Biochem. J. (1967) **104**, 1040

Characterization and Metabolism of Ovine Foetal Lipids

By T. W. SCOTT, B. P. SETCHELL and J. M. BASSETT
Commonwealth Scientific and Industrial Research Organization, Division of Animal Physiology, The Ian Clunies Ross Animal Research Laboratory, Prospect, New South Wales, Australia

(*Received* 23 *January* 1967)

1. Total phospholipid concentrations in liver, kidney and brain of the 140-day ovine foetus were only half of those in comparable maternal tissues. 2. Phosphatidylcholine was the predominant phospholipid in all foetal tissues examined. The most striking difference between foetal and maternal tissues in individual phospholipids was in the heart; foetal heart contained more ethanolamine plasmalogen than choline plasmalogen, whereas in adult tissue the concentration of these was reversed. Sphingomyelin content of foetal brain was only one-sixth of that of maternal brain tissue. 3. Oleic acid (18:1) was the predominant acid in the phospholipid extracted from foetal tissues, except in brain where palmitic acid (16:0) was slightly higher. In phospholipids from adult tissues there was a higher proportion of unsaturated fatty acids (linoleic acid, 18:2, and linolenic acid, 18:3) and a correspondingly lower proportion of oleic acid (18:1). The distribution of fatty acids in the neutral lipid fraction of foetal and maternal tissues was very similar; oleic acid (18:1) was generally the principal component. 4. ^{14}C derived from [U-^{14}C]-glucose and [U-^{14}C]fructose infused into the foetal circulation *in utero* was incorporated into the neutral lipids and phospholipids of heart, liver, kidney, brain and adipose tissue. 5. Phospholipid analysis revealed that the specific activity of phosphatidic acid was higher in liver than in other tissues. The specific activity of phosphatidylethanolamine was less than that of phosphatidylcholine in heart, but in other tissues they were about the same. The specific activities of phosphatidylinositol and phosphatidic acid in brain were very similar and were higher than the other components. The specific activity of phosphatidylserine was highest in liver and brown fat. 6. The pattern of incorporation of ^{14}C derived from [^{14}C]glucose and [^{14}C]fructose into foetal neutral lipids was similar. Diglyceride accounted for most of the radioactivity in brain, whereas triglyceride had more label in heart, liver, kidney and fat.

It has previously been shown that tissues from near-term foetal lambs (about 140 days) or newborn lambs contain less than 4% of lipid, whereas adult sheep tissues have 30–40% of total lipid (Alexander, 1962; Body & Shorland, 1964; Body, Shorland & Gass, 1966). However, phospholipid analysis on the total maternal and foetal tissues revealed little difference in the distribution of individual phosphatides. Analyses on the fatty acid composition of phospholipids and triglycerides of the whole animal showed that the adult contained more stearic acid, linoleic acid and linolenic acid and less oleic acid than the foetus (Body & Shorland, 1964; Shorland, Body & Gass, 1966), and Leat (1966) has shown a similar difference in the fatty acid composition of the lipids extracted from maternal and foetal liver and heart.

Since most of these studies were on lipid extracts from the whole animal, it was thought worth while to characterize the phospholipids of a wider range of individual foetal tissues. The studies were extended to compare the fatty acid composition of neutral lipids and phospholipids isolated from ovine foetal and maternal heart, liver, kidney, brain and perirenal fat. Moreover, the incorporation of ^{14}C from [^{14}C]glucose and [^{14}C]fructose into foetal lipids has been studied by infusing these precursors directly into the circulation of the foetus *in utero* to show that the ovine foetus is capable of lipid synthesis.

EXPERIMENTAL

Animals. Six pregnant Border Leicester × Merino cross-bred ewes (3 years old) weighing 35–45 kg. were housed individually in pens and fed (1200 g./day) on chaffed lucerne hay and oats (1:1).

