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METABOLISM OF RED-CELL LIPIDS

II. CONVERSIONS OF LYSOPHOSPHOGLYCERIDES

SUMMARY

Lysolecithin is converted by lysed rabbit erythrocytes into lecithin and glycerylphosphorylcholine. The formation of lecithin by two reactions was demonstrated: by a transacylation requiring the addition of ATP and CoA, and by a dismutation of lysolecithin, not involving the incorporation of exogenous fatty acid but accompanied by the formation of glycerophosphorylcholine. Under conditions favorable for fatty acid incorporation the first-mentioned pathway was found to be predominant. In addition, lysolecithin appears to be degraded by a lysophospholipase (EC 3.1.1.5).

INTRODUCTION

LANDS and co-workers[1-4] demonstrated that an enzyme system from microsomes catalyzes the transacylation of isomeric lysophosphoglycerides so as to form diacyl analogs which have a specific distribution of saturated and unsaturated fatty acids. The enzymic reacylation of lyso-derivatives has been confirmed for a diversity of phospholipid classes and has been detected in different animal tissues[5-9]. OLIVEIRA AND VAUGHAN[10] and VAN DEENEN *et al.*[11] reported that red-cell hemolysates are also capable of acylating lysophosphoglycerides and this observation was recently confirmed by ROBERTSON AND LANDS[12]. As discussed in a previous paper[13] this type of reaction is likely to account for the incorporation *in vitro* of fatty acids into red-cell phosphoglycerides and is perhaps relevant to the renewal of fatty acid constituents in circulating cells. Further studies[14] on the metabolism of lysolecithin by red-cell ghosts showed that, in addition to the energy-requiring acylation, two other reactions are operative.

MATERIALS AND METHODS

[^{32}P]*Lecithin and* [^{32}P]*phosphatidylethanolamine*

These compounds were prepared by incubating 300 mg of rat-liver slices from a young animal with 0.5 ml Krebs–Ringer bicarbonate buffer[15] (containing a small

amount of phosphate), 5 mg of glucose, 0.5 mg of CTP, 0.5 mg of sodium acetate and 2 mV of $KH_2{}^{32}PO_4$ (carrier-free) for 6 h in a CO_2–O_2 (4:96, v/v) atmosphere at 37°. The mixture was homogenized in a Potter–Elvehjem homogenizer and extracted twice according to BLIGH AND DYER[16]. The extract was washed twice with 1 M phosphate solution and evaporated to dryness. Chromatography on a silicic acid column was performed with chloroform–methanol mixtures, and the radioactivity of the eluent was continuously registered. The fractions were identified by means of paper chromatography. The isolated phospholipids had a high specific activity and the radiochemical purity was about 95%. Small quantities with a higher degree of purity were obtained by utilizing thin-layer chromatography on 1-mm layers of silicic acid with starch (SiO_2-S, purchased from Machery, Düren, Germany). The plates were developed with chloroform–methanol–water–concentrated ammonia (65:25:4:0.4, v/v) for 3 h at 0°. The radioactive spots, after location by autoradiography, were transferred to a small column and eluted with methanol. The radiochemical purity, as verified by several procedures, was about 99%.

[^{32}P]Lysophosphatidylcholine and [^{32}P]lysophosphatidylethanolamine

These compounds were prepared by enzymic hydrolysis with snake-venom phospholipase A (EC 3.1.1.4), according to established procedures. The enzymic hydrolysate was purified either by column or by thin-layer chromatography on silica. The radiochemical purity was 95–99%.

Lecithin containing ^{14}C-labeled fatty acid

Erythrocyte hemolysates were incubated with radioactive fatty acid in plasma and the phospholipids isolated as described previously[13]. The quantities used in a typical experiment were: 0.5 ml of rabbit erythrocytes, 0.5 ml of plasma, 1 mg of lysolecithin, 1 mg of glucose, 400 I.U. of penicillin, 400 I.U. of streptomycin, 20 μC of ^{14}C-labeled fatty acid and 0.5 ml of a solution containing 1 μmole of CoA per ml and 50 μmoles of ATP per ml. After ultrasonic vibration the mixture was incubated for 20 h at 37°. The incorporation for linoleic acid and oleic acid was over 90%, for palmitic acid about 50%. After extraction the lipids were separated as described for ^{32}P-labeled phospholipids.

