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Lipid Biosynthesis in Liver Slices of the Foetal Guinea Pig

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Lipid synthesis as measured by the incorporation of acetate or ^3H_2O into slices of foetal liver, is much higher than in slices of adult liver and shows a peak at about two-thirds of gestation. At this time the synthesis from glucose was low and reached a peak 10 days later. The changes in the activity of ATP citrate lyase, which mirrored acetate incorporation, and the effect of glucose and pyruvate on acetate incorporation into lipid suggests that some of the lipid synthesis occurs via intramitochondrial acetyl-CoA production from acetate. Despite this, lipid synthesis was not inhibited by (−)-hydroxycitrate. The low rate of synthesis from glucose at two-thirds of gestation is ascribed to the low activity of pyruvate carboxylase at this time, and a role for a phosphoenolpyruvate carboxykinase in providing oxaloacetate for lipogenesis is proposed. The activity of fatty acid synthetase broadly agreed with the changes in lipid synthesis, whereas the activity of acetyl-CoA carboxylase was barely sufficient to account for the rates of lipid synthesis *in vivo*. Acetate and short-chain fatty acids are likely to be the major precursors for lipid synthesis *in vivo*.

During foetal development rapid growth necessitates the production of large quantities of lipid. This is supplied both from the mother by transfer across the placenta (Van Duyne *et al.*, 1962; Van Duyne, 1965; Connor & Lins, 1967; Hershfield & Nemeth, 1968; Kayden *et al.*, 1969; Portman *et al.*, 1969; Szabo *et al.*, 1969) and through rapid rates of synthesis by the foetal tissues (Popják, 1954; Villee & Hagerman, 1958; Villee & Loring, 1961; Fain & Scow, 1966; Roux, 1966; Roux & Yoshioka, 1967; Taylor *et al.*, 1967; Ballard & Hanson, 1967*a*; Hanson & Ballard, 1968; Smith & Abraham, 1970; Jones, 1973). However, the changes in the rate of, control of and the supply of substrate for lipid biosynthesis in the foetal liver have not been investigated in detail. The foetal liver is a useful tool for the analysis of some of the factors controlling fatty acid synthesis. Except for late in gestation, it has a low glycogen concentration so that dilution of labelled glucose through glycogen breakdown is not a major problem (Salmon *et al.*, 1974). In addition, naturally occurring changes in the activity of enzymes presumed to be associated with lipid biosynthesis can be compared with changes in the rate of lipid synthesis from various substrates.

The rate of lipid biosynthesis *in vivo* and *in vitro* in the foetal guinea-pig liver is relatively high two-thirds of the way through gestation (Jones, 1973; Jones & Firmin, 1976) and in part may be maintained by the supply of short-chain fatty acids across the placenta. The present paper describes some of the factors controlling lipid biosynthesis in the liver of the foetal guinea pig at different stages of gestation. The presence of different pathways of fatty acid biosynthesis is discussed and a role for phosphoenolpyruvate carboxykinase in lipogenesis proposed.

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Experimental

Animals

Guinea pigs of the Dunkin–Hartley strain were mated and the time of conception was determined (±1.5 days) as described by Elvidge (1972). Pregnant animals were killed by a blow to the head and foetuses removed through a cut in the uterus. Foetal and maternal tissues were collected after bleeding from the neck.

Chemicals

NAD^+, NADH, $NADP^+$, CoA, malonyl-CoA, ATP, phosphenolpyruvate, L-malate, isocitrate, glucose 6-phosphate, oxaloacetate, triethanolamine hydrochloride and mercaptoethanol were purchased from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. Enzymes and 6-phosphogluconate were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K. Glutathione was obtained from Cambrian Chemicals, Croydon, Surrey, U.K., and dithiothreitol from Calbiochem, London W.1., U.K. Liacetyl-CoA was obtained from P.-L. Biochemicals, Milwaukee, Wis., U.S.A., and scintillation chemicals were from Koch–Light laboratories, Colnbrook, Bucks., U.K. 1,2,3-Benzenetricarboxylate was obtained from R. N. Emanuel, Wembley, Middx., U.K. Tris hydrochloride, sucrose

and H_2SO_4 were of Aristar grade, and all other routine chemicals were of analytical reagent grade and obtained from BDH Chemicals, Poole, Dorset, U.K. [1,3-^{14}C]Malonyl-CoA was obtained from N.E.N. Chemicals G.m.b.H., Dreieichenhain, West Germany. All other radioactive chemicals were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. L-Carnitine chloride was a gift from Dr. M. Umehara, Otsuka Pharmaceutical Co., Osaka, Japan. (−)-Hydroxycitrate was a gift from Dr. Y. S. Lewis, Central Food Technological Research Institute, Mysore, India.

Incubation of tissue

Slices of maternal or foetal liver (approx. 100mg, 0.35mm thick) were incubated for 30–90min at 37°C with shaking (110 oscillations/min) in 25ml conical flasks containing 3ml of Krebs bicarbonate saline (NaCl, 124mM; KCl, 5mM; KH_2PO_4, 1.2mM; $MgSO_4$, 1.3mM; $CaCl_2$, 2.8mM; $NaHCO_3$, 26mM; glucose, 10mM) together with the radioactive precursors (see below) and under an atmosphere of O_2+CO_2 (95:5). The incorporation was stopped with 3ml of 10% (w/v) KOH and the tissue lipids were saponified by heating in a boiling-water bath for 2h. After saponification the extract was acidified with 10M-H_2SO_4 and fatty acids and non-saponified lipids were extracted with 3×10ml of light petroleum (b.p. 40–60°C). The combined extract was washed with 3×30ml of 5% (w/v) acetic acid (or other precursor) and dried over anhydrous Na_2SO_4. A 10ml portion of the organic extract was evaporated to dryness in a glass counting vial, then 10ml of a toluene-based scintillation fluid [6g of 2,5-diphenyloxazole and 0.25g of 1,4-bis-(5-phenyloxazol-2-yl)benzene in 1 litre of toluene] added. The recovery of [1-^{14}C]-palmitate in the extraction procedure was 89.3±4.2 (6)%.

Fatty acid decarboxylation

Long-chain fatty acids from some of the incubations were decarboxylated in the Schmidt reaction by the method of Brady *et al.* (1960) adapted from that of Phares (1951). In the decarboxylation procedure 94.3±2.9 (6)% of the radioactivity in 1 μCi of [1-^{14}C]palmitate was recovered.

