Lipid Metabolism and Mobilization in the Guinea Pig during Pregnancy

By COLIN T. JONES

Nuffield Institute for Medical Research, University of Oxford, Headley Way, Oxford OX3 9DS, U.K.

(Received 26 November 1975)

The liver of the foetal guinea pig accumulates a large quantity of triacylglycerol late in gestation at the same time that adipose-tissue mass grows at its maximum rate and foetal adipose-tissue lipoprotein lipase activity and sensitivity to lipolytic hormones has substantially declined. The fatty acid for triacylglycerol synthesis is not synthesized in the foetal liver and it is unlikely that it originates from any of the foetal tissues. Before the accumulation of hepatic triacylglycerol the concentration of free fatty acids increases in both the umbilical vein and the maternal inferior vena cava. This occurs at a time when the triacylglycerol lipase activity in maternal adipose tissue is elevated and the rate of lipolysis, but not of fatty acid esterification, is higher than earlier in gestation or than in the non-pregnant state. It is proposed that the increase in lipolysis in maternal adipose tissue, brought about by an increase in circulating lipolytic hormones, mobilizes fatty acid, which passes to the foetus and is partly stored as hepatic triacylglycerol. The foetal liver effectively removes both long- and short-chain fatty acids from umbilical-vein blood. The rate of placental fatty acid transfer is more than adequate to account for the triacylglycerol accumulation.

Several species, notably the human and the guinea pig, have a high body fat content at birth (Widdowson, 1950). The origin of this lipid is not clear. The foetal tissues, particularly liver and adipose tissue, have high rates of lipogenesis and probably supply a major portion of foetal needs for a large part of gestation (Popjak, 1954; Vilee & Hagermann, 1958; Vilee & Loring, 1961; Fain & Scow, 1966; Taylor *et al.*, 1967; Ballard & Hanson, 1967; Roux & Yoshioka, 1970; Jones, 1973; Jones & Ashton, 1976a; Jones & Firmin, 1976). However, in the latter half of pregnancy maternal plasma lipids increase (Burt, 1960; McKay & Kaunitz, 1963; Scow *et al.*, 1964; Otway & Robinson, 1968; Herrera *et al.*, 1969; Fairweather, 1971; Bohmer *et al.*, 1972), probably as a result of an increase in their mobilization from adipose tissue (Knopp *et al.*, 1970; Schoni *et al.*, 1975). The permeability of the placenta in many species to fatty acids (Szabo & Grimaldi, 1970) suggests that the increase in maternal plasma lipids may be associated with an increase in the supply of lipids to the foetus from the maternal circulation. Although attempts have been made to quantify this contribution (Fain & Scow, 1966; Hull, 1975; Elphick *et al.*, 1975), none have provided satisfactory estimates.

In the foetal guinea pig, hepatic lipid accumulation occurs at a time when the rate of fatty acid biosynthesis is declining and it is possible that much of the stored lipid is of maternal origin. To investigate this the changes in lipid turnover in maternal and foetal adipose tissue, liver and plasma have been followed in the latter half of gestation.

Experimental

Materials

Animals. Guinea pigs of the Dunkin–Hartley strain were reared, and the time of mating was determined as described by Elvidge (1972). They were fed on Dixons Diet 18 and hay *ad lib* and drinking water was supplemented with ascorbic acid (approx. 100 mg/l; 100 mg/day per animal). Non-pregnant females used weighed approx. 250 g.

Chemicals. Enzymes were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K. Lipids were from Applied Science Laboratories, Richmond, Surrey, U.K. Bio-Rad resins were from Bio-Rad Laboratories, Bromley, Kent, U.K. Fatty acid-free bovine serum albumin was from Miles Laboratories, Slough, Bucks., U.K., and scintillation chemicals were from Koch–Light Laboratories, Colnbrook, Bucks., U.K., or Hopkin and Williams, Romford, Essex, U.K. Radioactive chemicals were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. All other chemicals were purchased from British Drug Houses, Poole, Dorset, U.K.

Methods

Animals. Pregnant guinea pigs were killed with a blow to the neck, the uterus was exposed and foetuses were removed through a uterine incision. Foetal and maternal tissues were collected after bleeding from the neck.

Fatty acid determination. Blood from the umbilical vein or artery, or the inferior vena cava, or the foetal heart was collected into syringes containing approx. 1 unit of heparin. Blood sampling commenced within 10–15 s of stunning the pregnant animal and was usually complete within 30 s; the maximum time taken from maternal death was 60 s. After centrifugation at 1500g and 2°C for 15 min, free fatty acids were extracted from plasma by the method of Dole (1956). Free fatty acids were determined by the colorimetric method of Duncombe (1963) by using palmitate as a standard. Palmitate recovery in the Dole (1956) extraction was 92.3 ± 4.7 (6)% ($\pm$ s.d.); no significant phospholipid extraction occurred.

Acetate determination. Blood from the umbilical vein or artery, or blood obtained from foetal cardiac puncture, was collected, within 60 s of maternal death, into plastic syringes containing about 1 unit of heparin. After centrifugation for 15 min at 1500g and 2°C, plasma acetate concentration was determined as previously described (Jones & Ashton, 1976a).