1-[1-^{14}C]Palmitoylglycero-3-phosphorylcholine

Lecithin having [^{14}C]palmitic acid located mainly at the 1-position of the molecule was degraded with snake-venom phospholipase A and purified by chromatography on thin-layer plates.

A mixture of [^{32}P]lysophosphatidylcholine and 1-[1-^{14}C]palmitoylglycero-3-phosphorylcholine served for double-labeled lysolecithin. Besides 1-[1-^{14}C]palmitoylglycero-3-phosphorylcholine and 1-palmitoylglycero-3-[^{32}P]phosphorylcholine this mixture may contain small amounts of radioactive 1-acylglycerol-3-phosphorylcholine containing other fatty acids. However, according to COLLINS[17] ^{32}P-incorporation into liver lecithin prevails in lecithin molecules containing palmitic acid; lecithin molecules with stearic acid revealed a 10-fold smaller incorporation. Both saturated fatty acids are located predominantly at the 1-position[18,19]; rat liver contains about 55% palmitic acid and 40% stearic acid at this position. From these observations it seems probable that the ^{32}P-label of lysolecithin obtained from liver lecithin after

treatment with snake venom, is located mainly in lysolecithin molecules having palmitic acid as fatty acid constituent.

The conditions used for the incubation of the erythrocytes with labeled substrates and the methods for identification of the lipid products have been described before[13]. The water-soluble products *e.g.* glycerophosphorylcholine, glycerophosphorylethanolamine, phosphorylcholine were identified by paper chromatography in propanol–ammonia–water (6:3:1, v/v), phenol–water (a saturated solution of phenol), and *tert.*-butanol–picric acid–water (80:4:20, v/w/v)[20]. Chromatograms were counted in a Baird and Tatlock chromatogram scanner. Qualitatively, ^{32}P and ^{14}C can be distinguished by means of an Al-foil of 30 mg/cm^2. ^{14}C-radiation is totally absorbed by the foil; ^{32}P-radiation passes for about 70%. For quantitative experiments the spots of the chromatograms were cut out and measured in a liquid scintillation counter[16]. ^{14}C and ^{32}P were counted in different channels and the ^{14}C counts corrected for the ^{32}P contribution in the ^{14}C channel. For calculations of $^{14}C/^{32}P$ ratios of lecithin, corrections for the small amounts of impurities of the labeled substrates were made.

RESULTS

The formation of lecithin by conversion of lysolecithin after incubation with a red-cell lysate under conditions suitable for fatty acid incorporation is illustrated in

