SYNTHESIS OF ODD-NUMBERED FATTY ACIDS BY SUB-CELLULAR FRACTIONS FROM THE LIVER OF THE FETAL GUINEA PIG

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1. Introduction

Preparations of fetal guinea-pig liver can incorporate phosphoenolpyruvate into fatty acids by a pathway which does not involve pyruvate as an intermediate, but which depends on the activity of the mitochondrial enzyme phosphoenolpyruvate carboxylase [1]. The present report amplifies these findings by demonstrating that the utilization of phosphoenolpyruvate leads to the formation of long-chain, odd-numbered fatty acids (mainly 15:0 and 17:0), particularly when the system is supplemented with HCO_3^- and malonyl CoA. The original suggestion [1] that propionyl CoA is formed and that it can act as primer unit for fatty acid synthesis is now supported by further evidence.

2. Materials and methods

The animals used, the preparation of a combined 100 000 × *g* supernatant and mitochondrial fraction, and the methods of incubation were as described [1,2]. The incubation mixture included one of a series of potential precursors of fatty acids, namely (1) [2-^{14}C]malonyl CoA, (2) [2-^{14}C]malonyl CoA plus propionate, (3) [2-^{14}C]methylmalonyl CoA plus malonyl CoA, (4) [1-^{14}C]propionyl CoA plus malonyl CoA, (5) [1-^{14}C]phosphoenolpyruvate, (6) [1-^{14}C]phosphoenolpyruvate plus malonyl CoA, or (7) $H^{14}CO_3^-$ plus malonyl CoA and phosphoenolpyruvate. After incubation for 30 min, 0.2 vol incubation mixture was saponified and, following acidification, the fatty acids were extracted into light petroleum (b.p. 40–60°C) and their radioactivity determined as before [1,2]. The reaction in the remainder of the incubation mixture was stopped by the addition of 20 vol. chloroform/methanol (2:1, v/v) [3]; the resulting mixture was immediately filtered and the filter washed with 4 vol. 0.74% (w/v) KCl. The chloroform phase was washed three times with 10 vol. methanol/water (1:1, v/v) and evaporated to dryness in vacuum. The residual lipid was dissolved in 6 ml chloroform, which was then stored in the dark until it was analysed further. Following removal of the chloroform in a stream of N_2, methyl esters of the component fatty acids were prepared by treating the mixture with a methanolic solution of sodium methoxide as in [4]. Aliquots of the resulting solution were taken for direct determination of the composition and radioactivity of the fatty acid methyl esters in a Pye 104 gas chromatograph which was coupled to a Panax radio-gas detector. The liquid phase of the column was 15% (w/w) EGSS-X on a support of acid-washed Celite and the carrier gas was 5% CO_2 in argon at a flow rate of 50 ml/min. The oven temperature was 176°C and the effluent gas was split 25:1 in favour of the oxidising furnace, the remainder passing to the

flame-ionization detector. The oxidising furnace, which was operated at 700°C, consisted of a stainless steel tube containing copper oxide. The counting efficiency for ^{14}C of the Panax proportional counter was 80–90%.

The $NaH^{14}CO_3$ and some of the [1-^{14}C]phosphoenolpyruvate were obtained from the Radiochemical Centre, Amersham, the [2-^{14}C]malonyl CoA, [1-^{14}C]-propionyl CoA, [2-^{14}C]methylmalonyl CoA and some of the [1-^{14}C]phosphoenolpyruvate from New England Nuclear, Dreieichenhain, FRG. Propionyl CoA was obtained from P. L. Biochemicals, Milwaukee, USA. All other chemicals were obtained from British Drug Houses, Poole, or Sigma, Kingston-on-Thames.

3. Results and discussion

When the combined mitochondrial and cytosolic fractions from the livers of 49–52 day fetal guinea pigs were incubated with [1-^{14}C]malonyl CoA the principal non-volatile, lipid products were the even-numbered fatty acid 16:0 and the odd-numbered acids 15:0 and 17:0 together with a small amount of 18:0 (fig.1a, table 1). The propionyl CoA required for the synthesis of 15:0 and 17:0 must have been derived from endogenous substrate or one of other components of the incubation mixture, namely 2-ketoglutarate, malate, alanine or glucose-6-phosphate. When propionate was added the synthesis of odd-numbered fatty acids (13:0, 15:0, 17:0) was