Enzyme extraction and assay

ATP citrate lyase. Liver was homogenized in a glass homogenizer with a motor-driven Teflon pestle with 9vol. of 0.4M-KCl in 20% (v/v) ethanol, and then centrifuged at 30000*g* and 2°C for 30min (Srere, 1959). ATP–citrate oxaloacetate-lyase (EC 4.1.3.8) activity was determined in the supernatant with a coupled-enzyme assay (Takeda *et al.*, 1969). Up to 0.1ml of supernatant was added to 125mM-Tris/HCl, pH8.4, containing 10mM-$MgCl_2$, 10mM-mercaptoethanol, 20mM-tripotassium citrate, 0.3mM-CoA, 0.2mM-NADH, 10mM-ATP, 1mM-KCN and 0.5 unit of malate dehydrogenase. The reaction was started with ATP and the decrease in E_{340} recorded at 25°C; it was normally linear for up to 30min. Enzyme blanks without CoA or ATP showed little change.

Acetyl-CoA carboxylase. Liver was homogenized in 9vol. of 0.2M-sucrose containing 20mM-triethanolamine hydrochloride, pH7.4, 1mM-EDTA and 2mM-glutathione, and then centrifuged for 30min at 30000*g* and 2°C. Acetyl-CoA–carbon dioxide ligase (ADP) (EC 6.4.1.2) activity was determined on the supernatant by the radiochemical carboxylation assay described by Vagelos *et al.* (1963) and Gregolin *et al.* (1968). The extract was preincubated with 5vol. of 80mM-Tris/HCl, pH7.5, 40mM-mercaptoethanol, 8.0mM-tripotassium citrate and 10mM-$MgCl_2$ at 25°C for 30min. The reaction system was completed by the addition of 8mM-ATP, 40mM-$NaH^{14}CO_3$ (1 μCi) and started with 0.2mM-acetyl-CoA. This was incubated in a final volume of 1ml for 30min at 25°C then stopped by the addition of 0.05ml of 5M-$HClO_4$. The protein precipitate was removed and 0.1–0.2ml of the supernatant was evaporated to dryness in a scintillation vial. The residue was dissolved in 1ml of water then 10ml of dioxan-based scintillation fluid [7g of 2,5-diphenyloxazole, 0.3g of 1,4-bis-(5-phenyloxazol-2-yl)benzene and 100g of naphthalene in 1 litre of dioxan] added. The incorporation was linear with time for at least 30min and dependent on the presence of acetyl-CoA and Mg^{2+}. Malonyl-CoA decarboxylase activity was very low.

Fatty acid synthetase. Liver extracts were prepared by homogenization with 10vol. of 0.32M-sucrose containing 1mM-EDTA followed by centrifugation for 60min at 100000*g* and 2°C. The supernatant fractions contained 85–93% of the total fatty acid synthetase activity which was determined by a modification of the method of Wakil *et al.* (1958). The reaction system contained 100mM-potassium phosphate, pH7.0, 2mM-NADPH, 5mM-$MgCl_2$, 5mM-dithiothreitol, 0.1mM-acetyl-CoA, 0.2mM-malonyl-CoA, 0.2 μCi of [1,3-^{14}C]malonyl-CoA in 0.5ml. Incubation was carried out under N_2 for 15min at 37°C. Reactions were stopped with 0.5ml of 10% (w/v) ethanolic KOH and lipids saponified by boiling for 2h. After saponification incorporation into lipid was determined as described above. It was almost linear with time for at least 30min and dependent on the presence of NADPH and acetyl-CoA.

Glucose 6-phosphate dehydrogenase. Liver was homogenized in 3vol. of 154mM-KCl, pH7.0, containing 0.66mM-EDTA, diluted 1:10 with homogenizing buffer. The homogenate was spun at 30000*g* and 2°C for 45min. D-Glucose 6-phosphate–$NADP^+$ oxidoreductase (EC 1.1.1.49) activity in the

supernatant was determined in 50mM-triethanolamine/HCl, pH7.5, containing 5mM-EDTA, 0.5mM-NADP and 0.66mM-glucose 6-phosphate by following the increase in E_{340} at 25°C. The presence of 6-phosphogluconate dehydrogenase in the tissue extracts was estimated to increase apparent glucose 6-phosphate dehydrogenase activity in the foetal liver by 1.61±0.2 (8) and the maternal liver by 1.43±0.17 (8). Enzyme activity was corrected for this additional NADPH production.

6-Phosphogluconate dehydrogenase. 6-Phospho-D-gluconate–$NADP^+$ oxidoreductase (EC 1.1.1.43) activity was extracted and determined as described above for glucose 6-phosphate dehydrogenase but with 1mM-6-phosphogluconate as substrate.

Isocitrate dehydrogenase. Liver extracts were prepared by homogenization with 4vol. of 0.88M-sucrose, pH7.0, containing 1mM-EDTA and 0.3mM-dithiothreitol followed by centrifugation at 40000*g* and 2°C for 60min. *threo*-D_s-Isocitrate–$NADP^+$ oxidoreductase (decarboxylating) (EC 1.1.1.42) was assayed by the method of Cleland *et al.* (1969). The reaction system contained 33mM-Tris/HCl, pH7.4, 0.33mM-EDTA, 1.3mM-$MnSO_4$, 1mM-KCN, 0.1mM-$NADP^+$, 0.1mM-dithiothreitol and 39mM-*threo*-D_s-(+)-isocitrate; final vol. 3ml. The reaction was started with isocitrate and increase in E_{340} followed at 25°C.

Malate dehydrogenase. Liver extracts were prepared as described for glucose 6-phosphate dehydrogenase. L-Malate–NAD^+ oxidoreductase (EC 1.1.1.37) was determined by the method of Ochoa (1955) on supernatant fractions in 50mM-Tris/HCl, pH7.4, and 0.3mM-NADH (NADPH). The reaction was started with 0.3mM-oxaloacetate and the decrease in E_{340} followed at 25°C.

'Malic' enzyme. Liver extracts were prepared by homogenization in 9vol. of 0.25M-sucrose and centrifugation at 30000*g* and 2°C for 30min. L-Malate–$NADP^+$ oxidoreductase (decarboxylating) (EC 1.1.1.40) activity was determined as described by Hsu & Lardy (1969).