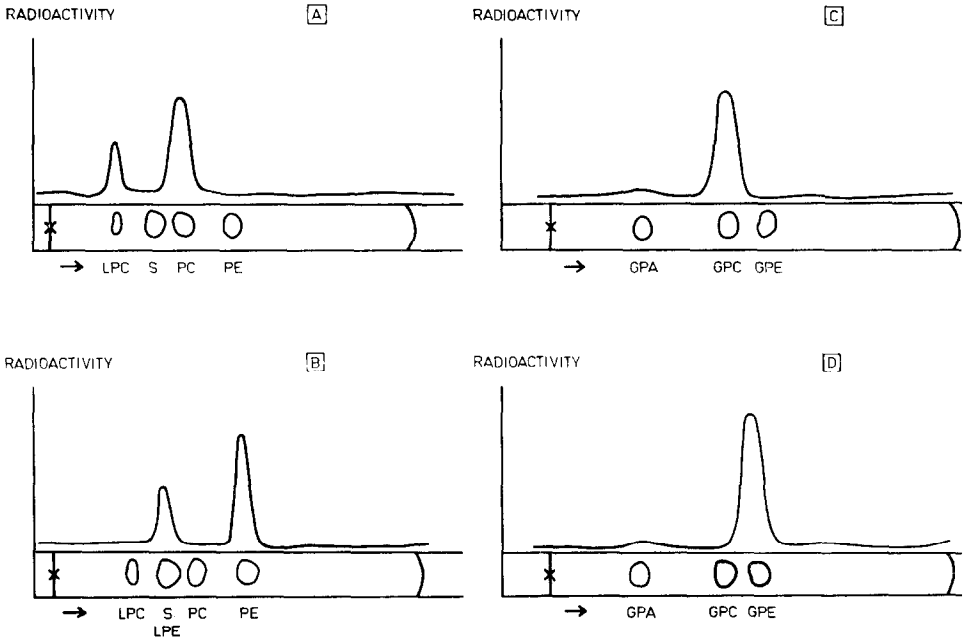

Fig. 1. Distribution of ^{32}P-activity on paper chromatograms of hemolysed rabbit erythrocytes after incubation with [^{32}P]lysophospholipids. A: Radioactivity of phospholipids after incubation with [^{32}P]lysolecithin. B: Radioactivity of phospholipids after incubation with [^{32}P]lysophosphatidylethanolamine. C: Radioactivity of the water-soluble fraction after incubation with [^{32}P]-lysolecithin. D: Radioactivity of the water-soluble fraction after incubation with [^{32}P]lysophosphatidylethanolamine.
Abbreviations: LPC, lysolecithin; S, sphingomyelin; PC, lecithin, PE, phosphatidylethanolamine, LPE, lysophosphatidylethanolamine; GPA, glycerophosphate; GPC, glycerophosphorylcholine; GPE, glycerophosphorylethanolamine.

Fig. 1. Similarly labeled phosphatidylethanolamine was found to be produced when ^{32}P-labeled lyso-analog was added to this system. However, examination of the water-soluble fraction showed significant radioactivity to be present, particularly after incubation of lysates of rabbit erythrocytes with lysolecithin. By means of paper chromatography the compounds were demonstrated to be identical to glycerophosphorylcholine and glycerophosphorylethanolamine respectively. Control experiments showed that the amounts of the water-soluble phosphodiesters produced are not attributable to non-enzymic breakdown. Hence, lysolecithin and lysophosphatidylethanolamine are utilized not only for the production of the diacyl-analogs, but the red cell is capable of degrading these lytic compounds to some extent as well. It was of interest to estimate this lysophospholipase (EC 3.1.1.5) activity by assaying the production of phosphodiesters after incubation of lyso-derivatives with red-cell lysates without the addition of CoA and ATP. Under these conditions, unfavorable for fatty acid incorporation into the red-cell phosphoglycerides, a very pronounced breakdown of the lyso-derivatives resulted. The post-hemolytic residues from both human and rabbit erythrocytes appeared to contain a fairly active lysophospholipase (Table I). The

TABLE I

DEGRADATION OF LYSOPHOSPHOGLYCERIDES BY ERYTHROCYTE HEMOLYSATES

Experimental conditions: 0.5 ml of hemolysed erythrocytes, 0.5 ml of Ringer solution, 1 mg of lysolecithin or lysocephalin and trace amounts of labeled substrates were incubated for 5 h at 37° after sonication. The figures are the mean of at least 3 experiments, and were corrected for the decomposition of lysolecithin (10–15%) occurring in the solution if erythrocytes were omitted. The range of the observed individual values is given between parenthesis.

Animal	*Substrate*	*% glycerophosphoryl derivative formed*
Rabbit	[^{32}P]lysolecithin	75 (60–80)
Human	[^{32}P]lysolecithin	65 (60–75)
Ox	[^{32}P]lysolecithin	7 (5–10)
Ox	[^{32}P]lysophosphatidylethanolamine	15 (10–30)

figures given in the table refer to a 5-h incubation period, but it has to be noted that a post-hemolytic residue of rabbit erythrocytes hydrolyses as much as 70% of the lysolecithin within 20 min (Fig. 2). Red-cell lysates from ox were found to be less active than hemolysates of rabbit erythrocytes (Table I).

