C^{14} Incorporated	
		Expt. 1	Expt. 2
		mμmoles	*mμmoles*
Palmitoyl CoA 85 mμmoles	None	0.85	2.5
Palmitic acid 5 mμmoles	None	0	0
Palmitic acid 5 mμmoles	ATP, CoA†	0.15	0.08

* The complete reaction mixture contained 125 μmoles of phosphate buffer, 3 μmoles of $MgCl_2$, 0.4 ml of ghosts, and other additions as indicated, in a total volume of 3 ml. Incubation was carried out for 1 hr. Calculation of mμmoles incorporated was based on specific activity of added substrate.

† Amounts indicated in legend for Table 2.

did not stimulate incorporation of palmitate-C^{14} into lecithin. Since only mμmoles of fatty acid were added or incorporated in these experiments, even chromatographically undetectable amounts of lysolecithin in the ghosts might well provide sufficient substrate for esterifications. We have attempted in several ways to demonstrate formation of lysolecithin from lecithin, endogenous or added to the medium, and have never obtained any evidence of phospholipase activity in ghosts.

Ghosts stored at −20° for several weeks retained full ability to incorporate labeled fatty acids into phospholipids. No activity was lost on lyophilization. On the other hand acetone powders were completely inactive. Numerous unsuccessful attempts were made to solubilize from the membranes the enzymes responsible for the incorporation of fatty acids into lecithin. Lysolecithin was added when acetone powders, sonicated or desoxycholate-treated ghosts were assayed. Added lysolecithin might have been required if the endogenous substrate had been removed or physically separated from the enzymes by the procedures employed.

Experiments with Rat and Sheep Erythrocyte Ghosts

In preliminary experiments (1) it appeared that ghosts prepared from rat erythrocytes incorporated somewhat more fatty acid into phospholipids than did human ghosts. Further studies have indicated that there is little if any difference in the activity of ghosts from the two species. Data from one experiment with linoleic acid-C^{14} are presented in Table 7. The specific activities of phospholipids from human and rat ghosts were similar and almost ten times as high as the specific activity of sheep ghost phospholipids. In some studies, particularly with palmitate-C^{14} the difference in incorporation into human and into sheep ghosts was even more striking. Chromatography of phospholipids from sheep ghosts that had been incubated with labeled fatty acid demonstrated small amounts of radioactivity in the phosphatidylethanolamine and phosphatidylserine areas, but not in the lecithin area. (In agreement with earlier reports (19–21) we found no detectable amounts of lecithin on these chromatograms.)

TABLE 7 SPECIES DIFFERENCES IN INCORPORATION OF LINOLEIC ACID-1-C^{14} INTO GHOST PHOSPHOLIPIDS*

Ghosts from Red Cells of	C^{14} Incorporated
	cpm/μg lipid P
Human	3340
	3200
Rat	2945
	3210
Sheep	336
	340

* Incubation conditions described in legend for Table 1.

Incorporation of palmitate-C^{14} by ghosts prepared from so-called high-potassium sheep red cells (22) and from low-potassium cells was compared.[3] No significant differences were observed. In one experiment the specific activity of the phospholipids after 1 hr of incubation was 7.5 cpm/μg P in the high-potassium ghosts, and 8.9 cpm/μg P in the low-potassium ghosts. Chromatography revealed no gross qualitative or quantitative differences between the phospholipids of the two types of ghosts.

Experiments with Whole Blood

When whole blood plus C^{14}-labeled fatty acid and small amounts of albumin and glucose was incubated at 37° in oxygen, incorporation of radioactivity into phospholipids of the red cell membranes proceeded at a constant rate for 3 hr (Fig. 3). These preliminary experiments were designed to determine whether the reactions that we had studied in ghosts were of quantitative importance in the incorporation of fatty acids into membrane phospholipids of the intact erythrocyte. The largest fraction of the incorporated fatty acid was found in the β-position of lecithin just as it was after incubations of the ghosts.

DISCUSSION

On the basis of the studies reported above it is suggested that the major pathway for incorporation of free fatty acids into phospholipids by human erythrocyte membranes involves two reactions.

1. Fatty acid + CoA + ATP → fatty acyl CoA + AMP + pyrophosphate
2. Fatty acyl CoA + lysolecithin → lecithin

The demonstrated requirement for ATP and the marked

[3] Samples of sheep blood were obtained through the courtesy of Dr. Joseph Hoffman.

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stimulation by CoA are in accord with Reaction 1. In addition, incorporation of C^{14} from 1-C^{14}-palmitoyl CoA was shown to proceed in the absence of added cofactors. The importance of Reaction 2 is suggested by the observation that most of the labeled fatty acid incorporated into phospholipids is recovered in the lecithin fraction, exclusively in the β-position. No effect of added lysolecithin on incorporation of palmitate-C^{14} was found. It seems likely, however, in view of the very small amounts of fatty acid incorporated, that even chromatographically undetectable amounts of lysolecithin, present in the ghosts or formed during the incubation, could provide saturation amounts of substrate for the acylation reaction. Van Deenen and co-workers (7) were able to demonstrate the conversion of lysolecithin-P^{32} to lecithin-P^{32} by washed rabbit erythrocytes. They also reported that the washed cells supplemented with glucose, 2 mg/ml, incorporated linoleic acid-C^{14} into the β-position of lecithin, and to a lesser extent into phosphatidyl ethanolamine. Lauric acid was not incorporated. In the studies reported here, human ghosts incorporated linoleic acid considerably more rapidly than they did oleic, palmitic, or stearic acids. Synthesis of lecithin by acylation of lysolecithin was first studied by Lands (23) in liver microsomes. In studies of the acyl transferase from this source, Lands and Merkl (24) compared the utilization of several fatty acyl CoA compounds with α'-acylglycerophosphoryl choline as the acceptor. CoA esters of unsaturated fatty acids reacted more rapidly than did derivatives of saturated acids. A similar specificity of an acyl transferase in erythrocyte membranes might be responsible for the preferential incorporation of linoleic acid.