Acetate determination. Blood samples were collected from the umbilical vein of the foetus or the inferior vena cava of the maternal guinea pig, centrifuged at 1500*g* and 2°C for 15min and plasma was collected. Protein was precipitated from the plasma by the addition of 0.5vol. of 1M-$HClO_4$ and the supernatant, after neutralization with 2M-$KHCO_3$, was used for acetate determination. Acetate concentrations were measured by using acetate thiokinase prepared from *Escherichia coli*. A plasma extract (0.2–0.5ml) was added to 66mM-triethanolamine, pH7.6, containing 1.5mM-$MgCl_2$, 6mM-ATP, 1mM-phosphoenolpyruvate, 0.4mM-NADH, 0.1mg of pyruvate kinase and 0.05mg of lactate dehydrogenase; final vol. 3ml. The reaction was started with 0.5mg of acetate thiokinase and the fall in E_{340} observed. Blanks omitting the acetate extract were included. The reaction was slow and was followed for 2h. By this time, in the acetate concentration range measured (0.05–0.25μmol) 90–95% of the reaction was complete.

Measurement of radioactivity

Radioactivity was determined in a Phillips PW 4510 liquid-scintillation counter. Counting efficiency was estimated by the channels-ratio method by using an external standard and a series of carbon tetrachloride-quenched [^{14}C]hexadecane standards.

Units of enzyme activity and kinetic analysis

The unit of enzyme activity used is μmol of substrate consumed or product synthesized/min, at the temperature of the assay. Michaelis constants were determined by the method of Lineweaver & Burk (1934) by using the weighting factors described by Wilkinson (1961).

Expression of results

Where appropriate the results are expressed as means±S.D., with the number of observations in parentheses.

Results

Incorporation into lipids

Short-chain fatty acid incorporation. The incorporation of [1-^{14}C]acetate into the lipid of slices of foetal guinea-pig liver was almost linear with time for at least 90min and was maximum at 2–3mM-acetate; above 3mM the incorporation declined. At 30 days the incorporation of acetate into lipid was low. It progressively rose to peak values at 45–50 days then declined so that by term it was very low (Fig. 1). After birth the incorporation remained low, as it was in the maternal liver [18.4±4.3 (22)nmol/90min per g wet wt.]. The presence of 5mM-L-carnitine chloride, 0.1 unit of insulin or 0.9mM-oleate (albumin-bound) had no significant effect on the acetate incorporation. The incorporation was substantially decreased by a range of inhibitors (Tables 1 and 2) but not by 1mM-(−)-hydroxycitrate. A decrease in the glucose concentration in the medium decreased acetate incorporation. With no added glucose, incorporation at 42–47 days was 17.9±7.6 (6)% of that observed at 10mM-glucose. Developmental changes in acetate incorporation similar to those reported above have been observed *in vivo* (Jones, 1973). The carboxylate carbon of the fatty acids, isolated either after injection of [1-^{14}C]acetate into the foetus or the pregnant guinea pig or after incubation of foetal liver slices with [1-^{14}C]acetate, contained between 12 and 16% of the total radioactivity. This suggests that mainly fatty acid synthesis *de novo* is being followed.

The pattern of incorporation of [U-^{14}C]propionate into lipid by foetal liver slices was similar, with values of 1.15±0.34 (5) and 0.38±0.1 (6) μmol/90min per g at 45–68 and 56–57 days respectively.

^3H_2O incorporation. The incorporation of ^3H_2O into lipid by slices of foetal liver, although significantly higher than acetate incorporation, showed a similar developmental change (Table 3). Incorporation in the presence of acetate was significantly higher than that with glucose alone. The ^3H_2O incorporation into lipids was decreased 60–90% by the metabolic inhibitors used (Tables 1 and 2) but not by (−)-hydroxycitrate.

Glucose incorporation. It has been reported that the pattern of [U-^{14}C]glucose incorporation into lipid was significantly different from that of [1-^{14}C]acetate, with low incorporation at 45 days, increasing two- to three-fold by 55–57 days then falling as term

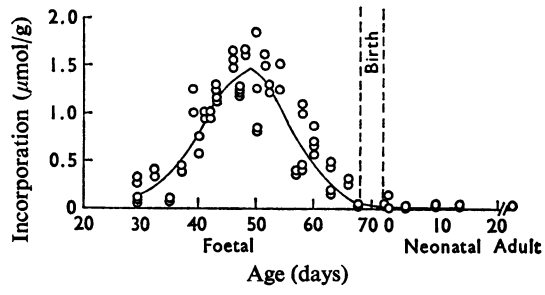

Fig. 1. *Incorporation of [1-^{14}C]acetate into lipid by the foetal, neonatal and adult liver*

Liver slices were incubated in 3ml of Krebs bicarbonate saline containing [1-^{14}C]acetate (1 μCi) for 90min at 37°C. Lipids were saponified, extracted and counted for radioactivity as described in the Experimental section. The curve was fitted by eye.

Table 1. *Inhibition of [^{14}C]acetate incorporation into lipids by the foetal guinea-pig liver*

Liver slices were incubated in 3ml of Krebs–bicarbonate saline containing 3.3mM-sodium [1-^{14}C]acetate (1 μCi) for 90min at 37°C. Inhibitors were present throughout the period of incubation. Lipids were saponified, extracted and counted for radioactivity as described in the Experimental section. The results are the means±S.D. of five incubations.

Additions	Incorporation (μmol/90min per g)	Inhibition (%)
Control	1.25 ±0.26	—
2,4-Dinitrophenol (0.2mM)	0.038 ±0.002	97.2± 2.1
2,4-Dinitrophenol (0.2mM) +NaF (5mM)	0.0052±0.0004	98.6± 1.4
1,2,3-Benzenetricarboxylate (10mM)	0.192 ±0.038	85.3± 4.8
(2mM)	0.392 ±0.057	67.6± 4.1
Quinolinate (10mM)	0.35 ±0.06	71 ±10.6
(2mM)	0.77 ±0.067	39.3± 7.4

Table 2. *Inhibition of lipid synthesis in the foetal liver by kynurenate*

Liver slices were incubated in 3ml of Krebs bicarbonate saline containing 3.3mM-[1-^{14}C]sodium acetate (1 μCi), 10mM-[U-^{14}C]glucose (1 μCi), 1mM-[U-^{14}C]leucine (1 μCi) or 2mCi of ^3H_2O. Kynurenate was present throughout the period of incubation. Lipids were saponified, extracted and counted for radioactivity as described in the Experimental section. The results are the means±S.D. of four to six incubations. Gestational age was 43–46 days.