The competition between the catabolic and anabolic reactions of lysolecithin and lysophosphatidylethanolamine were investigated with the lysates from rabbit and ox erythrocytes respectively (Tables II and III). Incubation of a trace amount of [^{32}P]lysolecithin with the lysate of rabbit erythrocytes gave rise to a considerable breakdown into glycerophosphorylcholine without the formation of any detectable quantity of radioactive lecithin. Upon addition of CoA and ATP less water-soluble phosphodiester was formed, and the remaining quantity of lysolecithin was considerably decreased, a significant part of the radioactivity being recovered in the lecithin fraction. Dilution of the tracer amount of lysolecithin (only a few micrograms) with unlabeled lysolecithin in the presence of the same concentration of ATP and CoA resulted in a decrease of the conversion of lysolecithin into lecithin. Under these conditions the fatty acids available for the acylation reaction possibly limit this reaction in favor of the enzymic degradation of lysolecithin. Qualitatively the same

results were recorded when lysophosphatidylethanolamine was subjected to incubation with a lysate from ox red cells (Table III). Without added ATP and CoA only a very small quantity of radioactive phosphatidylethanolamine was detectable, while a significant part of glycerophosphorylethanolamine was formed. It may be noted, however, that this degradation was less pronounced than the breakdown of lysolecithin by the lysate of rabbit erythrocytes. In the presence of ATP and CoA the

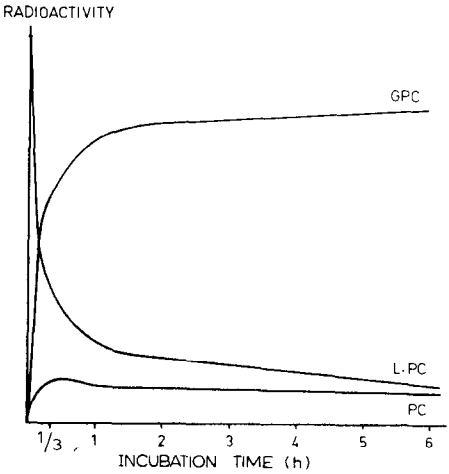

Fig. 2. Time course of the conversion of [^{32}P]lysolecithin by hemolysed rabbit erythrocytes. Incubation mixtures contained 0.5 ml of hemolysed erythrocytes, 0.5 ml of Ringer solution, 1 mg of lysolecithin and a tracer amount of [^{32}P]lysolecithin. Abbreviations are denoted in Fig. 1.

TABLE II

FORMATION OF LECITHIN AND GLYCEROPHOSPHORYLCHOLINE BY HEMOLYSATES OF RABBIT ERYTHROCYTES

A solution containing 0.5 ml of hemolysed rabbit erythrocytes, 0.5 ml of Ringer solution, 40 μg of linoleic acid, and a trace amount of [^{32}P]lysolecithin was incubated with additions indicated above for 5 h. Lysolecithin: 1 mg; CoA and ATP: 0.1 ml of a solution containing per ml 50 μmole of ATP and 1 μmole of CoA. The figures are the mean of at least 4 experiments, the individual measurements differing within the given range.

Additions	*Distribution of radioactivity*		
	Lecithin	*Lysolecithin*	*Glycerophosphorylcholine*
	(%)	(%)	(%)
None	0	30 (20–40)	70 (60–80)
CoA and ATP	38 (30–42)	8 (5–12)	55 (50–60)
CoA and ATP and lysolecithin	17 (9–25)	8 (4–15)	75 (70–85)
Inactive lysolecithin	8 (4–12)	12 (4–21)	80 (70–90)

acylation of lysophosphatidylethanolamine dominated its degradation, even when the tracer substance was diluted with unlabeled carrier.