Surgical preparation of animals and infusion of ^{14}C-*labelled substrate.* Each ewe was anaesthetized with thialbarbitone sodium and anaesthesia maintained with fluothane (Halothane; Imperial Chemical Industries Ltd., Macclesfield, Cheshire). The abdomen was opened with a paramedial incision extending for about 10cm. anteriorly from the pubic symphysis. With the uterus still in the abdominal cavity, a small blunt dissection was made in the uterine wall over a cotyledon, which was brought out through the opening and turned over to reveal the foetal artery and vein. The vein was identified by its colour and catheterized with an expanded polyvinyl chloride catheter (internal diam. 0·5mm., external diam. 0·8mm. at smaller end; Dural Plastics, Dural, N.S.W., Australia). The catheter could always be inserted about 10–15cm. without difficulty. The cotyledon was returned to the uterus, which was closed with a purse-string suture. The catheter was brought out through the incision and filled with 10% (w/v) EDTA (disodium salt) to prevent clotting.

Next morning, a priming dose of [^{14}C]glucose or [^{14}C]-fructose was given to the foetus through the catheter, then a constant infusion of sterile pyrogen-free aq. 0·9% NaCl (Farmer Hill Pty. Ltd., Sydney, N.S.W., Australia) containing the radioactive substrate was given at a rate of 0·15ml./min. with a peristaltic pump (Technicon, Chaucey, N.Y., U.S.A.). The infusion was continued for 5hr., and at the end of this time the ewe was anaesthetized with pentobarbitone sodium and the foetus quickly delivered by caesarian section. Both ewe and foetus were then killed with an overdose of pentobarbitone sodium.

Collection of foetal and maternal tissues. Heart, liver, kidney, brain and perirenal fat were removed from the foetus and ewes and 1–2g. of tissue was weighed. The tissues were immediately frozen on solid CO_2 before extraction.

Extraction and estimation of lipids. Frozen tissues were homogenized in chloroform–methanol (2:1, v/v; 20ml./g.) and the extract was filtered through glass wool. The filtrate was washed once with 0·2 vol. of water and thrice more with theoretical upper phase as defined by Folch, Lees & Sloane-Stanley (1957). Radioactivity was assayed by the techniques described by Scott, Jay & Freinkel (1966*a*) and phosphorus was estimated by the method of Fiske & Subbarow (1925). Individual phospholipids were separated by the chromatographic procedures of Dawson, Hemington & Davenport (1962). To determine the specific radioactivity of individual phospholipids, the chromatograms were dipped in a solution of quinine sulphate dihydrate (0·025%, w/v) in ethanol as described by Dawson & Dittmer (1961) and the phosphate esters were located by their fluorescence in u.v. light. The areas were cut out, placed in scintillation vials and counted in an automatic liquid-scintillation spectrometer (Packard Instrument Co. Inc., La Grange, Ill., U.S.A.). After counting, the strips were removed and washed with diethyl ether (Scott *et al.* 1966*a*) before phosphorus estimation.

Neutral lipids were separated from phospholipids by silicic acid (Mallinckrodt) column chromatography (Scott, Good & Ferguson, 1966*b*). Individual neutral lipids were separated on thin-layer plates (silica gel G; E. Merck A.-G., Darmstadt, Germany). The samples were applied on to the plates by using the device of Scott & Beeston (1966) and developed in a solvent system of light petroleum (b.p. 40–60°)–diethyl ether–acetic acid (90:10:1, by vol.). The spots were located by lightly spraying the plates with 2,7-dichlorofluorescein (0·2%, w/v) in ethanol and areas of gel corresponding to triglyceride, diglyceride, monoglyceride and cholesterol were aspirated into counting vials for radioactive assay (Goldrick & Hirsch, 1963). Recoveries of radioactivity were in the range 85–90%.

