A NEW PATHWAY FOR THE SYNTHESIS OF FATTY ACIDS: A ROLE FOR PHOSPHOENOLPYRUVATE CARBOXYLASE IN LIPOGENESIS

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1. Introduction

Largely through studies on the rat liver, acetyl CoA for lipid synthesis in the cytosol is thought to be formed predominantly within the mitochondrion. As it has a low mitochondrial permeability a scheme involving citrate transport from the mitochondrion and its subsequent breakdown to acetyl CoA and oxaloacetate by the action of ATP-citrate lyase has been proposed [1–5]. The rate of incorporation of radioactively labelled citrate into lipid by subcellular fractions of rat liver has supported this pathway [3,6], but an investigation into lipid synthesis in mouse tissues has not [7]. The rat liver pathway is also supported by observations on its sensitivity to the specific ATP-citrate lyase inhibitor, (−)-hydroxycitrate [8].

The fetal liver of the guinea pig at about two-thirds of gestation synthesises fatty acids at a high rate largely from short-chain fatty acids rather than glucose [9,10]. Its insensitivity to hydroxycitrate suggests that it may not entirely involve the normal mitochondrial pathway. This could partly be explained by cytosolic acetyl CoA production with acetyl CoA synthetase, but that would not explain the inhibition of lipid synthesis by quinolinate, the low acetyl CoA carboxylase activity in the fetal liver (~ 0.5 μmol fatty acid equivalents/h per g compared with fatty acid synthesis of about 1 μmol/h per g) or the totally different developmental pattern for the incorporation of pyruvate into lipid compared with that for glycerol and glucose [9,10]. The quinolinate inhibition may not be directly related to the flow of glucose carbon into lipid precursors, but this seems a possibility as quinolinate in vivo inhibits the production of oxaloacetate from phosphoenolpyruvate but not necessarily phosphoenolpyruvate production from oxaloacetate [11].

The present work describes the flow of glucose carbon into lipid. It suggests that this does not occur via pyruvate. The incorporation of phosphoenolpyruvate into lipid occurs at a high rate indicating that phosphoenolpyruvate carboxylase is probably involved in a pathway for lipid synthesis. Inhibition studies suggest that propionyl CoA is also an intermediate and a scheme involving conversion of oxaloacetate to succinyl CoA then its subsequent conversion to propionyl CoA is proposed.

2. Methods

The animals used and the methods of tissue preparation and incubation have been described previously [9]. Subcellular fractions of the fetal liver were prepared as described by DeDuve et al. [12] after homogenisation in 4 vol 0.25 M sucrose containing 1 mM EDTA, 1 mM glutathione, 5 mM $MgCl_2$ and 10 mM triethanolamine-HCl, pH 7.5. After washing the mitochondrial fraction it contained < 1% of the total lactate dehydrogenase activity and the cytosolic fraction contained < 0.5% of the total glutamate dehydrogenase activity. A combined cytosolic (0.05 ml) and washed mitochondrial fraction (0.05 ml) was incubated in 20 mM glycylglycine, pH 7.4, containing 30 mM $KHCO_3$, 9 mM dithiothreitol, 15 mM $MgCl_2$, 4 mM potassium phosphate, 5 mM 2-ketoglutarate, 5 mM alanine, 2 mM ATP,

4 mM glucose 6-phosphate, 1 mM NADPH, 1 mM NADH, 0.2 mM CoASH (final vol. 0.5 ml) for 20 min at 37°C. Preincubation with citrate had no effect on incorporation which was started by the addition of either 10 mM [1-^{14}C]phosphoenolpyruvate or 0.12 mM [1,3-^{14}C]malonyl CoA at zero time. In the malonyl CoA incorporations 0.1 mM acetyl CoA was also present. Reactions were stopped with 0.5 ml 10% (w/v) ethanolic KOH and lipids were saponified by boiling for 2 h. After acidification lipids were extracted with 3 × 2 ml light petroleum (bp 40–60°C) then washed with 3 × 10 ml water and dried over anhydrous Na_2SO_4. A sample was added to a toluene-based scintillation fluid and counted in a Phillips PW 4510 counter. The distribution of radioactivity in the lipid extracts was determined by t.l.c. on silica gel G with a two solvent system of isopropyl ether-acetic acid (96 : 4, v/v) followed by light petroleum–diethyl ether–acetic acid (90 : 10 : 1, v/v/v) [13]. The thin-layer plates were scanned with a Berthold Radio-chromatogram Scanner.