	Incorporation (μmol/90min per g)	Inhibition (%)
Acetate		
Control	1.32 ±0.34	—
10mM-Kynurenate	0.42 ±0.17	68.9± 9.4
40mM-Kynurenate	0.21 ±0.1	83.8± 6.3
Glucose		
Control	0.24 ±0.71	—
10mM-Kynurenate	0.1 ±0.04	59.1±17.6
40mM-Kynurenate	0.044±0.019	80.8± 7.2
Leucine		
Control	0.042±0.011	—
10mM-Kynurenate	0.037±0.026	8.8± 4.6
40mM-Kynurenate	0.034±0.019	14.7±14.2
^3H_2O		
Control	3.61 ±0.98	—
10mM-Kynurenate	1.48 ±0.62	58.7± 7.3
40mM-Kynurenate	0.86 ±0.4	74.7±11.7

Table 3. *Incorporation of ^3H_2O into lipid by the liver of foetal, neonatal and maternal guinea pigs*

Liver slices were prepared and incubated in 3ml of Krebs bicarbonate saline for 90min at 37°C. The incubation medium contained 2mCi of ^3H_2O and 10mM-glucose with or without 3mM-acetate. At the end of the incubation incorporation was stopped and lipids were saponified, extracted, washed and counted for radioactivity as described in the Experimental section. The results are the means±S.D. of four to six observations. Glucose concentration was 10mM and acetate, 3.3mM. $^{14}C/^3H$ ratio for the glucose substrate was obtained from the μg-atoms of glucose C incorporated into lipid during incubation with [U-^{14}C]glucose (see the text).

Gestation (days) Substrate ...	Incorporation (μg-atoms of H/90min per g) Glucose	$^{14}C/^3H$	Glucose+acetate	$^{14}C/^3H$
Foetal				
44–47	2.4 ±1.1	0.31	4.5 ±1.02	0.53
55–57	1.8 ±0.72	0.8	2.4 ±0.84	0.48
63–65	0.87±0.43	0.2	1.08±0.33	0.41
Neonatal	0.21±0.1	0.1	0.36±0.18	0.2
Maternal	0.18±0.07	0.11	0.24±0.11	0.15

Table 4. *Interactions between glucose, pyruvate and acetate incorporation into lipid by the foetal guinea-pig liver*

Liver slices were incubated in 3ml of Krebs–bicarbonate saline containing 10mM-[U-^{14}C]glucose, 3mM-[U-^{14}C]pyruvate (1μCi) and, or 3mM-[1-^{14}C]acetate (1μCi) for 90min at 37°C. Lipids were saponified, extracted and counted for radioactivity as described in the Experimental section. The results are the means±S.D. of four incubations.

Gestational age (days) ...	Incorporation (μmol/90min per g) 43–47	55–57
[^{14}C]Pyruvate	0.7 ±0.19	0.82±0.25
[^{14}C]Pyruvate+acetate	0.38±0.19	0.6 ±0.12
[^{14}C]Acetate	1.25±0.31	0.59±0.18
[^{14}C]Acetate+pyruvate	0.75±0.32	1.06±0.43
[^{14}C]Glucose	0.15±0.06	0.27±0.11
[^{14}C]Glucose+acetate	0.42±0.14	0.38±0.15

approached (Jones, 1973). The glucose incorporation was decreased by 91.1±5.3 (5)% in the presence of 2,4-dinitrophenol and NaF, but was not inhibited by 1mM-(−)-hydroxycitrate and was less sensitive to kynurenate inhibition than was acetate (Table 2). Incubation of slices with [U-^{14}C]glucose in the presence of 3.3mM-acetate produced an approximately threefold increase in glucose incorporation at 45–47 days and a smaller increase at 55–57 days (Table 4).

Pyruvate and glycerol incorporation

[U-^{14}C]Pyruvate incorporation into lipids by foetal liver slices was relatively high and did not show a significant change during gestation. It was decreased by 3.3mM-acetate; the inhibition was greatest at 45 days. Pyruvate also inhibited [1-^{14}C]acetate incorporation into lipid at about 45 days and increased it at 55–57 days (Table 4). [U-^{14}C]Glycerol incorporation occurred at a similar rate to glucose incorporation rising from 0.18±0.02 (5)μmol/90min per g at 45 days to 0.31±0.1 (5)μmol/90min per g by 55–57 days.

Leucine incorporation

Studies on leucine incorporation into lipid were included since it is incorporated predominantly into cholesterol by slices of foetal guinea-pig liver (C. T. Jones & G. F. Fellows, unpublished work). This is supported by the insensitivity of leucine incorporation to inhibition by the acetyl-CoA carboxylase inhibitor (Hashimoto *et al.*, 1971) kynurenate (Table 2). Leucine incorporation into lipid was low [42±11 (5)nmol/90min per g] at 45–48 days, it had almost doubled [78±14.2 (6)nmol/90min per g] by 55–57 days then fell to very low values just before term.

Plasma acetate

The plasma acetate concentration in the inferior vena cava of the pregnant guinea pig was 0.14±0.032 (16)mM between day 35 and day 55 of pregnancy then fell to 0.047±0.028 (10)mM. Over the period of gestation that could be studied (44–67 days) the foetal umbilical vein plasma acetate concentration remained almost constant with a mean value of 0.11±0.05 (23)mM.

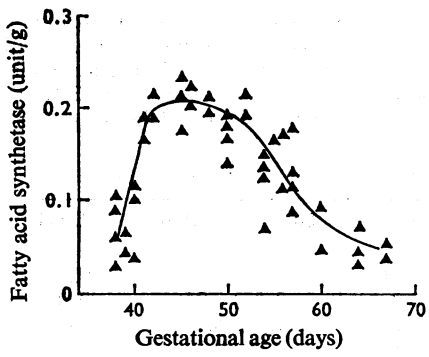

Fig. 2. *Activity of fatty acid synthetase in the liver of the foetal guinea pig*

Enzyme activity was determined on crude extracts of liver as described in the Experimental section.