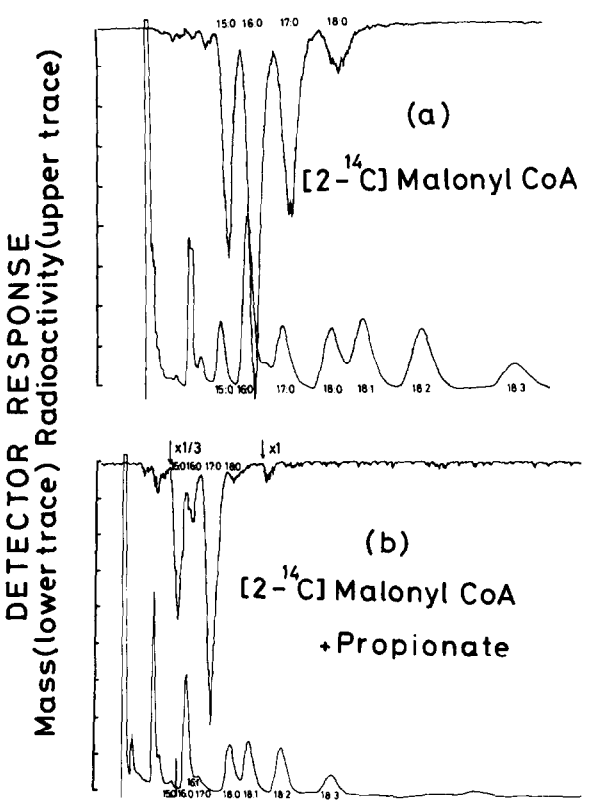

Fig.1. Gas–liquid chromatography of methyl esters of fatty acids after incubation of combined mitochondrial and cytosolic fractions of a 50 day fetal guinea pig liver with (a) [2-^{14}C]malonyl CoA; 15:0 and 17:0 were added to the extract, (b) [2-^{14}C]malonyl CoA plus 10 mM propionate. Other details as in table 1.

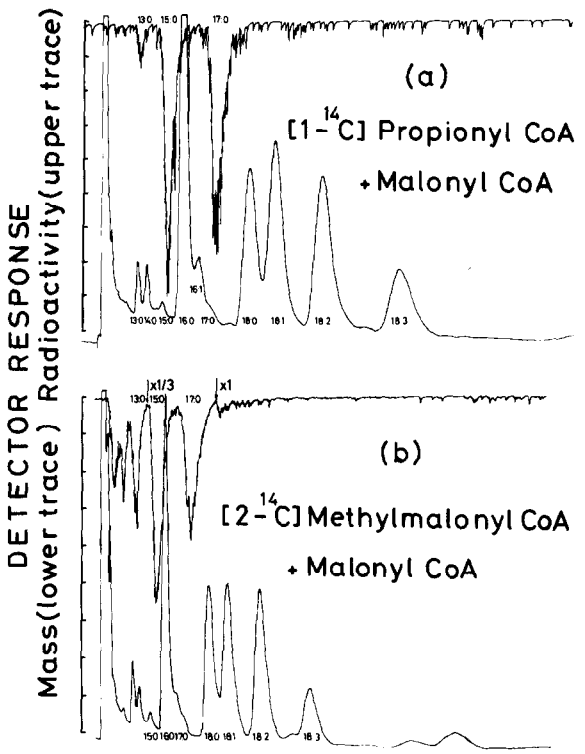

Fig.2. Gas–liquid chromatography of methyl esters of fatty acids after incubation of combined mitochondrial and cytosolic fractions of a 49 day fetal guinea pig liver with (a) [1-^{14}C]propionyl CoA, (b) [2-^{14}C]methylmalonyl CoA. Other details as in fig.1.

Table 1
Synthesis of fatty acids from various precursors by combined mitochondrial and cytosolic fractions from fetal guinea pig liver

Fatty acid	< 13:0	13:0	14:0	15:0	16:0	17:0	18:0	16:1	18:1	> 18:0
Precursor(s)	Incorporation (nmol/min/g)									
[2-^{14}C]Malonyl CoA, 0.12 mM	0.28 ± 0.03	0.88 ± 0.2	0.44 ± 0.22	19.4 ± 6.7	30.6 ± 4.6	20.2 ± 7.9	9.8 ± 3.4	–	–	–
[2-^{14}C]Malonyl CoA, 0.12 mM + propionate, 10 mM	0.48 ± 0.04	1.86 ± 1.4	1.82 ± 1.41	32.2 ± 4.9	15.3 ± 7.5	44.9 ± 8.9	4.34 ± 0.7	–	–	0.77 ± 0.25
[2-^{14}C]Methylmalonyl CoA, 0.1 mM + malonyl CoA, 0.1 mM	1.56 ± 1.5	1.79 ± 0.34	–	16.1 ± 3.9	–	16.6 ± 5.1	0.59 ± 0.2	–	–	0.17 ± 0.17
[1-^{14}C]Propionyl CoA, 0.1 mM + malonyl CoA, 0.1 mM	0.25 ± 0.27	0.31 ± 0.20	–	3.1 ± 1.5	–	2.6 ± 1.6	–	–	–	–

Results are the means ± SD of 2–4 incubations; (–) indicates that no incorporation was detected. Subcellular fractions of 49–52 day fetal guinea pig liver were prepared as described [6], then 0.05 ml mitochondrial fraction plus 0.05 ml of the 100 000 × *g* supernatant [1] were incubated for 20–30 min at 37°C in 0.5 ml containing 20 mM glycylglycine, 30 mM $KHCO_3$, 9 mM dithiothreitol, 15 mM $MgCl_2$, 4 mM potassium phosphate, 5 mM 2-ketoglutarate (or 5 mM malate), 5 mM alanine, 2 mM ATP, 4 mM glucose 6-phosphate, 1 mM NADPH, 1 mM NADH, 0.2 mM CoASH, 0.1 mM acetyl CoA and the precursors listed above. Fatty acids were extracted and separated as described under Materials and methods