3. Results and discussion

The maximum rates of lipid synthesis in the fetal guinea pig liver occur at 45–50 days [9,10] and the maximum rates of synthesis from glucose occur at

Table 1
Rates of incorporation of pyruvate carbon atoms into lipid by slices of fetal guinea pig liver

Precursor (2 mM)	Incorporation, nmoles/min per g			
	45–47 day fetus		56–57 day fetus	
	Incorporation	% Carbon source	Incorporation	% Carbon source
[1-^{14}C]pyruvate	0.65 ± 0.21	3.61 ± 1.10	0.46 ± 0.20	1.95 ± 0.89
[2-^{14}C]pyruvate	8.22 ± 2.60	44.1 ± 11.7	10.6 ± 3.70	46.3 ± 14.7
[3-^{14}C]pyruvate	9.78 ± 2.1	51.9 ± 14.3	12.8 ± 3.80	55.9 ± 13.5
[U-^{14}C]pyruvate	6.23 ± 2.41	100	7.59 ± 2.50	100

Slices were incubated in Krebs bicarbonate buffer containing 10 mM glucose and 2 mM [^{14}C]pyruvate (1 μCi) for 60 min. The results are the means of 5–6 incubations ± SD.

Table 2
Rates of incorporation of glucose carbon into lipid by slices of fetal guinea pig liver

Precursor (10 mM)	Incorporation, nmoles/min per g			
	45–47 day fetus		55–57 day fetus	
	Incorporation	% Carbon source	Incorporation	% Carbon source
[1-^{14}C]glucose	0.61 ± 0.15	4.40 ± 1.0	3.21 ± 0.55	15.8 ± 3.9
[2-^{14}C]glucose	2.00 ± 0.60	14.8 ± 3.6	2.32 ± 0.22	11.2 ± 1.6
[3,4-^{14}C]glucose	1.23 ± 0.37	19.7 ± 4.0	4.71 ± 1.24	45.8 ± 8.3
[6-^{14}C]glucose	3.85 ± 1.28	27.1 ± 5.9	3.00 ± 0.92	14.2 ± 2.5
[U-^{14}C]glucose	2.30 ± 0.48	100	3.45 ± 0.75	100
[5-^{14}C]glucose[a]	4.88 ± 1.59	34.0 ± 8.9	2.78 ± 0.86	13.0 ± 2.2

Slices were incubated in Krebs bicarbonate buffer containing 10 mM glucose (1 μCi) for 60 min. The results are the means ± SD of 5-6 incubations.
[a] Determined by subtraction.

55–58 days [9,14]. If pyruvate is the immediate precursor of acetyl CoA in a pathway of lipid synthesis from glucose then little incorporation of [1-^{14}C] pyruvate into fatty acid should occur. This is exactly what is seen in liver slices from 45–47 day and 56–57 day fetal guinea pigs (table 1). Similarly the incorporation of either [3-^{14}C]glucose or [4-^{14}C] glucose into lipid should be very low. However, while the incorporation of [3,4-^{14}C]glucose into lipid was lower at 45–47 days than either [2-^{14}C] - or [6-^{14}C]glucose it was higher at 55–57 days (table 2). This suggests that the major pathway from glucose to