These results indicate that the competition between utilisation of lyso-derivatives for synthesis of phosphoglycerides and their breakdown into water-soluble diesters is somewhat different for the red-cell lysates from different animal species, the addition of CoA and ATP always causing an increase of the anabolic reaction. However, it has to be noted that whereas the lysate of rabbit erythrocytes without added ATP and CoA did not acylate lysolecithin to form lecithin, dilution of the tracer

TABLE III

FORMATION OF PHOSPHATIDYLETHANOLAMINE FROM LYSOPHOSPHATIDYLETHANOLAMINE BY HEMOLYSATES OF OX ERYTHROCYTES

A solution containing 0.5 ml of hemolysed ox erythrocytes, 0.5 ml of Ringer solution, 40 μg of linoleic acid, and a trace amount [^{32}P]lysophosphatidylethanolamine was incubated with additions indicated above for five hours. Lyso-phosphatidylethanolamine was added (0.25 mg); CoA and ATP: 0.1 ml of a solution containing 50 μmoles ATP per ml and 1 μmole CoA per ml. The figures represent the average of two separate experiments.

Additions	*Distribution of radioactivity*		
	Phosphatidyl-ethanolamine (%)	*Lysophosphatidyl-ethanolamine* (%)	*Glycerophosphoryl-ethanolamine* (%)
None	3	54	43
CoA and ATP	67	22	11
CoA and ATP and lysophosphatidyl-ethanolamine	55	29	16
Unlabeled lysophosphatidyl-ethanolamine	8	50	42

substance with unlabeled lysolecithin did give rise to the production of a small amount of labeled lecithin (Table II). Under the same conditions the production of labeled phosphatidylethanolamine by lysates from ox erythrocytes was also stimulated by adding unlabeled lysophosphatidylethanolamine to the system (Table III). These observations suggested that, apart from the CoA- and ATP-requiring formation of diacylphosphoglycerides from lyso-analogs, another reaction may be involved. This view was supported by the time curves of the conversions (Fig. 2). The formation of lecithin from "diluted" lysolecithin without the addition of CoA and ATP resembles the breakdown of lysolecithin into glycerylphosphorylcholine, a very fast reaction reaching a plateau after about 20 min. By contrast, the ATP- and CoA-requiring incorporation of fatty acids into lysolecithins is a process which proceeds steadily with time[13].

The formation of lecithin from lysolecithin without the participation of free fatty acid CoA derivative was further studied in a fraction obtained after ultracentrifugation (1 h at 100 000 × *g*) of ultrasonically disrupted red cells. In the middle of the centrifuge tube appeared a dark layer probably consisting of membrane residues, and this layer contained most of the red cell lipids. The upper and lower layers, referred to as erythrocyte-hemolysate fraction, were practically free of lipids, but were enzymatically active. Although no separation of the different enzymes involved in the metabolism of lysolecithin was achieved, the low content of lipids in this fraction facilitated observations on the transformation of lysolecithin into lecithin, as illustrated by Fig. 3. Under the same conditions a quantitative comparison was made of the amount of [^{32}P]lysolecithin converted into lecithin and the incorporation of [^{14}C]-palmitic acid and [^{14}C]linoleic acid into the lecithin formed (Table IV). The results clearly showed that in this system, not enriched with ATP and CoA, a discrepancy exists between the amount of ^{14}C-labeled and ^{32}P-labeled lecithin produced. In these experiments the conversion of lysolecithin into lecithin significantly exceeded the incorporation of ^{14}C-labeled fatty acids, even when taking into consideration the dilution of the labeled fatty acids with the amounts freed by enzymatic hydrolysis of lysolecithin into glycerophorylcholine. Compared with the amount of lecithin formed, the incorporation of added palmitic acid and linoleic acid was too small (by a factor 60 and 8 times respectively) to account for an acylation reaction. Addi-