Table 2
Synthesis of fatty acids from phosphoenolpyruvate by combined mitochondrial and cytosolic fractions from fetal guinea pig liver

Fatty acid	< 13:0	13:0	14:0	15:0	16:0	17:0	18:0	16:1	18:1	> 18:0
Precursor(s)	Incorporation (nmol/min/g)									
[1-^{14}C]Phosphoenolpyruvate, 5 mM	0.37 ± 0.21	0.53 ± 0.32	–	1.8 ± 1.5	–	1.46 ± 0.7	–	–	–	–
[1-^{14}C]Phosphoenolpyruvate, 5 mM + malonyl CoA, 0.1 mM	1.8 ± 1.5	2.5 ± 0.7	–	16.8 ± 9.0	–	14.6 ± 7.2	–	–	–	–
$H^{14}CO_3^-$ + malonyl CoA, 0.1 mM + phosphoenolpyruvate 5 mM	4.2 ± 1.4	3.7 ± 0.6	–	23.5 ± 4.4	3.4 ± 2.4	16.2 ± 6.5	1.5 ± 0.3	–	–	–

Results are the means ± SD of 2–4 incubations; (–) indicates that no incorporation was detected. Incubation conditions were as given in the footnote to table 1, except that acetyl CoA was not included

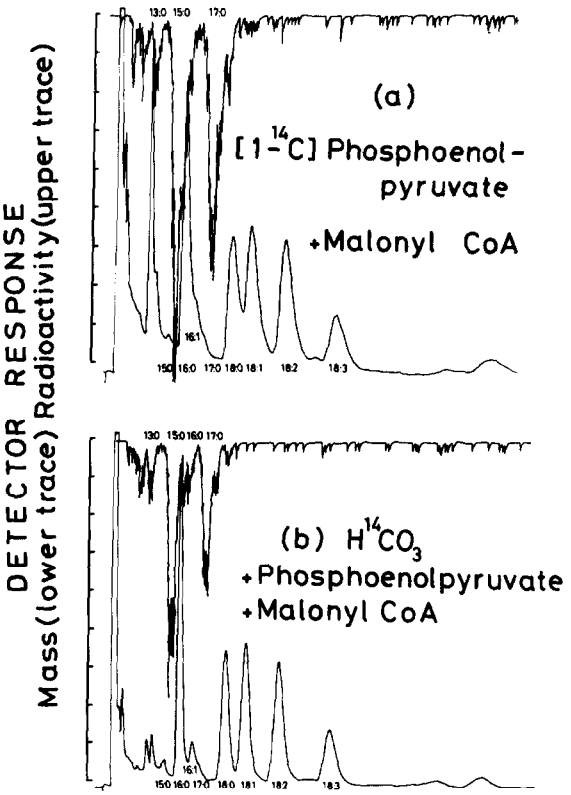

Fig.3. Gas–liquid chromatography of methyl esters of fatty acids after incubation of combined mitochondrial and cytosolic fractions of a 51 day fetal guinea pig liver with (a) [1-^{14}C]phosphoenolpyruvate, (b) $H^{14}CO_3^-$ + phosphoenolpyruvate. Other details as in fig.1 and tables 1 and 2.

enhanced, whilst that of even-numbered acids was diminished (fig.1b, table 1). Similar results were obtained when [1-^{14}C]propionyl CoA or [2-^{14}C]-methylmalonyl CoA was included, along with malonyl CoA, in the incubation mixture (fig.2, table 1), with the label appearing almost entirely in odd-numbered fatty acids. Thus methylmalonyl CoA was evidently decarboxylated to yield propionyl CoA.

When [1-^{14}C]phosphoenolpyruvate (alone or together with malonyl CoA) was employed as fatty acid precursor, the principal products of synthesis were again 13:0, 15:0 and 17:0 (fig.3a, table 2) at rates comparable to those with [2-^{14}C]malonyl CoA as the precursor. A similar pattern and rate of synthesis were also observed when the incubation system was supplied with $H^{14}CO_3^-$ together with unlabelled phosphoenolpyruvate and malonyl CoA (fig.3b, table 2), a result consistent with the production of oxaloacetate and its subsequent conversion to propionyl CoA. Evidence which strongly supports this proposed pathway derives from a recent (unpublished) observation that the presence of mercaptopicolinic acid (a known inhibitor of phosphoenolpyruvate carboxylase [5]) in the incubation medium causes a substantial reduction in the extent to which [1-^{14}C]phosphoenolpyruvate or $H^{14}CO_3^-$ in the presence of unlabelled phosphoenolpyruvate is incorporated into odd-numbered fatty acids.

In summary, the present results show that phosphoenolpyruvate can be utilized for the synthesis of odd-numbered fatty acids. The observations indicate that the probable pathway involves the production from phosphoenolpyruvate of oxaloacetate, followed by its metabolism in the tricarboxylic acid cycle to yield succinyl CoA which, in turn, can be converted to methylmalonyl CoA and then to propionyl CoA, the primer unit for the synthesis of odd-numbered fatty acids.

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