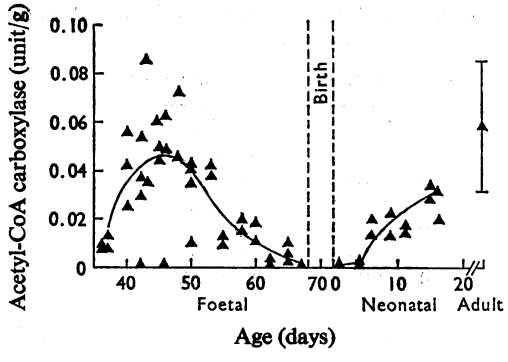

Fig. 3. *Activity of acetyl-CoA carboxylase in the liver of the foetal, neonatal and adult guinea pig*

Enzyme activity was determined in crude extracts of liver as described in the Experimental section.

Fatty acid synthetase

The activity of fatty acid synthetase in the liver of the foetal guinea pig was low before day 40. It subsequently rose to activities of approx. 0.2 unit/g which were maintained until just after 50 days gestation then gradually fell (Fig. 2). The apparent K_m for malonyl-CoA was 39.9 ± 16.2 (4) μM and for NADPH was 53.1 ± 24.6 (4) μM in the crude extracts of foetal liver.

Acetyl-CoA carboxylase

The activity of acetyl-CoA carboxylase throughout most of foetal, neonatal and adult life was very low. It appeared in significant activity in the foetal liver after day 40, reached a peak by 45–50 days then declined (Fig. 3). The mean activity in the maternal liver was 0.058 ± 0.027 (18) unit/g.

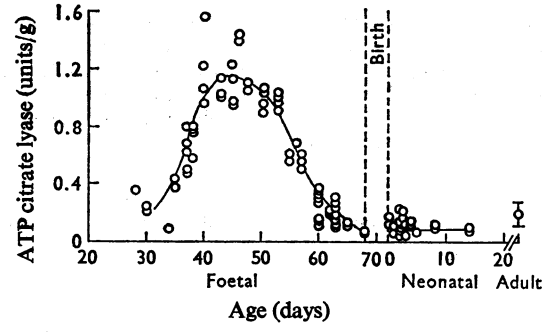

Fig. 4. *Activity of ATP citrate lyase in the liver of foetal, neonatal and adult guinea pigs*

Enzyme activity was determined in crude extracts of liver as described in the Experimental section.

ATP citrate lyase

Enzyme activity in the foetal liver rose between 30 and 40 days to values of 1–1.5 units/g. These were maintained until just after 50 days then progressively fell to very low values immediately before birth (Fig. 4). Neonatal liver activity was similar to that in the adult which was 0.199 ± 0.08 (30) unit/g. There were no significant changes during pregnancy. Michaelis constants for ATP citrate lyase activity in extracts of foetal and maternal liver were not significantly different and in the foetal liver were 71.1 ± 31.3 (5), 2.6 ± 0.7 (4) and 216 ± 98 μM for CoA, citrate and ATP respectively.

Glucose 6-phosphate dehydrogenase

Enzyme activity in the foetal liver rose progressively from about 0.25 unit/g at 30 days to about 1 unit/g by 50 days then gradually declined (Fig. 5). The neonatal liver activity was similar to that in the maternal liver. The mean maternal liver activity was 0.47 ± 0.18 (30) unit/g, and it showed no significant changes during pregnancy. The kinetics of the enzyme activity in foetal and maternal liver were similar with K_m values for the foetal liver enzyme of 28.7 ± 15.1 (4) and 27.1 ± 19.4 (4) μM for glucose 6-phosphate and $NADP^+$ respectively.

6-Phosphogluconate dehydrogenase

The activity in the foetal liver was almost constant between 35 days and term with a mean value of 1.07 ± 0.29 (35) units/g. The mean value in the maternal liver was 0.61 ± 0.25 (16) unit/g; it did not change significantly during pregnancy. The kinetic properties of maternal and foetal liver enzyme activities were similar. The K_m values for the foetal liver enzyme were

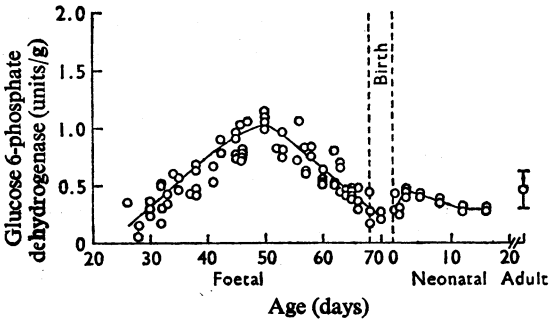

Fig. 5. *Activity of glucose 6-phosphate dehydrogenase in the liver of foetal, neonatal and adult guinea pigs*

Enzyme activity was determined in crude extracts of liver as described in the Experimental section.

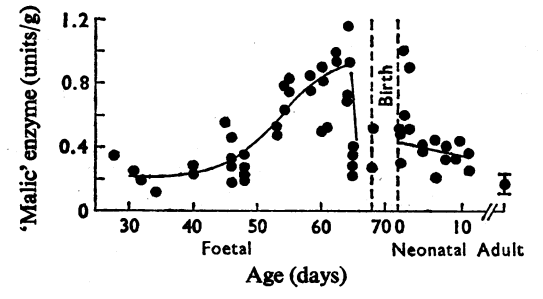

Fig. 6. *Activity of 'malic' enzyme in the liver of foetal, neonatal and adult guinea pigs*

Enzyme activity was determined in crude extracts of liver as described in the Experimental section.

114±51.2 (5) and 19±4.8 (5) μM for 6-phosphogluconate and $NADP^+$ respectively.

'Malic' enzyme

'Malic' enzyme activity in the foetal liver rose from about 0.2 unit/g at 30–40 days to about 0.8 unit/g by 60–64 days, it then fell immediately before term (Fig. 6). The mean activity in the maternal liver was 0.16±0.063 (18) unit/g. There were no significant differences in the kinetic properties of the maternal and foetal liver enzyme activities with K_m values for the foetal liver enzyme of 301±127 (5) and 8.1±4.2 (5) μM respectively for L-malate and $NADP^+$.

