values are not directly comparable, but there can be little doubt that, in lactating mammary gland, lipogenesis is much higher. It is likely that the distribution of 3H among various products and water will depend upon the relative rates of the biosynthetic processes and reactions which exchange the ³H with other protons.

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Department of Physiology, University of California. S. Abraham Berkeley, Calif. (U.S.A.), and JOSEPH KATZ* The Institute for Medical Research, Cedars of Lebanon Hospital, J. BARTLEY Los Angeles, Calif. (U.S.A.) I. L. CHAIKOFF ¹ S. J. WAKIL, J. Lipid Res., 2 (1961) 1. ² D. W. FOSTER AND B. BLOOM, Biochim. Biophys. Acta, 60 (1962) 189. ³ D. W. FOSTER AND B. BLOOM, J. Biol. Chem., 238 (1963) 888. ⁴ D. W. FOSTER AND B. BLOOM, J. Biol. Chem., 236 (1961) 2548.

- ⁵ I. A. Rose, J. Biol. Chem., 235 (1960) 1170.
- ⁶ S. Abraham, K. J. Matthes and I. L. Chaikoff, Biochim. Biophys. Acta, 49 (1961) 268.
- ⁷ J. Katz and J. Kornblatt, J. Biol. Chem., 237 (1962) 2466.
- ⁸ J. Katz, S. Abraham, J. Bartley and I. L. Chaikoff, unpublished observations.
- ⁹ J. Katz and H. G. Wood, *J. Biol. Chem.*, 238 (1963) 517.

 ¹⁰ S. Abraham, K. J. Matthes and I. L. Chaikoff, *J. Biol. Chem.*, 235 (1960) 2551.

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Fatty acid compositions of naturally occurring lysolecithins and lecithins*

The presence of lysolecithins in solvent extracts of egg, liver, heart, intestine, kidney, lung, spleen, brain, adrenals, blood, cytochrome and cytochrome oxidase preparations has been reported¹⁻¹⁴. Quantitative measurements have shown that the percentage of lipid P occurring as lysolecithins varies from 1 % in brain to 22 % in a cytochrome preparation from pig heart^{5,12}. Human blood plasma and serum phospholipids appear to contain 3-10 % lysolecithins^{4,7,9,10,13,14}, while rat blood plasma phospholipids contain 17.5 % lysolecithin 15. The fatty acid compositions of lysolecithins from human blood plasma⁸ and human blood serum^{9,16} have been reported and it appears that they contain more saturated fatty acids than the lecithins. If the saturated and unsaturated fatty acids occur in the lysolecithins predominantly at the α' - and β -positions, respectively, as in lecithins 17,18 , then the α -acyl lysolecithins are present in larger concentrations than the β -acyl lysolecithins. The present preliminary work was undertaken to investigate the structure and origin of the lysolecithins by comparing their fatty acid composition with that of the lecithins from the same source.

Lecithins and lysolecithins were isolated from hens' eggs, bovine plasma, bovine

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lung, yeast, and human plasma. Fresh bovine blood (containing I oz of 30 % Calgon per 30 lb of blood as an anticoagulant) was centrifuged at 2000 rev./min for 20 min and the plasma was obtained by decantation. Human blood (preserved with acid citrate dextrose) plasma was similarly prepared. Bakers yeast (Saccharomyces cerevisiae) was grown in a 40-gallon fermenter under aseptic conditions for 24 h. The cells were collected in a Sharples centrifuge and freeze-dried. To obtain the total lipids, all tissues were extracted with chloroform-methanol according to the method of BLIGH AND DYER¹⁹.

The method for the isolation of pure lecithins and lysolecithins was essentially that described by Niemiro and Przyjemski². In brief, the egg and lung lipids were placed on aluminum oxide columns (suitable for chromatographic adsorption), and chloroform—methanol (i:i, v/v) was used to elute choline-containing phospholipids and neutral lipids. The lecithins and lysolecithins were separated on silicic acid-impregnated papers using benzene—methanol (70:30, v/v) as the developing solvent. In order to obtain beef lung lecithin free of plasmalogens, the choline-containing phospholipids were incubated with acetic acid—water (90:10, v/v) at 37° for 17 h. The lecithin isolated from this mixture was used for fatty acid analyses. The papers were sprayed with Rhodamine 6G, the spots corresponding to lecithin and lysolecithin were cut out, placed in a soxhlet apparatus, eluted with methanol, and hydrolyzed with methanolic 0.5 N KOH overnight. The hydrolysates were acidified, the fatty acids extracted with light petroleum (b.p. 30-60°), taken to dryness under N₂, dissolved in ethyl ether and methylated with diazomethane.