Fatty acid analysis. Neutral lipids (2–50mg.) and phospholipids (3–10mg.) were saponified with 2N-NaOH (4ml.) for 120min. at 95–100°. The non-saponifiable material was removed by extracting twice with light petroleum (b.p. 40–60°) (6ml.). The aqueous phase was acidified with 5N-HCl and the long-chain acids were extracted into light petroleum (b.p. 40–60°). Methyl esters of the fatty acids were prepared by the method of Schlenk & Gellerman (1960) and were separated on a 5ft. column of diethylene glycol succinate (17%, w/w) on 80–100 mesh Chromosorb W, (Wilkins Instrument and Research Inc., Walnut Creek, Calif., U.S.A.) with a gas chromatograph (Aerograph 200 series). Peaks were identified and estimated as described by Ward, Scott & Dawson (1964).

Radioactive D-[U-^{14}C]glucose and [U-^{14}C]fructose were obtained from The Radiochemical Centre, Amersham, Bucks. No impurities were observed when the compounds were chromatographed on Whatman no. 1 paper with phenol saturated with water and butan-1-ol–ethanol–water (9:1:10, by vol.) as the solvent systems.

RESULTS

Phospholipid composition of foetal and maternal tissues (*Table* 1)

The phospholipid concentrations (based on mg. of phospholipid P/g. of tissue) of foetal liver, kidney and brain were half those occurring in corresponding maternal tissues. However, the phospholipid content of foetal heart was only slightly below that of the mother. The concentration of total phospholipid in foetal perirenal fat was almost the same as that in the kidney. In all of the foetal tissues examined phosphatidylcholine was the predominant phospholipid; as a percentage of the total phosphorus, it was slightly less in the corresponding maternal tissues. The most striking maternofoetal differences in the distribution of individual phospholipids were in the relative concentrations of ethanolamine plasmalogen and choline plasmalogen in heart. The ratio of ethanolamine plasmalogen to choline plasmalogen was 2:1 in foetal heart; this ratio was completely reversed (i.e. 1:2) in maternal heart (Table 1). The percentage of ethanolamine plasmalogen was lower in maternal than in foetal kidney, but the concentrations were similar. However, the concentration of ethanolamine plasmalogen was greater in maternal brain. The percentage of sphingomyelin in maternal brain was three times that of foetal brain, thus giving a sixfold increase in the concentration of this phospholipid. The distribution of phospholipids in perirenal fat closely resembled that in the foetal liver. There was little difference

Table 1. *Distribution of individual phospholipids in ovine foetal and maternal tissues*

Foetal lambs were 132 and 133 days old. Maternal tissues were obtained from the mother of lamb 2. Individual phospholipids are expressed as a percentage distribution of total phospholipid phosphorus.

	Heart			Liver			Kidney			Brain			Perirenal fat	
Phospholipid	Lamb 1	Lamb 2	Adult	Lamb 1	Lamb 2	Adult	Lamb 1	Lamb 2	Adult	Lamb 1	Lamb 2	Adult	Lamb 1	Lamb 2
Total (mg. of lipid P/g. wet wt. of tissue)	0·71	0·67	0·82	0·88	0·70	1·51	0·57	0·58	1·05	1·07	1·38	2·14	0·59	0·57
Phosphatidylcholine	39·8	38·4	25·5	50·2	42·5	39·7	37·4	36·3	34·1	54·8	44·6	37·3	45·9	48·9
Phosphatidylethanolamine	11·3	9·5	12·4	17·6	24·0	27·9	15·9	9·7	12·1	15·2	11·4	7·7	20·6	25·1
Phosphatidylserine	1·8	3·0	2·2	3·1	4·3	2·3	4·3	6·0	4·3	3·6	8·2	9·2	2·0	3·0
Phosphatidylinositol	2·3	5·1	4·7	4·7	6·5	8·2	3·4	4·1	7·1	2·5	2·8	2·1	0·5	4·3
Phosphatidic acid	0·3	0·8	0·4	2·0	1·0	0·8	0·9	1·2	1·7	0·5	1·4	2·6	0·5	1·4
Cardiolipin	3·8	5·9	5·8	3·6	2·8	1·1	—	—	0·7	—	0·7	2·0	2·3	0·5
Phosphatidylglycerol	—	—	1·1	1·6	—	5·3	—	—	0·8	0·5	—	—	0·7	0·2
Ethanolamine plasmalogen	15·9	13·6	11·0	2·0	2·1	3·6	11·2	10·0	5·3	10·2	13·5	16·5	4·6	7·3
Choline plasmalogen	8·1	7·0	20·4	0·7	0·2	0·8	1·3	0·8	1·1	0·5	0·9	0·9	2·1	1·2
Sphingomyelin	6·0	6·8	5·5	7·7	9·8	5·0	11·9	12·9	12·8	4·3	4·1	12·8	3·2	3·4
Alkyl ether lipid	4·3	3·6	3·2	3·3	2·7	2·1	3·4	3·0	2·4	2·2	4·1	3·0	3·9	3·2
Recovery (%)	93·6	93·7	92·2	96·5	95·9	96·8	89·7	84·0	82·4	94·3	91·7	94·1	86·3	98·5