Malate dehydrogenase

The cytoplasmic malate dehydrogenase activity in the foetal liver measured with NADH or NADPH showed no significant change between 36 days and term, the mean values were 196±85 (18) units/g and

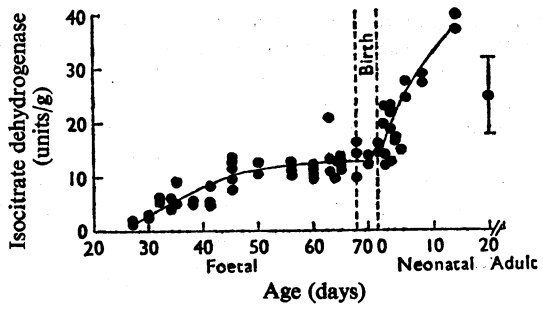

Fig. 7. *Activity of isocitrate dehydrogenase in the liver of foetal, neonatal and adult guinea pigs*

Enzyme activity was determined in crude extracts of liver as described in the Experimental section.

1.04±0.13 (29) units/g respectively. The mean maternal activities with NADH and NADPH were 342±121 (9) units/g and 1.27±0.08 (14) units/g; there were no significant changes during pregnancy.

Isocitrate dehydrogenase

Cytoplasmic $NADP^+$–isocitrate dehydrogenase in the foetal liver rose between 30 and 45 days to an activity of about 10 units/g which was maintained until birth. After birth the activity increased fourfold over the next 12 days (Fig. 7). The mean enzyme activity in the maternal liver was 25.1±7.3 (22) units/g. Enzyme activities in foetal and maternal liver had similar K_m values which in the foetal liver were 4.7±2.9 (5) and 20.6±5.4 (5) μM for isocitrate and $NADP^+$ respectively.

Discussion

Rates of lipid synthesis in the foetal liver

The acetate incorporation into lipid was largely (approx. 90%) into fatty acid and there were no major changes in the proportion of acetate or glucose incorporated into cholesterol between 45 and 57 days (C. T. Jones & G. F. Fellows, unpublished work). High rates of acetate incorporation into lipids have been reported for the liver of the foetal ox and rat (Villee & Hagerman, 1958; Roux, 1966; Taylor *et al.*, 1967; Ballard & Hanson, 1967*a*; Hanson & Ballard, 1968), human (Roux & Yoshioka, 1967) and rabbit (Popják, 1954; Roux, 1966). However, in those studies where glucose incorporation into lipid was also determined, it was not normally much lower than the rate of acetate incorporation. The maximum rate of fatty acid biosynthesis in the foetal guinea-pig liver, based on ^3H_2O incorporation, is about 0.2–0.3 μmol/h per mg. This is between one-quarter and one-half of the rate observed *in vivo* (Jones & Firmin, 1976) and no more than one-tenth of that reported

for the adult liver of other species (Guyinn *et al.*, 1972; Lowenstein, 1972; Brunengraber *et al.*, 1973; Wadke *et al.*, 1973; Salmon *et al.*, 1974).

The ratio of μg-atoms of $^{14}C/^{3}H$ observed during glucose and $^{3}H_2O$ incorporation into lipid by liver slices of foetuses younger than 57 days is higher than those previously reported for adult rat and mouse liver (Lowenstein, 1972; Brunengraber *et al.*, 1973; Clark *et al.*, 1974; Salmon *et al.*, 1974). This may be the result of a smaller dilution of intra- and extracellular [^{14}C]glucose specific radioactivity by unlabelled glucose produced through glycogen breakdown since the glycogen content of the foetal guinea-pig liver before 56 days is very low (<0.5 mg/g wet wt.).

Substrates for lipogenesis

If a theoretical $^{14}C/^{3}H$ ratio of 1 for ^{14}C-labelled substrate and $^{3}H_2O$ incorporation into lipid is assumed (Jungas, 1968; Brunengraber *et al.*, 1973; Clark *et al.*, 1974) then acetate represents at least 40–50% of the substrate used for lipid biosynthesis by slices in its presence. Glucose represents at least 15–80% of the substrate for lipid biosynthesis in the absence of acetate. At 44–47 days the rate of lipogenesis from glucose is insufficient to account for the additional source of substrate for lipogenesis in the presence of acetate and it is likely that endogenous substrate was used. At 55–57 days glucose and acetate could account for almost all the lipid synthesis by foetal liver slices. After 57 days increases in liver glycogen probably introduce [^{14}C]glucose dilution effects. These quantitative relationships for substrate supply for lipogenesis in liver slices may not necessarily apply to the liver *in vivo*.

The guinea pig possess a caecum capable of absorbing large quantities of short-chain fatty acids produced by bacterial fermentation (Hagen & Robinson, 1953) and germ-free guinea pigs have a substantially decreased growth rate (Newton & Dewitt, 1961). In other respects, for example the absence of glucokinase (A. Faulkner & C. T. Jones, unpublished work) and the presence of only low liver ATP citrate lyase activity, the adult guinea pig on the present feeding regime is like a ruminant. Both the foetal and maternal circulation have significant acetate concentrations and the foetal liver efficiently traps acetate passing the placenta (Jones, 1973). If umbilical blood flow is 5–10 ml/min (Shepherd & Whelan, 1951; Dawes, 1968) and all the umbilical vein acetate is removed by the foetal liver the supply of acetate to the foetal liver at 45 days should be about 0.25–0.5 μmol/min. This is more than adequate to account for the rates of lipogenesis observed *in vitro* and *in vivo*. The equally high rate of propionate incorporation into lipid by the foetal liver suggests that other short-chain fatty acids may be important lipid precursors (Groot, 1975). Besides the short-chain fatty acids the other substrates are likely to be glucose, pyruvate and lactate; the rate of amino acid incorporation (Jones, 1973) is low.

Pathways of lipogenesis in the foetal liver

Acetyl-CoA for lipid biosynthesis arises by two presumably independent pathways: either from acetate by the action of acetate thiokinase (Beinert *et al.*, 1953; Hele, 1954) or in the citric acid cycle. Acetate conversion into acetyl-CoA, which may be extra- or intra-mitrochondrial (Barth *et al.*, 1971), is a particularly important pathway for lipid biosynthesis in ruminants (Hanson & Ballard, 1967).