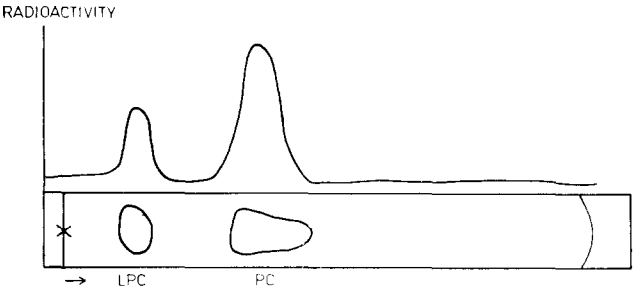

Fig. 3. Paperchromatographic demonstration of the formation of [^{32}P] lecithin from ^{32}P-lysolecithin by a fraction of hemolysed erythrocytes. Incubation conditions: 0,1 ml of the "supernatant" of hemolysed rabbit erythrocytes was mixed with 0,3 ml of Ringer solution containing a tracer amount [^{32}P]-lysolecithin and 2 mg of lysolecithin. The mixture was incubated for 40 min at 37°. For abbreviations compare Fig. 1.

TABLE IV

COMPARISON BETWEEN THE CONVERSION OF [^{32}P]LYSOLECITHIN INTO [^{32}P]LECITHIN AND THE FATTY ACID INCORPORATION INTO LECITHIN BY A FRACTION OF HEMOLYSED RABBIT RED CELLS WITHOUT ADDITION OF CoA AND ATP

0.1 ml of a fraction of hemolysed erythrocytes in 0.3 ml of Ringer solution was incubated for 40 min with 4 μmoles lysolecithin and trace amounts of [^{32}P]lysolecithin and 1-^{14}C-labeled fatty acids. The amounts of substances formed were calculated assuming a molecular weight of 500 for lysolecithin, and 250 for fatty acid. In the calculation it was presumed that all fatty acids liberated from lysolecithin by lysophospholipase are homogeneously mixed with added radioactive fatty acid and incorporated simultaneously into lecithin. With this supposition the calculated amount of lecithin formed by incorporation of fatty acid can only be some what too high, which would not alter the conclusion drawn in the text.

Labeled substrates	[^{14}C]*Lecithin formation (amounts in μmoles)*	[^{32}P]*Lysolecithin conversion (amounts in μmoles)*			
		[^{32}P]*Lecithin formation*	[^{32}P]*Glycerophosphorylcholine formation*	*Residual* [^{32}P]-*lysolecithin*	*Fatty acid liberated from lysolecithin*
[^{32}P]Lysolecithin and [1-^{14}C]palmitic acid	0.007	0.48	3.40	0.16	3.5
[^{32}P]Lysolecithin and [1-^{14}C]linoleic acid	0.053	0.44	3.40	0.12	3.5

tion of CoA and ATP to the system naturally abolished these differences, and as we reported previously[11], under such conditions a labeled lecithin is produced mainly by acylation of the fatty acids added.

These observations indicated that lecithin may be formed in the system described by utilising the fatty acids esterified in its precursor, *viz.* by a reaction 2 lysolecithin → lecithin + glycerophosphorylcholine. The reality of such a conversion was demonstrated by investigating the metabolism of lysolecithin labeled both with[^{32}P]-phosphate and a ^{14}C-labeled fatty acid constituent. Fig. 4 gives an example of the distribution of radioactivity among the lipids after incubation of the doubly-labeled lysolecithin with the supernatant fraction without CoA and ATP present; when the chromatograms were scanned for total radioactivity and ^{32}P-activity a great difference in the ratio ^{14}C/^{32}P was noted between the spots of the lysolecithin and lecithin. This effect is not likely to be brought about by an incorporation of [^{14}C]palmitic acid liberated enzymically from the lysolecithin substrate, since a surplus of unlabeled palmitic acid was added to dilute the radioactivity of the fatty acids freed. The results