Table 2. *Fatty acid composition of phospholipids extracted from ovine foetal and maternal tissues*

Fatty acid notation is that used by Ahrens *et al.* (1959). Values are expressed as percentage composition (w/w) of long-chain fatty acids isolated from lipid fractions. Foetal lambs were 132 and 133 days old. Maternal lipids were obtained from the mother of lamb 2.

	Heart			Liver			Kidney			Brain			Perirenal fat	
Fatty acid designation	Lamb 1	Lamb 2	Adult	Lamb 1	Lamb 2	Adult	Lamb 1	Lamb 2	Adult	Lamb 1	Lamb 2	Adult	Lamb 1	Lamb 2
12:0	—	—	—	—	Trace	Trace	—	—	—	0·1	0·4	0·2	Trace	0·3
14:0	0·4	0·6	0·4	0·5	0·6	0·2	0·5	1·0	0·2	2·3	1·2	0·7	0·2	0·6
14:1	—	Trace	0·4	—	Trace	0·1	Trace	0·4	0·1	—	—	—	—	—
15:0	0·4	Trace	0·6	0·3	0·6	0·4	Trace	0·4	0·2	—	—	0·2	Trace	—
16:0	17·0	15·9	12·8	13·3	19·8	15·7	20·4	19·6	16·0	22·2	29·8	19·2	14·2	18·3
16:1	3·2	5·4	5·1	4·1	5·7	1·0	4·7	5·3	3·6	4·8	4·9	3·0	1·8	2·1
17:0	0·6	1·4	1·3	2·3	1·6	1·2	1·6	0·8	1·1	0·7	1·0	0·9	0·6	0·3
17:1	0·4	1·8	2·6	1·3	0·8	0·4	0·5	1·0	1·1	0·3	1·6	1·7	0·2	0·8
18:0	12·1	11·3	13·3	18·3	19·6	29·5	11·6	10·5	16·3	13·2	13·8	16·5	13·2	12·6
18:1	36·4	38·9	14·3	29·4	28·3	23·6	35·8	31·7	23·7	19·2	22·5	33·4	43·5	46·0
18:2	4·5	3·0	23·6	3·5	1·7	9·2	2·7	1·7	13·7	0·8	Trace	2·2	1·1	0·8
18:3	—	—	1·7	1·0	Trace	1·8	—	—	1·6	—	—	1·3	—	—
19:0	2·5	3·2	—	2·9	1·7	1·5	2·6	2·2	1·9	0·3	1·9	3·3	1·0	1·1
20:0	—	—	0·9	—	—	—	2·5	2·8	1·6	—	—	—	—	—
20:4	11·4	9·5	15·6	11·0	11·3	9·7	11·4	13·0	14·2	13·3	8·0	6·8	16·8	10·1
22:0	5·0	4·4	—	4·1	3·2	—	—	—	—	1·4	1·8	—	5·0	4·9
Hydroxy acids?	—	—	—	—	—	—	—	—	—	14·4	9·0	9·5	—	—
Unknown acids > C_{18}	5·1	3·4	4·9	6·2	3·2	4·9	5·6	8·0	3·4	6·1	3·0	0·8	2·0	1·1

Table 3. *Fatty acid composition of neutral lipids extracted from ovine foetal and maternal tissues*

Further details are given in Table 2.