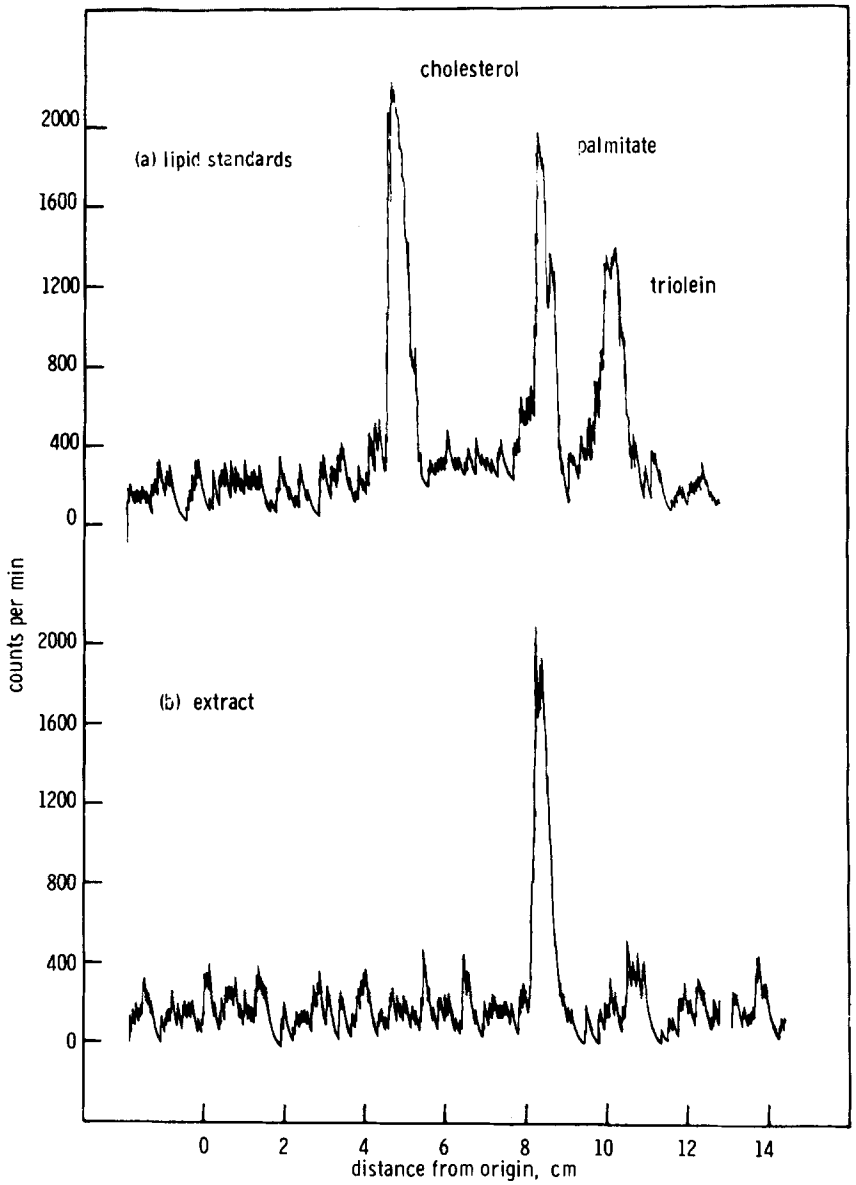

Fig.1. Thin-layer chromatography on silica gel G. of lipid standards and the lipids synthesised by subcellular fractions of fetal guinea pig liver. (a) chromatography of a mixture of [1-^{14}C]palmitate, glyceryl tri-[1-^{14}C]oleate and [4-^{14}C]cholesterol, (b) chromatography of a saponified lipid extract obtained from three cytosol-mitochondrial incubations with [1-^{14}C]phosphoenol-pyruvate. Thin layer plates were counted with a Radiochromatogram Scanner.

lipid does not involve pyruvate as an intermediate. The similarity of the developmental changes in glucose and glycerol incorporation compared with that of pyruvate [9] suggested that the pathway for lipid synthesis from glucose may diverge from the glycolytic pathway somewhere between glyceraldehyde 3-phosphate and pyruvate. In addition the inhibition of lipogenesis by quinolinate [9] and the relatively high activities of mitochondrial phosphoenolpyruvate carboxylase in the fetal liver [15] indicated that phosphoenolpyruvate might be that point of divergence.