Triacylglycerol determination. Liver was homogenized with 9 vol. of 100 mM-potassium phosphate (pH 6.2). A fraction of the homogenate was centrifuged (10000g, 10 min), and glycerol was determined in the supernatant. The remainder of the homogenate (or a plasma sample to which was added 5 vol. of 100 mM-potassium phosphate, pH 6.2) was extracted for 10 min with 6 vol. of redistilled chloroform (Itaya & Ui, 1965). To remove polar lipids, 6 ml of the chloroform extract was shaken with 0.5 g of silicic acid for 10 min. It was then evaporated to dryness under N_2 . Lipids were saponified at 60°C for 30 min after addition of 0.5 ml of 0.5 M-KOH in ethanol (95%, v/v), then 1 ml of 0.15 M-MgSO₄ was added and the Mg(OH)₂ formed removed by centrifugation (1500g, 10 min). Glycerol in the supernatant was determined enzymically by using glycerol kinase. Tripalmitin was used as a standard; its mean recovery was 94.6 ± 2.4 (16)% ($\pm$ s.d.). No significant phospholipid was detected after silicic acid extraction.

Lipid extraction. After injection of [$1-^{14}C$]palmitate (see below), maternal and foetal tissues were homogenized in 20 vol. of chloroform/methanol (2:1, v/v), cell debris was removed by filtration and the chloroform/methanol extracts were evaporated to dryness under N_2 . The extracts were saponified at 90°C for 2 h with 5 ml of 10% (w/v) KOH, acidified with 10 M-H₂SO₄ and fatty acids extracted with 3 × 10 ml of light petroleum (b.p. 40–60°C). The petroleum extract was dried with anhydrous Na₂SO₄ and a sample counted for radioactivity in 10 ml of scintillation fluid [6 g of 2,5-diphenyloxazole and 0.25 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene in 1 litre of toluene].

Long-chain fatty acids in the extracts from some tissues were decarboxylated in the Schmidt reaction by using the method of Brady *et al.* (1960), adapted from that of Phares (1951). Radioactive CO₂ was collected and counted for radioactivity as previously described (Jones & Ashton, 1976a).

Triacylglycerol lipase assay. Adipose tissue or liver was homogenized with 3 vol. of ice-cold 20 mM-Tris/HCl (pH 7.0)/250 mM-sucrose/1 mM-EDTA, then centrifuged at 40000g and 4°C for 30 min. Triacylglycerol lipase (EC 3.1.1.3) activity in the supernatant was assayed immediately by the method of Kelly (1968) as described by Schwartz & Jungas (1971). Glycerol tri[$1-^{14}C$]oleate (100 μ Ci/g) was dissolved in *n*-heptane and passed over a short Bio-Rad AG1-X8 column to remove fatty acids. Heptane was removed under N_2 and 100 mM-sodium phosphate, pH 7.4, containing 2.5 mM-MgCl₂, and fatty acid-free bovine serum albumin (83 mg/ml) was added to form a triolein suspension (50 mg/ml). This was sonicated (150 W, 4 × 30 s; MSE sonicator) and 0.5 ml of the emulsion added to diluted supernatant. After incubation at 37°C for 30 min, free fatty acids were extracted with 2.5 ml of heptane/propan-2-ol/1 M-H₂SO₄ (10:40:1, by vol.), 2 ml of heptane and 1 ml of glass-distilled water with shaking for 15 min. The organic phase was added to a glass scintillation vial with 400 mg of Bio-Rad RG1-X8 (previously washed with 50 vol. of 5 M-NaOH, 20 vol. of propan-2-ol and 20 vol. of heptane) and shaken for 15 min. The heptane phase was removed and the resin washed with 4 × 10 ml of heptane, then suspended in 0.2 ml of NCS tissue solubilizer and 10 ml of scintillation fluid [6 g of 2,5-diphenyloxazole; 0.25 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene; 1 litre of toluene]. After being left overnight, radioactivity in the scintillation vials was determined. Lipase activity was linearly dependent on extract volume and incubation time for at least 60 min. Controls containing no tissue extract were run with each assay.

Lipoprotein lipase activity. Enzyme activity was extracted by the method of Greten *et al.* (1968). Perirenal adipose tissue was homogenized with 20 vol. of acetone (–20°C), filtered in a Buchner funnel, then washed with 20 vol. of ice-cold acetone and 20 vol. of peroxide-free diethyl ether. The washed powder was dried under N_2 and stored at –20°C until assay. Before assay the dried extract was dissolved in 0.025 M-NH₃ (2 ml/g), pH 8.5, with shaking for 30 min at 4°C. After centrifugation at 10000g and 4°C for 30 min, the pellet was re-extracted and the supernatants were pooled for assay. To 0.5 ml of the supernatant was added 0.5 ml of an emulsion containing 3 mg of glycerol tri[$1-^{14}C$]oleate (100 μ Ci/g), 0.3 mM-NH₄Cl, pH 8.5, 30 mg of fatty acid-free albumin, 0.08 M-CaCl₂ and 0.25 ml of fresh human serum. After sonication the

emulsion was incubated for 30 min at 37°C. Liberated fatty acids were extracted and counted for radioactivity as described above. Hydrolysis was dependent on extract volume and incubation time for at least 60 min. The results are expressed in terms of μmol of triacylglycerol hydrolysed/g wet wt. tissue.

Adipose-tissue incubation. Epididymal fat-pads (50–100 mg) were incubated in 3 ml of Krebs bicarbonate saline (10 mM-glucose, 26 mM- NaHCO_3 , 1.2 mM- KH_2PO_4 , 124 mM- NaCl , 5 mM- KCl , 1.3 mM- MgSO_4 , 2.25 mM- CaCl_2), with shaking (100 oscillations/min), containing 40 mg of fatty acid-free bovine serum albumin/ml, under an atmosphere of $\text{O}_2 + \text{CO}_2$ (95:5) at 37°C for 60 min. At the end of the incubation free fatty acids and glycerol were extracted from the medium and assayed as described above.