	Heart			Liver			Kidney			Brain			Perirenal fat	
Fatty acid designation	Lamb 1	Lamb 2	Adult	Lamb 1	Lamb 2	Adult	Lamb 1	Lamb 2	Adult	Lamb 1	Lamb 2	Adult	Lamb 1	Lamb 2
10:0	0·4	0·2	0·1	—	—	—	0·2	0·3	—	0·1	0·5	—	0·2	0·2
12:0	0·5	0·3	0·1	1·2	0·4	0·6	1·0	0·7	0·5	0·2	0·7	0·4	1·1	0·4
14:0	1·9	2·0	2·7	5·1	5·0	2·3	2·9	2·4	2·0	0·7	2·0	1·0	1·4	1·5
14:1	0·4	1·3	0·3	—	—	1·0	0·3	0·5	0·3	0·2	0·5	0·3	0·4	0·5
15:0	0·5	1·1	0·4	1·6	0·3	0·8	0·3	0·8	0·3	0·2	0·3	0·3	0·7	0·4
15:1	0·1	0·6	0·3	—	—	0·5	Trace	0·3	0·2	0·1	0·3	1·8	0·5	0·4
16:0	19·5	20·2	20·4	20·6	25·1	28·3	21·6	25·4	28·9	13·2	13·6	9·4	14·4	22·2
16:1	3·8	3·7	2·6	7·6	3·0	5·0	5·7	3·9	4·3	4·0	2·8	2·1	3·4	3·5
17:0	0·9	0·8	1·8	4·5	0·8	1·5	1·3	1·1	0·8	0·8	0·6	0·4	0·5	1·5
17:1	1·0	2·8	1·1	—	—	1·3	0·5	0·7	0·5	0·6	0·5	2·0	0·4	0·6
18:0	15·1	13·6	28·6	9·8	14·0	12·5	12·0	11·8	13·7	21·3	18·2	30·5	11·9	12·6
18:1	50·5	48·0	37·0	41·2	45·0	37·3	40·3	43·9	35·0	23·3	18·5	31·4	52·9	45·5
18:2	2·1	1·3	3·4	6·3	5·8	5·3	3·3	1·6	6·5	2·8	0·9	2·2	4·3	3·7
18:3	—	—	0·8	—	—	0·8	0·5	0·8	1·3	Trace	0·6	2·0	2·1	1·2
20:4	0·4	1·5	0·4	0·2	Trace	2·1	3·7	3·0	2·5	9·6	4·1	8·4	3·6	2·2
22:0	1·5	1·2	—	—	—	—	3·7	1·9	—	—	—	—	—	—
Hydroxy acids?	—	—	—	—	—	—	—	—	—	18·6	22·5	7·8	—	—
Unknown acids $>C_{18}$	0·9	0·8	—	1·1	0·3	0·3	1·9	0·7	1·8	4·0	5·5	—	2·0	2·5

Table 4. *Incorporation of* [U-^{14}C]*glucose and* [U-^{14}C]*fructose into lipids extracted from foetal tissues*

[^{14}C]Glucose and [^{14}C]fructose were infused into the umbilical vein of the foetus for a 5 hr. period at the rate listed after giving a priming dose (p.d.). Values are expressed as ^{14}C counts/min./g. wet wt. of tissue.