Sheep ghosts contain little or no lecithin and incorporated no labeled fatty acid into it. The small amount of fatty acid that was incorporated into phospholipids by sheep ghosts in our experiments was recovered in phosphatidylserine and -ethanolamine areas after chromatography on silicic acid paper. Mulder et al. (8) found a similar pattern of incorporation with bovine red cells which also have a very low lecithin content. The failure of sheep ghosts to incorporate fatty acids into lecithin might be due either to lack of a necessary enzyme or to the absence of lysolecithin acceptor. It would be of interest in this regard to know whether the conversion of lysolecithin-P^{32} into lecithin-P^{32} can be demonstrated with sheep cells as it was with rabbit cells (8).

De Gier and Van Deenen (21), a few years ago, drew attention to a correlation between the lecithin content of the red cells of several species of animals and certain permeability properties of the membranes of these cells. It is possible that the lysolecithin–lecithin

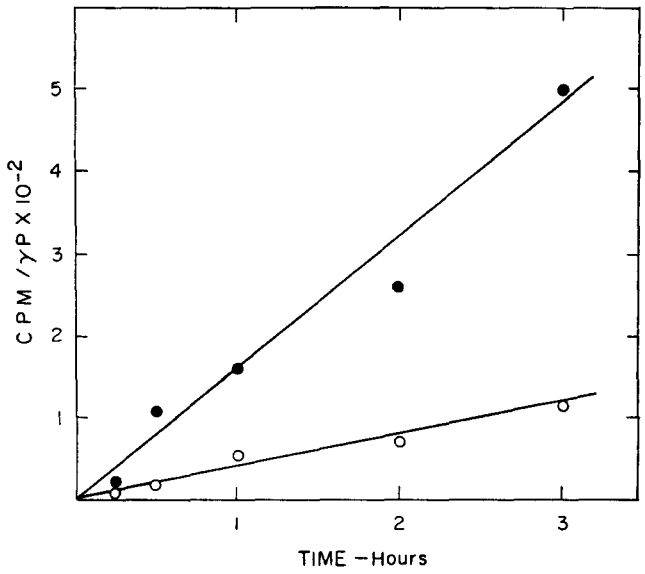

FIG. 3. Incorporation of labeled fatty acids into ghost phospholipids in whole blood as a function of incubation time. Conditions as described in Methods. ● = 2.5 mμmoles linoleic acid-1-C^{14} incubated with human blood; ○ = 3.4 mμmoles palmitic acid-1-C^{14} incubated with rat blood.

conversion, perhaps by altering the relative amounts of these phosphatides or by facilitating alterations in the fatty acid composition of lecithins in the membrane, might influence membrane function. The studies with whole blood, which demonstrated that these reactions were relatively as important in the intact cells as they were in the isolated membranes, were preliminary to an attempt to assess the activity of this sytem in various types of abnormal red cells. There are certain theoretical and obviously practical advantages in being able to study the cells in the intact, undisturbed state. In order to make meaningful comparisons of incorporation of labeled fatty acid into lecithin, however, it will probably be necessary to prepare ghosts before incubation since free fatty acids, lipases, etc., in whole blood will influence the amount of incorporation observed.

Whether or not any functional properties of the membrane are influenced, these reactions are very likely to be instrumental in the alterations in fatty acid composition of red cell phospholipids that can be induced by varying dietary fat (7, 8, 25). Farquhar and Ahrens (25) found that the linoleic acid content of human erythrocyte phospholipids varied between 5 and 27% (with reciprocal changes in oleic acid), depending on the amount of linoleic acid in the diet. The maximal changes were obtained after 4–6 weeks on a particular diet. In rabbits fed corn oil, Van Deenen and co-workers (7, 8) observed a rapid increase in the linoleic acid content of the red cell lecithin and phosphatidyl-ethanolamine fractions, again accompanied by a loss of oleic acid. Even prolonged feeding of coconut oil,

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on the other hand, did not increase the content of lauric acid, consistent with the failure of rabbit erythrocytes in vitro to incorporate lauric acid. If the lysolecithin to lecithin reaction is involved in these dietary effects, there must of course also be a mechanism for converting lecithin to lysolecithin. We have attempted in a number of ways to demonstrate phospholipase activity in ghosts, thus far without success. Perhaps the hydrolyzing enzyme is to be found in the plasma or within the red cell, rather than associated with the membrane itself, as are the enzymes that catalyze the acylation.

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