Incubation of mitochondrial and cytosolic fractions from the fetal liver with [1-^{14}C]phosphoenolpyruvate should produce little incorporation into lipid if metabolised by the mitochondrial pyruvate-acetyl CoA pathway. However [1-^{14}C]phosphoenolpyruvate was incorporated by a time, temperature and concentration dependent process at a rate that was as high as the rate of acetate or propionate incorporation and higher than the rate of glucose or pyruvate incorporation into lipid by liver slices. Also its rate of incorporation was not much less than that for malonyl CoA. The incorporation was predominantly into fatty acid (70–85%) as determined by t.l.c (fig.1). Its requirement for IDP and $KHCO_3$ and the inhibition by quinolinate indicates that phosphoenolpyruvate carboxylase catalyses the first step in the incorporation. The large reduction by propionyl CoA but not by acetyl CoA or malonyl CoA suggests that propionyl CoA is an intermediate in the pathway but acetyl CoA and malonyl CoA are not. The only known pathway in which C-1 carbon from phosphoenolpyruvate or the C-1, C-4 carbon of oxaloacetate would not be completely lost on incorporation involves tapping-off from the tricarboxylic acid or glyoxylate cycles. No significant incorporation of glyoxylate into lipid was observed, while the phosphoenolpyruvate incorporation was inhibited by malate, succinate and malonate but not by citrate or by the removal of 2-ketoglutarate. A decarboxylation pathway via malonate is unlikely as malonate incorporation into lipid was slow. Also a pathway involving the conversion of oxaloacetate to 3-hydroxybutyrate then to

Table 3
The incorporation of phosphoenolpyruvate into lipid by a combined mitochondrial and cytosolic fraction from the fetal guinea pig liver

Precursor	System	Incorporation, nmoles/min per g	
		44–48 days	52–55 days
10 mM [1-^{14}C]PEP	Complete	40.4 ± 7.2	79.2 ± 21.3
	– IDP	10.4 ± 6.1	20.6 ± 9.70
	– $KHCO_3$	8.6 ± 4.2	19.5 ± 8.4
	– CoA	21.2 ± 10.5	28.7 ± 9.1
	+ 10 mM Quinolinate	13.8 ± 7.1	22.6 ± 11.4
	+ 1 mM (–)-hydroxycitrate	37.4[a]	67.8[a]
	+ 10 mM citrate	36.9 ± 11.7	64.8 ± 15.5
	+ 10 mM malate	22.1 ± 8.4	33.2 ± 11.9
	+ 10 mM aspartate	24.6 ± 8.4	50.2 ± 9.7
	+ 10 mM succinate	17.6 ± 8.2	33.8 ± 16.6
	+ 10 mM malonate	20.3 ± 11.0	36.1 ± 11.7
	+ 0.1 mM acetyl CoA	38.7 ± 19.4	70.8 ± 24.5
	+ 0.1 mM malonyl CoA	49.2 ± 18.0	102 ± 29.4
	+ 0.1 mM propionyl CoA	2.80 ± 2.4	7.1 ± 5.4
	+ 10 mM propionate	20.6 ± 6.8	27.4 ± 8.3
0.12 mM [1,3-^{14}C]malonyl CoA		142 ± 51.3	115 ± 37.6

The results are the means ± SD of 4-6 incubations except for [a] which is the mean of 2 incubations.

acetoacetate, a possibility since Rous and Favarger [16] have indicated that acetoacetyl CoA may be a lipid precursor in mouse liver and adipose and in rat adipose, is unlikely as there is no detectable conversion of 3-hydroxybutyrate to acetoacetate in the 50 day fetal guinea pig liver. The production of acetoacetyl CoA from [1-^{14}C]phosphoenolpyruvate via acetyl CoA thiolase would involve loss of the C-1 carbon of phosphoenolpyruvate.

The most likely pathway involves the production of succinyl CoA in the tricarboxylic acid cycle, its subsequent conversion to propionyl CoA then the direct incorporation of propionyl CoA into fatty acid. Whether this involves the tricarboxylic acid cycle in the forward or reverse direction is not clear. The available evidence indicates that reversal is possible as the pathway of oxaloacetate → succinyl CoA is reversible and the mitochondrial NAD^+/NADH ratio in the fetal guinea pig liver, calculated from the glutamate dehydrogenase reaction, is low [17].

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