Acetyl-CoA produced in the mitochondrion needs to be transported to the cytosol for fatty acid synthesis and convincing evidence suggests that, in the rat liver, citrate is the immediate precursor of cytosolic acetyl-CoA (Srere, 1959, 1965; Spencer & Lowenstein, 1962, 1966; Spencer *et al.*, 1964). The pathway probably involves the extramitochondrial generation of malate and pyruvate which both serve as precursors of intramitochondrial oxaloacetate for citrate synthesis thereby establishing a citrate–malate cycle (Kornacker & Ball, 1965; Walter *et al.*, 1966; Ballard & Hanson, 1967*b*). The enzymes associated with this pathway are cytosolic ATP citrate lyase and 'malic' enzyme and mitochondrial pyruvate carboxylase. In species where the activity of these enzymes in the liver is low, such as ruminants, lipid synthesis occurs at a much higher rate from acetate than from glucose (Hanson & Ballard, 1968; Patel & Hanson, 1974); during foetal life these species may have a hepatic pathway closer to that of the adult rat (Hanson & Ballard, 1968; Brierley *et al.*, 1969). The intermediary metabolism of the 45-day foetal guinea pig lies somewhere between that of the adult rat and that of the ruminant, with low pyruvate carboxylase activity (Jones & Ashton, 1972), relatively high activities of 'malic' enzyme and ATP citrate lyase and high rates, relative to glucose, of acetate and propionate incorporation into lipid. The low rate of glucose incorporation is probably explained by the virtual absence of pyruvate carboxylase. It seems likely that a substantial proportion of acetyl-CoA production is intramitochondrial because of the high activity of ATP citrate lyase. Mitochondrial short-chain acyl-CoA synthetase activity has been demonstrated in the guinea-pig liver (Groot, 1975). If a citrate pathway for lipid synthesis exists to a significant extent in the 45-day foetal liver, its oxaloacetate requirement cannot be supplied from pyruvate, and other sources such as malate, aspartate or phosphoenolpyruvate may be used. Experiments on subcellular fractions indicate that phosphoenolpyruvate is more likely to fulfil this role than either aspartate or malate (C. T. Jones, unpublished work). Phosphoenolpyruvate carboxykinase is high in the mitochondrion at this time despite the absence of gluconeogenesis (Jones &

Ashton, 1972) and the enzyme may thus have a lipogenic function. This is supported by quinolinate inhibition of lipogenesis, a probable inhibitor of phosphoenolpyruvate carboxylation (Veneziale *et al.*, 1967). Garber & Salganicoff (1973) have suggested that mitochondrial oxaloacetate may be maintained by phosphoenolpyruvate under some conditions.

If oxaloacetate is produced from phosphoenolpyruvate at significant rates then glucose should, as was observed, stimulate acetate incorporation, although some of the increase may have arisen through an increase in NADPH production.The inability of pyruvate to stimulate acetate incorporation until 55–57 days of gestation demonstrates the low activity of the pyruvate carboxylation pathway at 45 days. The contrasting effects of acetate in inhibiting pyruvate incorporation and stimulating that of glucose suggests that pyruvate and glucose are not necessarily incorporated into lipid via the same pathways. This is supported by the way glucose and glycerol incorporation increases and pyruvate incorporation shows little change between 45 and 57 days. That the fatty acid synthesis pathways may differ from those in the adult rat liver is further suggested by the insensitivity of incorporation to the ATP citrate lyase inhibitor (−)-hydroxycitrate (Watson *et al.*, 1969; Watson & Lowenstein, 1970), although incorporation was sensitive to a citrate-transport inhibitor 1,2,3-benzenetricarboxylate (Robinson *et al.*, 1971; Kleinke *et al.*, 1973; Cheema-Dhadli *et al.*, 1973) and inhibitors of acetyl-CoA carboxylase (Barth *et al.*, 1973). Thus the nature of the pathways from glucose to fatty acid and the significance of the citrate–malate cycle remains to be established in the liver of the foetal guinea pig.

Acetyl-CoA carboxylase activity in the foetal liver is barely adequate to account for the rates of lipogenesis measured by ^3H_2O incorporation *in vitro* and *in vivo*. If all fatty acid is synthesized via this pathway its low activity is consistent with its suggested role in controlling the rate of lipogenesis (Lane & Moss, 1971). In the foetal rat liver there is little change in fatty acid synthetase activity (Volpe & Kishimoto, 1972) which is much higher than activated acetyl-CoA carboxylase (Lockwood *et al.*, 1970).

The similarity of the changes in acetate and ^3H_2O incorporation into lipid with the changes in the activities of the key lipogenic enzymes indicates that acetate rather than glucose incorporation is a good index of the changes in lipid synthesis in the developing guinea-pig liver.

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References

Ballard, F. J. & Hanson, R. W. (1967*a*) *Biochem. J.* **102**, 952–958

Ballard, F. J. & Hanson, R. W. (1967*b*) *J. Lipid Res.* **8**, 73–79

Barth, C., Sladek, M. & Decker, K. (1971) *Biochim. Biophys. Acta* **248**, 24–33.

Barth, C. A., Hackenschmidt, H. J., Weis, E. E. & Decker, K. F. A. (1973) *J. Biol. Chem.* **248**, 738–799

Beinert, H., Green, D. E., Hele, P., Hift, H., Vonkorff, R. W. & Ramakrishnan, C. V. (1953) *J. Biol. Chem.* **203**, 35–45

Brady, R. O., Bradley, R. M. & Trams, E. G. (1960) *J. Biol. Chem.* **235**, 3093–3103

Brierley, R. V., Jeacock, M. K. & Shepherd, D. A. L. (1969) *Biochem. J.* **114**, 70P

Brunengraber, H., Boutry, M. & Lowenstein, J. M. (1973) *J. Biol. Chem.* **248**, 2656–2669

Cheema-Dhadli, S., Halperin, M. L. & Leznoff, C. C. (1973) *Eur. J. Biochem.* **38**, 98–102

Clark, D. G., Rognstad, R. & Katz, J. (1974) *J. Biol. Chem.* **249**, 2028–2036

Cleland, W. W., Thompson, V. W. & Barden, R. E. (1969) *Methods Enzymol.* **13**, 30–33

Connor, W. E. & Lins, D. S. (1967) *J. Lipid Res.* **8**, 558–564

Dawes, G. S. (1968) in *Foetal and Neonatal Physiology* pp. 66–78, Year Book Medical Publishers, Chicago