The methyl esters were estimated by gas-liquid chromatography as previously described²⁰.

After the initial experiments with egg and bovine-lung lecithins and lysolecithins were completed Renkonen²⁴ reported that lecithins were broken down to lysolecithins on alumina columns so the lysolecithins may not be representative of those formed in the tissues. Therefore, the lecithins and lysolecithins were isolated by spotting the total lipids on thin-layer plates using chloroform—methanol—water (65:25:4, v/v) as the developing solvent and spraying with Rhodamine 6G. The lecithins and lysolecithins were scraped off the plates into soxhlet thimbles and analysed as previously reported. A typical thin-layer plate was photographed and is shown in Fig. 1.

As reported by Renkonen²⁴, considerable breakdown of lecithins occurs on passage through an alumina column, so the lysolecithins would not be representative of the naturally occurring compounds. The fatty acid analyses of egg lysolecithins (Table I) revealed that they were 26 % unsaturated but after passage through an alumina column they were 53 % unsaturated, indicating that the lecithins were preferentially hydrolysed at the α -position on alumina. Although bovine-lung lecithin appeared to be almost 50% hydrolysed (Fig. 1) on alumina, the composition of the fatty acids in the lysolecithins did not appear to be altered.

The results in Table I show that the lysolecithins in all the tissues, except bovine plasma, are more saturated than the lecithins. It therefore appears that the lysolecithins are mixtures of α - and β -isomers with a predominance of the α -acyl compound, except in bovine plasma.

Since Lands²¹ has shown that (an) enzyme(s) exist(s) in rat-liver particles which preferentially incorporate(s) unsaturated fatty acids in the β -position and the satu-

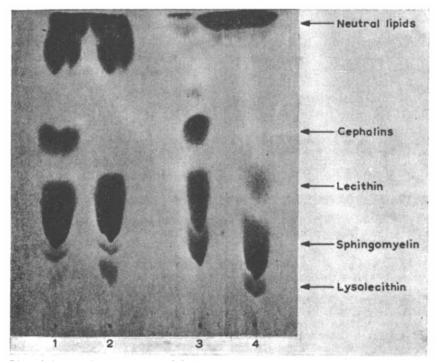


Fig. 1. Photograph of a typical thin-layer chromatogram: 1, total egg lipids; 2, egg lipids eluted from an alumina column with chloroform—methanol (1:1, v/v); 3, total bovine-lung lipids; 4, bovine-lung lipids eluted from an alumina column with chloroform—methanol (1:1, v/v).

TABLE I

Source -	Total fatty acids (moles %)								
	14:0	16:1	16: o	18: 2 + 18: 3	18: 1	18: o	20:3*	20:4	Sat./Unsat
Egg									
Lecithin, direct		1.4	31.0	17.8	36.o	11.0	_	3.0	42/58
Lecithin, alumina		1.3	29.3	17.5	36.o	13.0	_	2.9	
Lysolecithin, direct		2.I	48.3	6.0	18.3	25.4			73/27
Lysolecithin, alumina	_	2.3	32.1	10.9	40.2	14.6	_	_	
Bovine lung									
Lecithin, direct	1.8	3.6	49.2	8.8	15.6	13.0		8.0	64/36
Lecithin, alumina	3.2	4.8	51.3	7.9	14.6	13.4	_	4.7	
Lysolecithin, direct	6.0	3.3	54.6	6.7	9.0	13.3		7.0	74/26
Lysolecithin, alumina	6.4	4.4	51.7	5.7	11.9	14.0	_	5.8	
Yeast									
Lecithin, direct		32.0	38.6	3.2	17.2	8.9	_		48/52
Lysolecithin, direct	_	15.3	40.8	4.3	22.4	15.0	_	_	56/44
Human plasma									
Lecithin, direct	_	1.6	44.4	16.2	17.1	17.2		3.5	62/38
Lysolecithin, direct	_	2.4	57.6	9.4	11.1	19.7		< 1.0	76/24
Bovine plasma									
Lecithin, direct		0.7	25.8	19.0	20.3	22.3	6.9	5.0	48/52
Lysolecithin, direct	_	1.2	24.8	17.3	23.4	22.8	6.0	4.5	47/53