Expt. no.	Age of foetus (days)	Substrate	Lipid	Heart	Liver	Kidney	Brain	Perirenal fat
1	133	Glucose 5 μc (p.d.) + 0·05 μc/min.	Total	102	184	54	53	247
2	132	Glucose 5 μc (p.d.) + 0·05 μc/min.	Total	112	29	20	77	170
3	132	Glucose 25 μc (p.d.) + 0·23 μc/min.	Neutral	162	104	214	316	3260
			Phospholipid	486	905	179	670	507
4	133	Fructose 5 μc (p.d.) + 0·05 μc/min.	Total	47	500	66	69	531
5	137	Fructose 25 μc (p.d.) + 0·23 μc/min.	Neutral	70	49	57	55	333
			Phospholipid	69	203	101	137	100
6	134	Fructose 10 μc (p.d.) + 0·09 μc/min.	Neutral	55	58	40	102	1419
			Phospholipid	88	230	71	192	186

between foetal and maternal tissues in the percentage distribution of most other phospholipids.

Fatty acid composition of ovine foetal and maternal tissues

(i) *Phospholipid fatty acids* (*Table* 2). Oleic acid (18 : 1) was the predominant fatty acid in foetal heart, liver, kidney and particularly in perirenal fat. The percentage of palmitic acid (16 : 0) in brain exceeded that of oleic acid (18 : 1) in the foetus but not in the mother (Table 2). The outstanding difference between foetal and maternal phospholipids was the much greater amount of linoleic acid (18 : 2) and, to a smaller degree, linolenic acid (18 : 3) in maternal tissues. In phospholipids of maternal liver and kidney, there was a greater proportion of stearic acid (18 : 0) when compared with the foetal values. There was also a smaller proportion of oleic acid (18 : 1) in maternal heart, liver and kidney when compared with the corresponding foetal tissue. There were no consistent differences between foetal and maternal tissues in the proportions of palmitic acid (16 : 0) and arachidonic acid (20 : 4). In maternal and foetal brain, the phospholipids contained appreciable proportions of unknown acids, tentatively identified as hydroxy acids.

(ii) *Neutral lipid fatty acids* (*Table* 3). Differences in the degree of saturation of fatty acids in the neutral lipid components were less pronounced than in the phospholipid fractions. In fact, the proportion of linoleic acid (18 : 2) in most tissues was strikingly similar. However, the proportion of oleic acid (18 : 1) in the neutral lipids of maternal heart was well below the foetal value and that of stearic acid (18 : 0) was greater. The distribution of fatty acids in neutral lipids of foetal and maternal liver and kidney was very similar. Oleic acid (18 : 1) was the principal fatty acid of foetal perirenal fat and accounted for about 50% of the total. There were also appreciable proportions of hydroxy acids (?) in the neutral lipid fraction extracted from foetal and adult brain.

Biosynthesis of foetal lipids

(i) *Incorporation of* ^{14}C *derived from* [U-^{14}C]*glucose and* [U-^{14}C]*fructose into foetal lipid* in vivo (*Table* 4). Although there was considerable variation between animals, ^{14}C from both [^{14}C]glucose and [^{14}C]fructose was incorporated into lipid in all tissues. The most active incorporation of ^{14}C from [^{14}C]glucose (per g. of tissue) was in perirenal fat (Expts. 1–3, Table 4). Further fractionation of the lipids in this tissue revealed that about 80% of the radioactivity was in the neutral lipids; in most other tissues the proportion of radioactivity was greater in the phospholipids, particularly in liver and brain (Expt. 3, Table 4). Similar results were obtained with [U-^{14}C]fructose (Expts. 4–6, Table 4), perirenal fat incorporating more ^{14}C radioactivity than any other foetal tissue. The distribution of radioactivity in the neutral lipids and phospholipids was very similar to that observed with glucose as precursor.

(ii) *Specific radioactivities of individual phospholipids* (*Table* 5). Since radioactivity was only determined in the deacylated phospholipids any ^{14}C incorporated into fatty acids will not have been detected. The foetal phospholipids obtained from Expt. 3 (see Table 4) were analysed further to determine the specific activities of individual phosphatides. The specific activity of heart phosphatidylethanolamine was very low and well below that of phosphatidylcholine; similar results have been obtained with heart slices when incubated with [^{32}P]orthophosphate (T. W. Scott & G. Alexander, unpublished work). This was in contrast with most other foetal tissues, where the specific activities of these two lipids were almost the same.