Elvidge, H. (1972) *J. Inst. Anim. Technicians* **23**, 111–117

Fain, J. N. & Scow, R. O. (1966) *Am. J. Physiol.* **210**, 19–25

Garber, A. J. & Salganicoff, L. (1973) *J. Biol. Chem.* **248**, 1520–1529

Gregolin, C., Ryder, E. & Lane, M. D. (1968) *J. Biol. Chem.* **243**, 4227–4235

Groot, P. H. E. (1975) *Biochim. Biophys. Acta* **380**, 12–20

Guyinn, R. W., Veloso, D. & Veech, R. L. (1972) *J. Biol. Chem.* **247**, 7325–7331

Hagen, P. & Robinson, K. W. (1953) *Aust. J. Exp. Biol.* **31**, 99–104

Hanson, R. W. & Ballard, F. J. (1967) *Biochem. J.* **105**, 529–536

Hanson, R. W. & Ballard, F. J. (1968) *Biochem. J.* **108**, 705–713

Hashimoto, T., Isano, H., Iritani, N. & Numa, S. (1971) *Eur. J. Biochem.* **24**, 128–139

Hele, P. (1954) *J. Biol. Chem.* **206**, 671–676

Hershfield, M. S. & Nemeth, A. M. (1968) *J. Lipid Res.* **9**, 460–478

Hsu, R. Y. & Lardy, H. A. (1969) *Methods Enzymol.* **13**, 230–235

Jones, C. T. (1973) in *Foetal and Neonatal Physiology: Proc. Sir Joseph Barcroft Centenary Symp.* (Comline, K. S., Cross, K. W., Dawes, G. S. & Nathanielsz, P. W., eds.), pp. 403–409, Cambridge University Press, Cambridge

Jones, C. T. & Ashton, I. K. (1972) *Biochem. J.* **130**, 22P–23P

Jones, C. T. & Firmin, W. (1976) *Biochem. J.* **154**, 159–161

Jungas, R. L. (1968) *Biochemistry* **7**, 3708–3717

Kayden, H. J., Dancis, J. & Money, W. L. (1969) *Am. J. Obstet. Gynecol.* **104**, 564–572

Kleinke, J., Sauer, H. & Soling, H. D. (1973) *FEBS Lett.* **29**, 82–87

Kornacker, M. S. & Ball, E. G. (1965) *Proc. Natl. Acad. Sci. U.S.A.* **54**, 899–904

Lane, M. D. & Moss, J. (1971) *Metab. Pathways* 3rd Edn., **5**, 23–54
Lineweaver, H. & Burk, D. (1934) *J. Am. Chem. Soc.* **56**, 658–666
Lockwood, E. A., Bailey, E. & Taylor, C. B. (1970) *Biochem. J.* **118**, 155–162
Lowenstein, J. M. (1972) *J. Biol. Chem.* **246**, 629–632
Newton, W. L. & Dewitt, W. B. (1961) *J. Nutr.* **75**, 145–151
Ochoa, S. (1955) *Methods Enzymol.* **1**, 735–739
Patel, M. S. & Hanson, R. W. (1974) *Mech. Ageing Dev.* **3**, 65–74
Phares, E. J. (1951) *Arch. Biochem. Biophys.* **33**, 173–194
Popják, G. (1954) *Cold Spring Harbor Symp. Quant. Biol.* **19**, 200–208
Portman, O. W., Behrman, R. E. & Soltys, P. (1969) *Am. J. Physiol.* **216**, 143–147
Robinson, B. H., Williams, G. R., Halperin, M. L. & Leznoff, C. C. (1971) *J. Biol. Chem.* **246**, 5280–5286
Roux, J. F. (1966) *Metab. Clin. Exp.* **15**, 856–864
Roux, J. F. & Yoshioka, T. (1967) *Clin. Obstet. Gynecol.* **13**, 595–620
Salmon, D. M., Bowen, N. L. & Hems, D. A. (1974) *Biochem. J.* **142**, 611–618
Shepherd, J. T. & Whelan, R. F. (1951) *J. Physiol. (London)* **115**, 150–157
Smith, S. & Abraham, S. (1970) *Arch. Biochem. Biophys.* **136**, 112–121
Spencer, A. F. & Lowenstein, J. M. (1962) *J. Biol. Chem.* **237**, 3640–3648
Spencer, A. F. & Lowenstein, J. M. (1966) *Biochem. J.* **99**, 760–765
Spencer, A., Corman, L. & Lowenstein, J. M. (1964) *Biochem. J.* **93**, 378–388
Srere, P. A. (1959) *J. Biol. Chem.* **234**, 2544–2547
Srere, P. A. (1965) *Nature (London)* **205**, 766–770
Szabo, A. J., Grimaldi, R. D. & Jung, W. F. (1969) *Metab. Clin. Exp.* **18**, 406–415
Takeda, Y., Suzuki, F. & Inoune, H. (1969) *Methods Enzymol.* **13**, 153–160
Taylor, C. B., Bailey, E. & Bartley, W. (1967) *Biochem. J.* **105**, 717–722
Vagelos, P. R., Alberts, A. W. & Martin, D. B. (1963) *J. Biol. Chem.* **238**, 533–540
Van Duyne, C. M. (1965) *Biol. Neonat.* **9**, 115–127
Van Duyne, C. M., Havel, R. J. & Felts, J. M. (1962) *Am. J. Obstet. Gynecol.* **84**, 1069–1074
Veneziale, C. M., Walter, P., Kneer, N. & Lardy, H. A. (1967) *Biochemistry* **6**, 2129–2138
Villee, C. A. & Hagerman, D. D. (1958) *Am. J. Physiol.* **194**, 457–464
Villee, C. A. & Loring, J. M. (1961) *Biochem. J.* **81**, 488–494
Volpe, J. J. & Kishimoto, Y. (1972) *J. Neurochem.* **19**, 737–753
Wadke, M., Brunengraber, H., Lowenstein, J. M., Dolhun, J. J. & Arsenault, G. P. (1973) *Biochemistry* **12**, 2610–2624
Wakil, S. J., Tilchener, E. B. & Gibson, D. M. (1958) *Biochim. Biophys. Acta* **29**, 225–243
Walter, P., Paetkau, V. & Lardy, H. A. (1966) *J. Biol. Chem.* **241**, 2523–2532
Watson, J. A. & Lowenstein, J. M. (1970) *J. Biol. Chem.* **245**, 5993–6002
Watson, J. A., Fang, M. & Lowenstein, J. M. (1969) *Arch. Biochem. Biophys.* **135**, 209–217
Wilkinson, G. N. (1961) *Biochem. J.* **80**, 324–332