HOFMANN[11] has suggested that lysophosphatidyl choline, in addition to bile salts, may aid the absorption of fat by forming soluble micelles with fatty acids and monoglycerides in the intestinal lumen. In sheep, the absorption of fat is absolutely dependent on the presence of bile in the gut[5] and it is possible that the large amounts of phospholipid which enter the duodenum in the bile may facilitate the uptake of fat into the cells of the intestinal mucosa.

Department of Experimental Pathology, Australian National University, Canberra (Australia) E. P. ADAMS T. J. HEATH

[1] R. BLOMSTRAND, *Acta Chem. Scand.*, 14 (1960) 1006.
[2] G. B. PHILLIPS, *Biochim. Biophys. Acta*, 41 (1960) 361.
[3] B. BORGSTRÖM, *Acta Chem. Scand.*, 11 (1957) 749.
[4] R. BLOMSTRAND AND P. EKDAHL, *Proc. Soc. Exptl. Biol. Med.*, 104 (1960) 205.
[5] T. J. HEATH AND B. MORRIS, *Brit. J. Nutr.*, 1963, in the press.
[6] D. B. ZILVERSMIT AND A. K. DAVIS, *J. Lab. Clin. Med.*, 35 (1950) 155.
[7] W. C. VOGEL, W. M. DOIZAKI AND L. ZIEVE, *J. Lipid Res.*, 3 (1962) 138.
[8] H. M. BREGOFF, E. ROBERTS AND C. C. DELWICHE, *J. Biol. Chem.*, 205 (1953) 565.
[9] T. J. HEATH, E. P. ADAMS AND B. MORRIS, submitted for publication.
[10] I. STERN AND B. SHAPIRO, *J. Clin. Pathol.*, 6 (1953) 158.
[11] A. F. HOFMANN, in P. DESNUELLE, *Enzymes of Lipid Metabolism*, Pergamon Press, London, 1961, p. 158.

Received August 16th, 1963

Biochim. Biophys. Acta, 70 (1963) 688–690

SC 2344

The origin of hydrogen in fatty acids formed by lactating rat mammary gland

It is generally accepted that, of the 4 hydrogens in each ($-CH_2-CH_2-$) group in fatty acids, the two on the odd-numbered carbons are derived from reduced pyridine nucleotides, predominantly TPNH[1]. Of the 2 hydrogens on the even-numbered carbons, one is derived from acetyl-CoA and the other from water[1].

FOSTER AND BLOOM[2,3] have shown that more than half of the hydrogens on the even-numbered carbons of the fatty acids formed by rat-liver slices is derived from water, indicating an exchange between the methyl hydrogen atoms of acetyl-CoA and the protons of water.

The present report deals with incorporation of ^3H and carbon from acetate and glucose into fatty acids by lactating rat mammary gland preparations. In this tissue, labilization of the methyl hydrogen (that derived from acetate or glucose, and appearing on the even-numbered carbons of the fatty acid) also occurs. But in this case the exchange with the protons of water is less than that observed with liver slices. The value for the $^3H/^{14}C$ ratio was about 0.3 (Table I) in fatty acids synthesized by mammary gland slices from [2-^3H,^{14}C]acetate in the presence of glucose or from [6-^3H,^{14}C]glucose in the presence of acetate. The values that have been reported for liver slices, however, are 0.2 for acetate and 0.1 for glucose[2,4]; the difference between the acetate and glucose ratios for liver was attributed by FOSTER AND BLOOM[2,4] to labilization of the 6-^3H from glucose at the phosphoenol pyruvate stage[5]. The reason

for the lack of labilization of hydrogen at this stage in lactating mammary gland is not apparent.

WAKIL[1] has proposed that all 3 hydrogens attached to the methyl carbon of

TABLE I

CONVERSION OF [1-^{3}H,^{14}C]GLUCOSE, [6-^{3}H,^{14}C]GLUCOSE AND [2-^{3}H,^{14}C]ACETATE TO CO_2 AND FATTY ACIDS BY LACTATING RAT MAMMARY GLAND SLICES

250 mg of lactating rat (18–19 days post partum) mammary gland slices were incubated for 3 h at 37° in 2.5 ml of Krebs–Henseleit bicarbonate buffer (pH 7.3) containing 25 μmoles of labeled or unlabeled glucose and acetate as indicated below. Gas phase was 95% O_2–5% CO_2. The fatty acids and CO_2 were isolated and assayed for isotopic activity as described by ABRAHAM *et al.*[6]. The values reported below are the averages of 2 closely agreeing determinations from experiments with 2 rats.

Substrate		*% of added label recovered in:*		*Fatty acid $^3H/^{14}C$ ratio*
Labeled	*Unlabeled*	*CO_2*	*Fatty acids*	
[1-^{3}H]Glucose	Acetate		21	2.6
[1-^{14}C]Glucose	Acetate	23	8	
[6-^{3}H]Glucose	Acetate		8	0.3
[6-^{14}C]Glucose	Acetate	2.3	27	
[2-^{3}H]Acetate	Glucose		5.4	0.3
[2-^{14}C]Acetate	Glucose	1.0	19	