Determination of radioactivity. Radioactivity was determined in a Philips PW 4510 liquid-scintillation counter. Counting efficiency was estimated by the channels-ratio method by using an external standard and a series of CCl_4 -quenched [^{14}C]hexadecane standards.

Units of enzyme activity. The unit of enzyme activity is μmol of substrate consumed or product synthesized/min at the temperature of the assay.

Expression of results. Where appropriate the results are expressed as means $\pm$ s.d. with the numbers of observations in parentheses. Significance was estimated by using Student's *t* test.

Results

Plasma free fatty acid

The free fatty acid concentration in the inferior-vena-cava plasma of guinea pigs showed a significant ($P < 0.001$) increase between day 45 of pregnancy and term (Fig. 1a). At days 45–54 the mean value was 0.32 ± 0.12 (10) $\mu\text{mol/ml}$ and then rose to 0.60 ± 0.18 (21) $\mu\text{mol/ml}$ by 60 days to term. The concentration in the umbilical-vein plasma of foetal guinea pigs showed a similar ($P < 0.001$) although more pronounced increase (Fig. 1b). Before 50 days the mean concentration was 0.17 ± 0.046 (7) $\mu\text{mol/ml}$ and this increased to 0.68 ± 0.14 (21) $\mu\text{mol/ml}$ by 60 days to term. The maternal and foetal concentrations followed each other closely, with maternal/foetal ratios of 1.8 ± 0.42 and 1.04 ± 0.32 at the two age ranges.

Free fatty acid clearance

After the intraperitoneal injection of $4 \mu\text{Ci}$ of [^{14}C]palmitate into pregnant guinea pigs, radioactivity was recovered largely in the maternal liver, lung and adipose tissue and in the foetal liver lipids (Table 1). There was an increase with time in the incorporation into foetal adipose-tissue, brain and

lung lipids. Decarboxylation of the radioactive saponified lipids removed 73.8 ± 12.6 (6)% and 88.5 ± 9.6 (6)% of the label in lipids isolated from maternal and foetal liver respectively. Some 88–94% of the radioactivity in the saponified-lipid fraction was recovered as fatty acid after t.l.c.

The ability of the foetal liver to remove short-chain fatty acids from the umbilical vein has previously been suggested (Jones, 1973). This ability to remove fatty acids is shown not only by the above results but also by the large concentration gradient of acetate and fatty acid across the foetal liver (Table 2). Both acetate and free fatty acids are transported across the guinea-pig placenta (Table 2). If umbilical blood flow is about 5–10 ml/min (Dawes, 1968), then placental acetate transfer is 0.5–1 and 0.2–0.5 $\mu\text{mol/min}$ and placental free fatty acid transfer is 0.5–1 and 2–4 $\mu\text{mol/min}$ at 47–53 and 58–64 days respectively.

Liver, adipose-tissue and plasma triacylglycerols

During the period that the umbilical-vein concentration of free fatty acids was high, a large accumulation of triacylglycerols occurred in the

Fig. 1. Plasma free fatty acid concentrations in maternal (a) and foetal (b) guinea pigs

Plasma from the umbilical vein of foetal and the inferior vena cava of maternal guinea pigs was obtained, and free fatty acids were determined as described in the Experimental section.

Table 1. *Distribution of [1-¹⁴C]palmitate after injection into pregnant guinea pigs*

An intraperitoneal injection of 4 μ Ci of [1-¹⁴C]palmitate (2 μ mol in 0.5 ml of 0.9% NaCl, 4% fatty acid-free bovine serum albumin) was given to guinea pigs 48–52 days pregnant. Then 5, 10 and 20 min later tissues from the maternal and all the foetal guinea pigs were extracted with chloroform/methanol (2:1, v/v) and lipid was saponified, extracted and counted for radioactivity as described in the Experimental section. The results are expressed as the means of the percentage of the total injection in each tissue, $\pm$ S.D. of four experiments. The foetal values represent results for pooled tissue from whole litters.

	Radioactivity (% of initial injection)		
	5 min	10 min	20 min
Maternal			
Liver	31.2 $\pm$ 8.3	25.6 $\pm$ 10.6	18.7 $\pm$ 7.9
Lung	4.7 $\pm$ 2.1	7.3 $\pm$ 5.2	3.6 $\pm$ 3.1
Kidney	3.6 $\pm$ 3.2	4.1 $\pm$ 3.2	2.3 $\pm$ 1.7
Brain	2.1 $\pm$ 1.7	2.0 $\pm$ 1.5	1.5 $\pm$ 0.9
Adipose tissue	10.5 $\pm$ 3.9	14.2 $\pm$ 8.4	16.3 $\pm$ 5.2
Foetal			
Liver	14.2 $\pm$ 5.3	16.3 $\pm$ 9.3	14.1 $\pm$ 4.9
Lung	1.1 $\pm$ 1.0	1.4 $\pm$ 1.0	1.7 $\pm$ 0.8
Kidney	0.5 $\pm$ 0.4	0.4 $\pm$ 0.25	0.35 $\pm$ 0.2
Brain	0.4 $\pm$ 0.15	0.6 $\pm$ 0.23	2.0 $\pm$ 0.6
Adipose tissue	1.3 $\pm$ 0.7	4.2 $\pm$ 2.6	5.7 $\pm$ 3.9

foetal liver (Fig. 2a). Most of the triacylglycerol disappeared after birth. The triacylglycerol content of the maternal liver was low [mean 1.02 $\pm$ 0.21 (28) μ mol/g] and did not change during the latter half of pregnancy. At the time of triacylglycerol accumulation by the foetal liver a rapid growth of the perirenal adipose tissue occurred, about half of which was lost after birth (Fig. 2b).