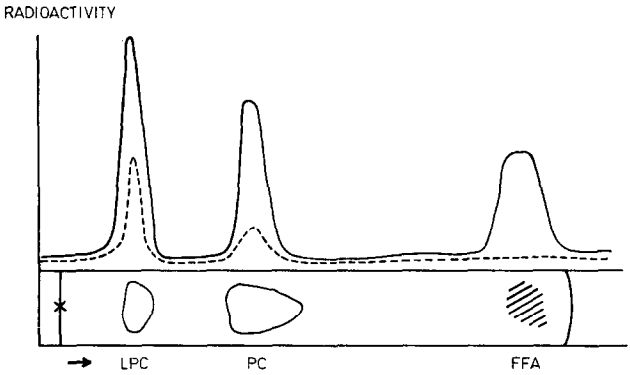

Fig. 4. Conversion of 1-[1-^{14}C]palmitoylglycerol-3-[^{32}P]phosphorylcholine by rabbit-erythrocyte hemolysates. Reproduction of the distribution of radioactivity on a paper chromatogram scanned with a chromatogram scanner measuring both ^{14}C and ^{32}P activity. The ^{32}P activity (indicated by the dotted curve) was measured through an aluminium absorber of 30 mg/cm^2. Incubation conditions are given in Table V. No ATP and CoA were added and the mixture was incubated for 30 min at 37°. For abbreviations see legend to Fig. 1.

TABLE V

RATIO OF ^{14}C/^{32}P IN LECITHIN FORMED FROM 1-[1-^{14}C]PALMITOYL-2-ACYLGLYCERO-3-[^{32}P]PHOSPHORYLCHOLINE BY RABBIT-ERYTHROCYTE HEMOLYSATES

Experimental conditions: 0.1 ml hemolysed erythrocyte fraction or 0.5 ml hemolysed erythrocytes mixed with 0.5 ml of Ringer solution, 0.5–2 mg of lysolecithin, 1–5 mg of palmitic acid, 0.1 mg of linoleic acid, 1-[1-^{14}C]palmitoylglycero-3-phosphorylcholine and 1-acylglycerol-3-[^{32}P]phosphorylcholine in trace amounts were incubated at 37° for periods varying from 20 min to 5 h. In the third group of experiments 0.1 ml of a solution containing 1 μmole CoA per ml and 50 μmoles ATP per ml was added, and the incubation was carried out for 5 h at 37°. The B/A values of the first groups of experiments (a–e) exceed the theoretical value of 2. This discrepancy may be caused by a systematic experimental error, or by the different origin of the [^{32}P]- and [^{14}C]lysolecithins as was described under materials.

Substrate	*^{14}C/^{32}P ratio of lysolecithin (A)*	*^{14}C/^{32}P ratio of lecithin (B)*	*B/A*
Hemolysed erythrocyte (a)	7.05	15.2	2.2
Fraction without ATP and CoA (b)	6.75	18.2	2.7
Hemolysed erythrocytes (c)	8.2	17.9	2.2
Without ATP and CoA (d)	8.1	17.3	2.1
(e)	0.36	0.84	2.3
Mean of a–e			2.3
Hemolysed erythrocytes (f)	3.74	5.03	1.3
With ATP and CoA (g)	3.71	4.45	1.2
(h)	3.20	4.60	1.4
Mean of f–h			1.3

of similar experiments carried out on a quantitative base are compiled in Table V. A comparison of ^{14}C/^{32}P ratio in the lysolecithin substrate and the lecithin shows that, under conditions not favorable for the incorporation of fatty acids, this characteristic is about twice as high in the diacyl-derivative as it was in the lyso-compound. Apparently both fatty acid constituents from lecithin are completely derived from the lysolecithin. Addition of CoA and ATP alters this pattern. Under these conditions the ^{14}C/^{32}P ratio of the lecithin produced resembles more closely that of the substrate studied, thus indicating that an esterification of the lysolecithin with exogenous fatty acids occurred in addition.