acetate are retained when acetyl-CoA is incorporated into the terminal, methyl end of the fatty acid chain and that 2 of these 3 hydrogens are lost during chain elongation via malonyl-CoA. Thus, the value for the $^3H/^{14}C$ ratio in fatty acids depends upon chain length. For example, the value for the $^3H/^{14}C$ ratio in the case of decanoic acid is $(1 + 4 \cdot 0.333)/5 = 0.47$, and for palmitate, $(1 + 7 \cdot 0.333)/8 = 0.42$. The predominant fatty acid formed from glucose or acetate by lactating rat mammary gland is decanoic acid[6,7]. In this tissue (Table I), therefore, 0.30/0.47, or about 64%, of the maximal yield of the ^3H bound to the methyl carbon of acetate or C-6 of glucose (which yields C-3 of pyruvate) is recovered in fatty acids. Thus only 36% of the H bound to the methyl carbon of acetate exchanges with the hydrogens of water. Data reported elsewhere[8] are consistent with this conclusion. Such labilization may occur between the H of the β-C of malonyl-CoA and that of water as suggested by FOSTER AND BLOOM[2]. In Table II the values for the $^3H/^{14}C$ ratio in the fatty acids synthesized from acetate by mammary-gland homogenates were about 0.3 and the values for liver homogenates were about 0.16. Thus, the difference in labilization of acetate-bound H can also be demonstrated in homogenates of these two tissues.

From the data in Table I we can also estimate H transfer from glucose to odd-numbered C atoms of the fatty acids via TPNH. The ^3H bound to C-1 of glucose can be incorporated into fatty acids by two pathways: (1) via the Embden–Meyerhof by conversion to pyruvate and acetyl-CoA and (2) by transfer via TPNH formed in the pentose cycle, followed by reduction of the keto-acyl derivatives to fatty acids.

TABLE II

FATTY ACID SYNTHESIS FROM [2-^{3}H,^{14}C]ACETATE BY HOMOGENATES OF LACTATING RAT MAMMARY GLAND AND LIVER

Tissue homogenates were incubated for 2 h with 6 μmoles of labeled acetate under optimal conditions as described by ABRAHAM *et al.* for liver[10] and for lactating rat mammary glands[6]. Fatty acids were isolated as described elsewhere[6]. The values recorded below are the averages of 2 closely agreeing determinations from experiments with 3 rats each.

Tissue	*Homogenate fraction incubated*		*% of added label recovered in fatty acids from:*		*Fatty acid $^3H/^{14}C$ ratio*
	Fraction	*Protein (mg)*	*[2-^{3}H]Acetate*	*[2-^{14}C]Acetate*	
Mammary gland	particle-free supernatant	11	10	30	0.33
Liver	particle-free supernatant	21			
	plus microsomes	4.5	3.3	21	0.16

The value for the $^3H/^{14}C$ ratio by Pathway 1 is likely to be the same for glucose labeled in positions 1 and 6. The incorporation of [1-^{3}H]glucose via Pathway 1 can be calculated from the incorporation of the ^{14}C of [1-^{14}C]glucose into fatty acids and the value for the $^3H/^{14}C$ ratio observed with [6-^{3}H,^{14}C]glucose. When glucose was the labeled substrate, 8 % $\times$ 0.3, or 2.4 %, of the ^{3}H bound to C-1 was incorporated into fatty acids via Pathway 1, and 21 % $-$ 2.4 %, or about 19 %, via Pathway 2.

From these experiments we can approximate the radiochemical TPNH yield via the pentose cycle. In the conversion of [1-^{3}H,^{14}C]glucose to hexose 6-phosphate, the fates of the carbon and the hydrogen are identical, and the dilution of ^{14}C and ^{3}H via the pentose cycle would therefore be the same[9]. The yield of $^{14}CO_2$ from C-1 of glucose by decarboxylation corresponds to the formation of TPNH from [1-^{3}H]-glucose. The $^{14}CO_2$ yield via the pentose cycle will be less than the total $^{14}CO_2$ yield from [1-^{14}C]glucose, since the total contains some formed via the Embden–Meyerhof pathway. Thus, the $^{14}CO_2$ yield via the pentose cycle will be less than the $^{14}CO_2$ yield from [1-^{14}C]glucose, but more than the difference in the $^{14}CO_2$ yields from [1-^{14}C]- and [6-^{14}C]glucose[9]. The $^{14}CO_2$ yield via the pentose cycle was between 23 % and 21 (23 minus 2) % (Table I). The radiochemical TPNH yield was therefore about 22 %. This yield was accompanied by a recovery of 19 % of ^{3}H in fatty acids via Pathway 2. The remainder of the TPNH hydrogen (3 %) may either have been used for other reductive processes or lost via oxidation or exchange processes, to appear ultimately in water. While the latter calculation is not a precise estimate of the labilization of the TPNH tritium, it does not exceed 3/22, or 14 %. Experiments reported elsewhere[8] on incorporation of ^{3}H from ^3H_2O into fatty acids by lactating rat mammary glands and adipose tissue slices also indicate a limited exchange between the hydrogens of water and those of fatty acids.

Recently FOSTER AND BLOOM[3] suggested that, in the transfer of the reductive hydrogen of TPNH by rat-liver slices to odd-numbered C atoms of fatty acids, essentially complete equilibration with water occurs. The difference between lactating rat mammary gland and liver may be related to their different lipogenic activities. In the experiments of FOSTER AND BLOOM[4] the incorporation of [1-^{14}C]glucose into fatty acids was less than 0.1 %, as compared with 8 % in our experiments (Table I). Because of the presence of large amounts of endogenous carbohydrate in liver, these

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.

This investigation was supported by grants from the National Science Foundation to I.L.C. and the U.S. Public Health Service (No. A-3682) to J.K.

Department of Physiology, University of California, Berkeley, Calif. (U.S.A.), and The Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles, Calif. (U.S.A.)

S. ABRAHAM
JOSEPH KATZ*
J. BARTLEY
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.

Received June 24th, 1963

* Established Investigator of the American Heart Association.

SC 2333

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 reported[1–14]. 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 plasma[8] 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

* Issued as *NRC Bull.* No. 7669.