An increase in foetal umbilical-vein plasma triacylglycerol concentration occurred 4–5 days after the increase in liver triacylglycerol and was not significant until 64–65 days (Fig. 3). The concentrations in foetal liver and plasma were not significantly correlated between 55 and 62 days ($P > 0.1$). The triacylglycerol concentration in the maternal plasma did not change; the mean value was 1.34 $\pm$ 0.47 (33) nmol/ml of plasma.

Triacylglycerol lipase activity in liver and adipose tissue

Triacylglycerol lipase activity was low in maternal perirenal tissue throughout most of pregnancy (Fig. 4a). Between 56 and 60 days the activity was significantly ($P < 0.001$) higher [mean 0.06 $\pm$ 0.015 (9) unit/g] than at other times [mean 0.015 $\pm$ 0.007 (17) unit/g]. Lipase activity was not increased by

Table 2. *Removal of fatty acid and acetate by the liver and their production by the placenta of the foetal guinea pig*

Foetal blood was collected from the umbilical vein, artery or heart. Free fatty acid and acetate were determined in separated plasma as described in the Experimental section. The results are the means $\pm$ S.D. of 6–20 determinations.

Gestational age (days)	Plasma acetate (μ mol/ml)		
	Umbilical vein	Umbilical artery	Heart
48–53	0.12 $\pm$ 0.04	0.025 $\pm$ 0.009	0.02 $\pm$ 0.01
59–62	0.096 $\pm$ 0.039	0.046 $\pm$ 0.02	0.04 $\pm$ 0.019
	Plasma free fatty acid (μ mol/ml)		
	Umbilical vein	Umbilical artery	Heart
47–51	0.19 $\pm$ 0.04	0.1 $\pm$ 0.03	0.09 $\pm$ 0.03
58–64	0.7 $\pm$ 0.15	0.33 $\pm$ 0.12	0.18 $\pm$ 0.13

Fig. 2. *Accumulation of triacylglycerol in the liver (a) and growth of perirenal tissue (b) of the foetal guinea pig*

For details see the Experimental section. M, maternal.

Fig. 3. Plasma triacylglycerol concentrations in foetal guinea pigs

For details see the Experimental section.

Fig. 4. Triacylglycerol lipase activity in the perirenal adipose tissue of maternal (a) and foetal (b) guinea pigs

Enzyme activity was determined at 37°C in adipose-tissue extracts as described in the Experimental section.

Fig. 5. Triacylglycerol lipase activity in the liver of maternal and foetal guinea pigs

Enzyme activity was determined at 37°C in the supernatant fraction obtained after centrifuging liver homogenates for 30min at 40000g as described in the Experimental section. M, maternal.

administration of cyclic AMP (1mM) to the extract. Triacylglycerol lipase activity in foetal perirenal adipose tissue progressively declined between 45 and 60 days, then remained low until birth (Fig. 4b).

The activity in the maternal liver was 0.24 ± 0.05 (14) unit/g and did not change during pregnancy. In the foetal liver it increased between 35 and 50–60 days to a peak value of 0.4unit/g, then declined (Fig. 5).

Lipoprotein lipase activity in adipose tissue

The lipoprotein lipase activity in maternal perirenal adipose tissue was 0.23 ± 0.54 (16) unit/g; it did not change during the latter half of pregnancy. In foetal perirenal adipose tissue it showed a progressive decline from about 0.25unit/g at 50 days to about 0.05unit/g at term (Fig. 6). Enzyme activity in extracts from maternal and foetal tissue was inhibited by 80–90% by protamine sulphate (3mg/ml) and 1M-NaCl.

Lipolysis in isolated adipose tissue

Glycerol [0.23 ± 0.11 (6) $\mu\text{mol/g}$] and free fatty acid [0.4 ± 0.21 (6) $\mu\text{mol/g}$] concentrations were low in foetal epididymal adipose tissue. The concentrations in tissue of the non-pregnant female, 0.46 ± 0.19 (6) $\mu\text{mol/g}$ and 0.74 ± 0.3 (6) $\mu\text{mol/g}$ respectively, were significantly higher. During pregnancy there was evidence of an increase in the rate of lipolysis, as the tissue concentrations of glycerol and free fatty acid were respectively 0.72 ± 0.24 (6) and

1.43 ± 0.5 (6) $\mu\text{mol/g}$ at 45–50 days and 1.31 ± 0.34 (6) and 2.53 ± 0.89 (6) $\mu\text{mol/g}$ at 55–60 days. This suggested increase in lipolysis in pregnancy was also observed in adipose tissue incubated in Krebs bicarbonate saline for 60 min. If the rates of lipolysis are calculated for the control incubations (Table 3) as described by Vaughan (1962), they were 3.04 ± 1.1 (6), 5.76 ± 1.3 (6) and 8.9 ± 2.4 (6) $\mu\text{mol/h}$ per g for non-pregnant female and 45–50- and 55–60-day-pregnant guinea pigs respectively. The rates of fatty acid esterification (calculated from the difference in fatty acid output, tissue plus medium, and the rates of lipolysis above) were not significantly different in the three groups, at 2–2.5 μmol of fatty acid esterified/h per g.