^{*} Tentative identification.

rated fatty acids in the α -position of a mixture of α - and β -acyl lysolecithins, these lysolecithins may be important intermediates in the biosynthesis *in vivo* of lecithins. Their presence in the blood plasma of the intact animal¹⁵ indicates that they are not artifacts or breakdown products of the lecithins^{13,15}.

It has been reported that the lecithins from bovine lung and yeast were entirely dipalmitoyl²² and dipalmitoleoyl²³ lecithins, respectively, but the present study shows that these fatty acids make up only approx. 60 % and 32 % of the fatty acids of these lecithins.

It was interesting to find that yeast lecithin from a 48-h culture had 60% palmitoleic acid, as compared to 32% in the 24-h culture (unpublished results).

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<sup>1</sup> C. H. LEA, D. N. RHODES AND R. D. STOLL, Biochem. J., 60 (1955) 353.
<sup>2</sup> G. V. Marinetti, R. F. Witter and E. Stotz, Federation Proc., 15 (1956) 308.
<sup>3</sup> E. Titus, H. Weiss and S. Haidju, Science, 124 (1956) 1205.
<sup>4</sup> G. B. PHILLIPS, Proc. Natl. Acad. Sci. U.S., 43 (1957) 566.
<sup>5</sup> G. V. Marinetti, J. Kochen, J. Erbland and E. Stotz, J. Biol. Chem., 229 (1957) 1027.

<sup>6</sup> G. B. PHILLIPS, Biochim. Biophys. Acta, 29 (1958) 594.
<sup>7</sup> G. V. Marinetti, M. Albrecht, T. Ford and E. Stotz, Biochim. Biophys. Acta, 36 (1959) 4.

8 C. E. Rowe, Biochem. J., 76 (1960) 471.

    G. J. Nelson, J. Lipid Res., 3 (1962) 71.
    E. GJONE, J. F. BERRY AND D. A. TURNER, J. Lipid Res., 1 (1959) 66.

11 H. P. SCHWARZ, L. DREISBACH, R. STAMBAUGH AND A. KLESCHICK, Arch. Biochem. Biophys.,
87 (1961) 171.
12 R. Niemiro and J. Przyjemski, Acta Biochim. Polon., 8 (1961) 449.
13 M. PAYSANT-DIAMENT, S. BADON AND J. POLONOASKI, in P. DESNUELLE, Enzymes of Lipid
   Metabolism, Pergamon Press, London, 1961, p. 250.

    W. G. Vogel, L. Zieve and R. O. Carleton, J. Lab. Clin. Med., 59 (1962) 335.
    H. A. I. Newman, Ching-Tong Lium and D. B. Zilversmit, J. Lipid Res., 2 (1961) 403.

16 W. M. F. LEAT, Biochem. J., 84 (1962) 85P.
17 N. H. TATTRIE, J. Lipid Res., 1 (1959) 60.
18 D. J. HANAHAN, H. BROCKERHOFF AND E. J. BARRON, J. Biol. Chem., 235 (1960) 1917.

    E. G. BLIGH AND W. J. DYER, Can. J. Biochem. Physiol., 37 (1959) 911.
    J. R. BENNETT AND N. H. TATTRIE, Can. J. Biochem. Physiol., 39 (1961) 1357.
```

22 S. J. THANNHAUSER, J. BENOTTI AND N. F. BONCODDO, J. Biol. Chem., 166 (1946) 669.

23 D. J. HANAHAN AND M. E. JAYKO, J. Am. Chem. Soc., 74 (1952) 5010.

Received February 15th, 1963

Revised manuscript received July 9th, 1963

21 W. E. M. Lands, private communication.

24 O. RENKONEN, J. Lipid Res., 3 (1962) 181.

Biochim. Biophys. Acta, 70 (1963) 693-696