DISCUSSION

The present investigation shows that, apart from utilisation of lysophosphoglycerides for the production of diacyl-derivatives, post-hemolytic residues from red cells are capable of degrading these substances into water-soluble phosphodiesters. These results were confirmed by the observations of HEEMSKERK AND VAN DEENEN[21], who showed that after treatment of disrupted rabbit erythrocytes with snake-venom phospholipase A the lyso-derivatives formed were further degraded to form glycerophosphorylcholine and glycerophosphorylethanolamine. In addition, it was demonstrated in both studies that significant quantitative differences may exist in this respect between red cells from different animal species.

Both the conversion of lysolecithin into lecithin and its breakdown into glycerophosphorylcholine each appear to proceed by at least two different pathways. The results obtained so far support the view that in red-cell lysates lysophosphoglycerides may be converted according to the following reactions.

$$\text{lysolecithin} + \text{fatty acid} \xrightarrow{\text{ATP}+\text{CoA}} \text{lecithin} \quad (1)$$

$$\text{lysolecithin} \rightarrow \text{lecithin} + \text{glycerophosphorylcholine} \quad (2)$$

$$\text{lysolecithin} \rightarrow \text{glycerophosphorylcholine} + \text{fatty acid.} \quad (3)$$

Reaction 1, a transacylation of lysolecithin by red cells requiring the participation of fatty acid–CoA derivatives, was already indicated by previous studies[10–13]. In addition to the indications derived from the stimulating effect of lyso-derivatives on fatty acid incorporation and the conversion of either ^{32}P- or ^{14}C-labeled lysolecithin into labeled lecithin, more conclusive evidence was produced in the present study by the use of a double-labeled lyso-compound. Although, because of interference by two other reactions, the ratio of $^{14}C/^{32}P$ in the lecithin formed could not be expected to be completely identical to the theoretical value, the results obtained indicated that, in the presence of ATP and CoA, red-cell lysates convert lysolecithins to a most significant extent according to this pathway. When ATP and CoA were omitted the production of lecithin is greatly reduced, if not completely stopped, but when the amount of lysolecithin added to the system was increased a small but detectable quantity of lecithin still appeared to be formed. Even the relative ineffectiveness of the red-cell lysate in incorporating fatty acids under these conditions, as well as the time relationships of lecithin formation, indicated that a second mechanism might be involved. By the use of double-labeled lysolecithin it became clear that Reaction 2 is responsible for this conversion. The necessity of diluting the trace amount of lysolecithin to augment the substrate concentration can perhaps be traced back to the requirement of the enzyme to act on a substrate present in a micellar organisation. Evidence for this type of reaction has been claimed by ERBLAND AND MARINETTI[22] using a rat-liver preparation, and by KOKKE *et al.*[23] studying formation of lecithin in a yeast supernatant fraction. Although this conversion gives rise to the production of an equivalent amount of glycerophosphorylcholine, circumstantial evidence indicates that in red-cell lysates the water-soluble phosphodiesters are not formed merely by Reaction 2, but that in addition a lysophospholipase (Reaction 3) is active. The amount of glycerophosphorylcholine found to be produced after incubation of lysolecithin with lysate of rabbit erythrocytes dominated the formation of lecithin. In our studies on the metabolism of lysolecithin in a yeast supernatant[23]

similar observations were made, but in this system phospholipase A was found to degrade the lecithin formed, thus giving an accumulation of glycerophosphorylcholine.

In the red-cell lysate, however, we were so far unable to detect any appreciable phospholipase A-like activity, this being in agreement with the observations of OLIVEIRA AND VAUGHAN[24], and ROBERTSON AND LANDS[12]. Furthermore, under certain conditions a lysate of rabbit red cells gave only degradation of lysolecithin into glycerophosphorylcholine without any detectable formation of lecithin, indicating the activity of a distinct enzyme.

As regards the physiological significance of Reactions 2 and 3 it may be speculated that the red cell, capable of carrying out Reaction 1, is equipped with systems for keeping the lysophosphoglyceride concentration within certain limits, in situations where failure of the transacylation reaction would give rise to an accumulation of these harmful substances.

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