The production of fatty acid and glycerol by maternal epididymal adipose tissue was substantially increased by a number of lipolytic hormones, but there was no difference in sensitivity at the two stages of pregnancy studied. Lipolysis in adipose tissue from non-pregnant females was less sensitive to stimulation by adrenocorticotrophin and placental lactogen but not by adrenaline (Table 3). Fatty acid production in epididymal adipose tissue from foetal guinea pigs was also stimulated by a range of lipolytic compounds at a rate, except in the presence of

Fig. 6. Lipoprotein lipase activity in perirenal adipose tissue of maternal and foetal guinea pigs

Enzyme activity was determined at 37°C in extracts of adipose tissue as described in the Experimental section. M, maternal.

Table 3. Stimulation of lipolysis in epididymal adipose tissue from foetal and adult guinea pigs

Epididymal fat-pads were incubated in 3 ml of Krebs bicarbonate saline with shaking for 60 min at 37°C. Free fatty acids and glycerol were extracted and determined as described in the Experimental section. The results are each the means $\pm$ s.d. of five or six incubations.

		Lipolysis (μmol released/g wet wt. of tissue)			
		Fatty acid		Glycerol	
Time of gestation (days)	...	45–50	55–60	45–50	55–60
Foetal					
Control		2.5 ± 1.6	0.7 ± 0.4	0.9 ± 0.7	1.1 ± 0.4
Adrenocorticotrophin (100 ng/ml)		6.4 ± 2.9	1.3 ± 0.8	3.0 ± 1.9	1.6 ± 1.0
Thyroid-stimulating hormone (1 $\mu\text{g}/\text{ml}$)		3.1 ± 2.6	1.2 ± 0.9	2.7 ± 1.6	1.3 ± 0.7
Adrenaline (100 ng/ml)		14.2 ± 6.5	8.6 ± 4.1	7.2 ± 3.4	3.1 ± 1.6
Adrenaline (100 ng/ml) + phentolamine (0.2 mM)		67 ± 21.6	24.1 ± 12.2	34.6 ± 11.1	9.9 ± 5.4
Placental lactogen (1 mg/ml)		7.3 ± 3.1	2.1 ± 0.9	3.6 ± 1.1	1.4 ± 0.5
Maternal					
Control		0.8 ± 0.4	1.9 ± 0.7	1.92 ± 0.6	3.1 ± 1.2
Adrenocorticotrophin (100 ng/ml)		45.3 ± 11.7	39.8 ± 10.6	15.9 ± 4.3	14.3 ± 3.9
Thyroid-stimulating hormone (1 $\mu\text{g}/\text{ml}$)		14.2 ± 7.6	19.5 ± 4.7	5.1 ± 1.6	6.9 ± 2.8
Adrenaline (100 ng/ml)		21.3 ± 5.6	25.6 ± 9.7	7.6 ± 2.5	8.9 ± 4.3
Adrenaline (100 ng/ml) + phentolamine (0.2 mM)		71.2 ± 31.4	65.3 ± 19.4	24.6 ± 8.8	22.6 ± 4.6
Placental lactogen (1 mg/ml)		51.2 ± 12.3	67.6 ± 21.2	17.9 ± 3.4	23.9 ± 5.1
Non-pregnant female					
Control		0.2 ± 0.17		1.05 ± 0.36	
Adrenocorticotrophin (100 ng/ml)		26.2 ± 8.4		9.2 ± 2.8	
Adrenaline (100 ng/ml) + phentolamine (0.2 mM)		65.2 ± 20.5		22.9 ± 6.7	
Placental lactogen (1 mg/ml)		20.6 ± 7.2		8.7 ± 2.5	

Table 4. *Fatty acid and glyceride glycerol synthesis in adipose tissue of the maternal and foetal guinea pig*

Epididymal fat-pads from maternal and foetal guinea pigs were incubated for 3 h in Krebs bicarbonate saline containing 1 μ Ci of [U - 14 C]glucose (10 mM). At the end of the incubation they were washed and lipid was extracted, saponified and counted for radioactivity as described in the Experimental section. The results are the means $\pm$ S.D. of five to seven incubations.

Time of gestation (days)	Incorporation (nmol/3h per g)			
	Fatty acid		Glyceride glycerol	
	Maternal	Foetal	Maternal	Foetal
30-40	2.1 $\pm$ 0.3	—	0.9 $\pm$ 0.14	—
45-50	2.46 $\pm$ 0.45	41.2 $\pm$ 12.7	0.76 $\pm$ 0.25	5.7 $\pm$ 0.9
54-58	3.99 $\pm$ 0.7	105 $\pm$ 33	0.61 $\pm$ 0.14	22.6 $\pm$ 4.8
63-66	0.7 $\pm$ 0.15	37.4 $\pm$ 12.6	1.42 $\pm$ 0.26	10.9 $\pm$ 2.7

adrenaline, that was less than in adult tissue. The foetal adipose tissue at 55-60 days was substantially less sensitive to lipolytic agents than at 45-50 days (Table 3).

Fatty acid and glycerol synthesis in maternal and foetal adipose tissue

The rate of synthesis of fatty acid and glycerol from glucose in the epididymal fat-pads of non-pregnant female guinea pigs was very low. During pregnancy both increased, with the maximum rate of fatty acid biosynthesis occurring at 54-58 days and that of glycerol synthesis at 63-66 days (Table 4). Rates of synthesis were much higher in the foetal fat-pads, with peak values for both at 54-58 days (Table 4).

Discussion

Most of the triacylglycerol in the term foetal guinea-pig liver was deposited between days 58 and 61 of gestation at a rate of about 50 μ mol/48-72 h per g. Although maximal rates of glyceride glycerol synthesis have been observed around this time (Jones & Ashton, 1976b), fatty acid synthesis determined either *in vitro* or *in vivo* had declined substantially from its peak values to rates of around 0.3 μ mol/h per g (Jones, 1973; Jones & Ashton, 1976a; Jones & Firmin, 1976). Thus the rate of fatty acid incorporation into triacylglycerol, which during this time was approx. 50-70 μ mol/24 h per g, was substantially higher than endogenous fatty acid synthesis, of 4-10 μ mol/24 h per g. Even if all the fatty acid synthesized was incorporated into triacylglycerol (approx. 80% is, as determined by 3 H $_2$ O incorporation into total and triacylglycerol fatty acid; C. T. Jones & G. F. Fellows, unpublished work), this could account for only a small proportion

of the triacylglycerol synthesized. Thus the additional fatty acid was supplied by lipid mobilization from other foetal tissues, or by placental synthesis, or by transfer from the maternal circulation.

In the guinea pig, placental fatty acid synthesis is low *in vitro* or *in vivo* (Jones, 1973, 1976; Jones & Firmin, 1976) and is unlikely to be a major source of fatty acids for the foetus. Besides the liver, the major site of lipid biosynthesis in the foetal guinea pig is the adipose tissue, where highest rates of synthesis occur at 55-60 days (Jones, 1973; Jones & Firmin, 1976). This coincides with the major growth phase of adipose tissue, which is predominantly if not exclusively of the brown-adipose type (Hirvonen, 1968). It occurs at a time when the lipoprotein lipase activity of the tissue has fallen to one-half to one-quarter of its value at 45-50 days. Similar falls in foetal adipose-tissue lipoprotein lipase activity have been observed in the rabbit (Harding, 1971, 1972). The rise in foetal plasma triacylglycerol after 60 days may be caused by a decline in their uptake by adipose tissue because of the fall in lipoprotein lipase activity. At the same time the lipid turnover in the foetal adipose tissue probably declines, as judged by the fall in the sensitivity of the tissue to lipolytic hormones and the substantial decline in triacylglycerol lipase activity in the tissue. The poorer response of foetal compared with maternal adipose tissue to the pituitary lipolytic hormones can be explained by the different sensitivities of brown and white adipose tissue (Fain *et al.*, 1967). Thus the rapid phase of foetal adipose-tissue growth is probably produced by a fall in triacylglycerol mobilization and an increase in lipid synthesis, but not by an increase in triacylglycerol uptake. Because of this it seems unlikely that lipid mobilization in the foetal adipose tissue *in vivo* should suddenly increase to become the major source of free fatty acids for hepatic triacylglycerol deposition between 58 and 61 days.

In the last third of human pregnancy there is an increase in maternal plasma free fatty acids associated with a decreased insulin sensitivity (Burt, 1960; Bleicher *et al.*, 1964; Fairweather, 1971), and this may also be related to the increased sensitivity of human adipose tissue to lipolytic agents during pregnancy (Schoni *et al.*, 1975). A fall in adipose-tissue lipogenesis is also observed (Galton & Wilson, 1970). Similarly in the pregnant rat there is first a phase of enhanced adipose-tissue and hepatic lipogenesis followed by a fall in both (Fain & Scow, 1966; Knopp *et al.*, 1970, 1973). During this phase of decreased synthesis there is an increase in maternal plasma free fatty acids and triacylglycerol (McKay & Kaunitz, 1963; Scow *et al.*, 1964; Otway & Robinson, 1968; Herrera *et al.*, 1969; Knopp *et al.*, 1973) that is associated with an increase in adipose-tissue lipolysis and a fall in

lipoprotein lipase activity (Otway & Robinson, 1968; Knopp *et al.*, 1970). The extent to which this lipid can pass to the foetus is not clear. The human placenta is permeable to fatty acid (Szabo *et al.*, 1969) and placental transport of fatty acids may be partly responsible for the high fat content of the human baby. In rats there is a poor placental permeability to fatty acids (Koren & Shafir, 1964) and a low body fat content at birth (Widdowson, 1950). In this species the hyperlipaemia during late pregnancy may be a preparation for milk production, as plasma triacylglycerols decrease when lipoprotein lipase activity in the mammary gland rises (Otway & Robinson, 1968; Hamosh *et al.*, 1970).

The placenta of the guinea pig is permeable to fatty acids, and an increase in the placental transport of fatty acids late in gestation has been suggested (Hershfield & Nemeth, 1968; Kayden *et al.*, 1969; Bohmer *et al.*, 1972). The higher adipose-tissue free fatty acid and glycerol concentrations from day 55 to 60 of pregnancy suggests that the rate of lipolysis at this time is higher than either earlier in pregnancy or in the non-pregnant state. This is supported by the increase in hormone-sensitive lipase activity in maternal adipose tissue between 55 and 60 days but not by an increase in the adipose-tissue sensitivity to lipolytic hormones (as found in pregnant women) except when compared with non-pregnant animals. It suggests that hormone-sensitive lipase measurements reflect enzyme activity *in vivo* and that the absence of an effect of cyclic AMP *in vitro* may be explained by the absence of protein kinase activity from the adipose-tissue extracts (Corbin *et al.*, 1970). Thus the increase in lipolysis is probably produced, in part at least, by an increase in circulating hormone concentration. Apart from placental lactogen, with a high plasma concentration during pregnancy (Kaplan & Grumbach, 1974; Kelly *et al.*, 1974) that stimulates lipolysis (Turtle & Kipnis, 1967), adrenocorticotrophin, corticosteroids and thyroid hormones promote lipolysis (Rudman, 1963; Rudman & Shank, 1966). The plasma concentrations of these three groups of hormones increase between days 50 and 60 of pregnancy in the guinea pig (Jones, 1974; C. T. Jones, unpublished work) and may be responsible for the increase in maternal plasma free fatty acids at that time. This mobilization of fatty acids from maternal adipose tissue is likely to be responsible for the increase in umbilical-vein free fatty acid concentration and, as discussed above, indicated by the high fatty acid concentration gradient across the placenta and liver. Mobilization of lipids from the foetal tissues is relatively less important. The foetal guinea-pig liver, through which all umbilical-vein blood flows (C. T. Jones & A. Faulkner, unpublished work), removes most of the short-chain and long-chain fatty acids passing through it. Thus when the free fatty acid concen-

tration in foetal umbilical-vein plasma rises, a major portion of the fatty acids is recovered in hepatic acylglycerols. Similar relationships between plasma free fatty acids and hepatic acylglycerol have been observed in adult rats (Van Harken *et al.*, 1969; Kohout *et al.*, 1971). Some of the hepatic fatty acid is probably subsequently transported to foetal adipose tissue and other tissues, as indicated by the tissue distribution of [14 C]palmitate. The increase in foetal plasma triglycerols after 60 days occurs at a time when the foetal adipose-tissue lipoprotein lipase activity is low and is coincident with the appearance of β -lipoproteins in foetal plasma (Bohmer *et al.*, 1972). Thus before 60–63 days the hepatic transport of acylglycerols may be relatively slow, but this does not appear to be primarily responsible for the accumulation of hepatic triacylglycerols. The deposition of hepatic triacylglycerols is, however, not simply a passive response to an increased placental transport of fatty acids, since foetal hypophysectomy substantially diminishes the triacylglycerol content of the foetal guinea-pig liver (C. T. Jones & M. Peddie, unpublished work).

In summary, before 50 days much of the lipid required by the foetus may be synthesized in the foetal liver and adipose tissue, with acylglycerol transport from the liver being less than in adult life. After 50 days there is an increase in the mobilization of fatty acids from maternal adipose tissue, probably promoted by increased pituitary-hormone secretion, and the associated rise in maternal-plasma free fatty acids increases placental transfer and the concentration in umbilical-vein plasma. Most of the free fatty acids transported by the placenta are initially removed by the foetal liver and result in substantial triacylglycerol accumulation at a time when fatty acid synthesis in the liver is low and in the adipose tissue is high. After 60 days the low foetal adipose-tissue lipoprotein lipase activity and the appearance of plasma β -lipoproteins cause a large increase in plasma triacylglycerols. The hepatic triacylglycerol stores are mobilized rapidly after birth and are probably essential metabolic fuel for the first hours of life of the newborn guinea pig (Widdowson & McCance, 1955).

This pathway of fatty acid transport from maternal adipose tissue to foetal liver is also suggested by the increase in lipids in foetal liver and adipose tissue that occurs during maternal starvation (Flexner & Flexner, 1950; Shelley & Thalmé, 1970; Edson *et al.*, 1975). The transport of fatty acids across the guinea-pig placenta should occur at a rate more than adequate to account for the triacylglycerol accumulation in the foetal liver, which, over the period of maximum deposition, was about $350 \mu\text{mol/liver}$. If the umbilical blood flow was about 10 ml/min (Dawes, 1968) and the fatty acid concentration difference between umbilical vein and artery was

0.3–0.4 μmol , the placental transport over a 48–72 h period would have been 7–15 mmol of fatty acid. This is about ten times that required for hepatic triacylglycerol synthesis, which may thus represent only 10% of the fatty acid transported at this time.

I am grateful to Professor G. S. Dawes for his interest and encouragement and to Mrs. Paula Webb for expert technical assistance. This work was supported by a grant from the Medical Research Council.

References

- Ballard, F. J. & Hanson, R. W. (1967) *Biochem. J.* **102**, 952–958
- Bleicher, S. J., O'Sullivan, J. B. & Freinkel, N. (1964) *N. Engl. J. Med.* **271**, 866–872
- Bohmer, T., Havel, R. & Long, J. A. (1972) *J. Lipid Res.* **13**, 371–382
- Brady, R. O., Bradley, R. M. & Trams, E. G. (1960) *J. Biol. Chem.* **235**, 3093–3103
- Burt, R. L. (1960) *Obstet. Gynecol.* **15**, 460–464
- Corbin, J. D., Reimann, E. M., Walsh, D. A. & Krebs, E. G. (1970) *J. Biol. Chem.* **245**, 4849–4851
- Dawes, G. S. (1968) *Foetal and Neonatal Physiology*, pp. 66–78, Year Book Medical Publishers, Chicago
- Dole, V. P. (1956) *J. Clin. Invest.* **35**, 150–154
- Duncombe, W. G. (1963) *Biochem. J.* **88**, 7–10
- Edson, J. L., Hudson, D. G. & Hull, D. (1975) *Biol. Neonat.* **27**, 50–55
- Elphick, M. L., Hudson, D. G. & Hull, D. (1975) *J. Physiol. (London)* **252**, 29–42
- Elvidge, H. (1972) *J. Inst. Anim. Technol.* **23**, 111–117
- Fain, J. N. & Scow, R. O. (1966) *Am. J. Physiol.* **210**, 19–25
- Fain, J. N., Reed, N. & Sapperstein, R. (1967) *J. Biol. Chem.* **242**, 1887–1894
- Fairweather, D. V. I. (1971) *J. Obstet. Gynaecol. Br. Commonw.* **78**, 707–711
- Flexner, J. B. & Flexner, L. B. (1950) *Anat. Rec.* **106**, 413–427
- Galton, D. J. & Wilson, J. P. D. (1970) *Clin. Sci.* **38**, 661–675
- Greten, H., Levy, R. I. & Frederickson, D. S. (1968) *Biochim. Biophys. Acta* **164**, 185–194
- Hamosh, M., Clary, T. R., Chernick, S. S. & Scow, R. O. (1970) *Biochim. Biophys. Acta* **210**, 473–482
- Harding, P. G. R. (1971) *Clin. Obstet. Gynecol.* **14**, 685–709
- Harding, P. G. R. (1972) in *Physiological Biochemistry of the Fetus* (Hodari, A. A. & Mariona, F., eds.), pp. 229–243, Charles C. Thomas, Springfield
- Herrera, E., Knopp, R. H. & Freinkel, N. (1969) *J. Clin. Invest.* **48**, 2260–2272
- Hershfield, M. S. & Nemeth, A. M. (1968) *J. Lipid Res.* **9**, 460–468
- Hirvonen, J. (1968) *Ann. Med. Exp. Biol. Fenn.* **46**, 576–581
- Hull, D. (1975) *Br. Med. Bull.* **31**, 32–36
- Itaya, K. & Ui, M. (1965) *J. Lipid Res.* **6**, 16–20
- Jones, C. T. (1973) in *Foetal and Neonatal Physiology* (Comline, K. S., Cross, K. W., Dawes, G. S. & Nathanielsz, P. W., eds.), pp. 403–409, Cambridge University Press, Cambridge
- Jones, C. T. (1974) *Endocrinology* **95**, 1129–1133
- Jones, C. T. (1976) *J. Reprod. Fertil.* **47**, 189–201
- Jones, C. T. & Ashton, I. K. (1976a) *Biochem. J.* **154**, 149–158
- Jones, C. T. & Ashton, I. K. (1976b) *Arch. Biochem. Biophys.* **174**, in the press
- Jones, C. T. & Firmin, W. (1976) *Biochem. J.* **154**, 159–161
- Kaplan, S. L. & Grumbach, M. M. (1974) in *Lactogenic Hormones, Fetal Nutrition and Lactation* (Josimovich, J. B., Reynolds, M. & Cobo, E., eds.), pp. 183–191, Academic Press, New York and London
- Kayden, H. J., Dancis, J. & Money, W. L. (1969) *Am. J. Obstet. Gynecol.* **104**, 564–572
- Kelly, P. A., Robertson, H. A. & Friesen, H. G. (1974) *Nature (London)* **248**, 435–437
- Kelly, T. F. (1968) *J. Lipid Res.* **9**, 799–800
- Knopp, R. H., Herrera, E. & Freinkel, N. (1970) *J. Clin. Invest.* **49**, 1438–1446
- Knopp, R. H., Saudek, C. D., Arky, R. A. & O'Sullivan, J. B. (1973) *Endocrinology* **92**, 984–988
- Kohout, M., Kohoutova, B. & Heimberg, M. (1971) *J. Biol. Chem.* **246**, 5067–5074
- Koren, Z. & Shafir, E. (1964) *Proc. Soc. Exp. Biol. Med.* **116**, 411–414
- McKay, D. G. & Kaunitz, H. (1963) *Metab. Clin. Exp.* **12**, 990–995
- Otway, S. & Robinson, D. S. (1968) *Biochem. J.* **106**, 677–682
- Phares, E. J. (1951) *Arch. Biochem. Biophys.* **33**, 173–184
- Popjak, G. (1954) *Cold Spring Harbor Symp. Quant. Biol.* **19**, 200–208
- Roux, J. F. & Yoshioka, T. (1970) *Clin. Obstet. Gynecol.* **13**, 595–620
- Rudman, D. (1963) *J. Lipid Res.* **4**, 119–129
- Rudman, D. & Shank, P. W. (1966) *Endocrinology* **79**, 565–571
- Schoni, M., Kaiser, D., Drack, E., Axmacher, U. & Elliott, J. A. (1975) *Eur. J. Clin. Invest.* **5**, 159–163
- Schwartz, J. P. & Jungas, R. I. (1971) *J. Lipid Res.* **12**, 553–562
- Scow, R. O., Chernick, S. S. & Brinley, M. S. (1964) *Am. J. Physiol.* **206**, 796–804
- Shelley, H. J. & Thalme, B. (1970) in *Stoffwechsel des Neugeborenen* (Joppich, G. & Wolf, H., eds.), pp. 178–199, Hippokrates Verlag, Stuttgart
- Szabo, A. J. & Grimaldi, R. D. (1970) *Adv. Metab. Disord.* **4**, 185–228
- Szabo, A. J., Grimaldi, R. D. & Jung, W. E. (1969) *Metab. Clin. Exp.* **18**, 406–411
- Taylor, C. B., Bailey, E. & Bartley, W. (1967) *Biochem. J.* **105**, 717–722
- Turtle, J. R. & Kipnis, D. M. (1967) *Biochim. Biophys. Acta* **144**, 583–593
- Van Harken, D. R., Dixon, C. W. & Heimberg, M. (1969) *J. Biol. Chem.* **244**, 2278–2285
- Vaughan, M. (1962) *J. Biol. Chem.* **237**, 3354–3358
- Villee, C. A. & Hagermann, D. D. (1958) *Am. J. Physiol.* **194**, 457–464
- Villee, C. A. & Loring, J. M. (1961) *Biochem. J.* **81**, 488–494
- Widdowson, E. M. (1950) *Nature (London)* **166**, 626–628
- Widdowson, E. M. & McCance, R. A. (1955) *Br. J. Nutr.* **9**, 316–321