Table 5. *Specific radioactivities of individual phospholipids isolated from ovine foetal tissues after infusion of* [U-^{14}C]*glucose*

Values are expressed as counts/min./mg. of phosphorus and represent the radioactivity present in the deacylated lipids. [U-^{14}C]Glucose, 25 μc priming dose, followed by 0·23 μc/min., was infused into the umbilical vein of the foetal lamb *in utero*.

Phospholipid	Heart	Liver	Kidney	Brain	Perirenal fat
Phosphatidylcholine	197	660	232	135	215
Phosphatidylethanolamine	24	465	248	127	287
Phosphatidylinositol	571	681	567	507	377
Phosphatidylserine	*	1727	132	101	854
Phosphatidic acid	*	4400	1050	452	*

* No ^{14}C radioactivity could be detected in these lipids.

Table 6. *Distribution of radioactivity in ovine foetal neutral lipids after infusion of* [U-^{14}C]*glucose and* [U-^{14}C]*fructose*

[U-^{14}C]Glucose and [U-^{14}C]fructose were infused into the foetal lambs *in utero* by the procedure outlined in the text and Tables 4 and 5. Values are expressed as percentages of the neutral-lipid radioactivity.

Expt. no.		Neutral lipid	Heart	Liver	Kidney	Brain	Perirenal fat
3	[U-^{14}C]Glucose	Monoglyceride	6·2	25·7	7·8	19·4	0·1
	25 μc (p.d.)+0·23 μc/min.	Diglyceride	8·7	14·6	17·2	53·1	12·4
		Triglyceride	81·4	48·5	73·5	10·5	86·7
		Cholesterol	3·7	11·2	1·5	17·0	0·8
5	[U-^{14}C]Fructose	Monoglyceride	13·3	20·5	9·6	10·0	0·2
	25 μc (p.d.)+0·23 μc/min.	Diglyceride	11·3	30·6	12·6	57·7	12·9
		Triglyceride	74·8	41·5	75·6	8·3	81·8
		Cholesterol	0·6	7·4	2·2	24·0	5·0

The specific activities of phosphatidylcholine and phosphatidylethanolamine were higher in liver than in any other tissue, but these values were still below those of phosphatidylinositol, phosphatidic acid and phosphatidylserine. The specific activity of liver phosphatidic acid far exceeded that of any other lipid in all the tissues examined. Phosphatidylserine had its highest specific activity in liver and perirenal fat, with much lower values in kidney and brain. In brain, the specific activities of phosphatidylinositol and phosphatidic acid were well above those of any other component.

The specific activities of maternal liver phospholipids from this experiment were examined and found to be 170, 183, 373 and 55 counts/min./mg. of P respectively for phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol. These values were below those of the corresponding foetal liver phospholipids (see Table 5).

(iii) *Distribution of* ^{14}C *radioactivity in neutral lipids* (*Table* 6). Most of the radioactivity in the brain neutral lipids was in the diglyceride fraction with only a small portion of radioactivity in the triglyceride. In contrast, in all other tissues examined, the radioactivity in the triglyceride fraction was greater than in any other component. In heart, kidney and perirenal fat, the triglyceride radioactivity accounted for more than 70% of the total; in liver this was less than 50% and there was a corresponding increase in the proportions of radioactivity in monoglyceride and diglyceride fractions. Very similar trends were observed when the foetus was infused with [U-^{14}C]fructose.

DISCUSSION

The results establish that there are differences between foetal and maternal tissues in the phospholipid content (cf. Body *et al.* 1966). First, there are definite differences between foetal and maternal heart in the distribution of the plasmalogens. The higher amount of choline plasmalogen in ovine adult heart agrees with results for bovine heart (Gray & Macfarlane, 1958; Thiele, Schröder & Berg, 1960; Dawson *et al.* 1962) and equine heart muscle (Thiele *et al.* 1960). The ovine foetal heart is unlike these but resembles rat heart, in which the concentration of ethanolamine plasmalogen is much greater than the choline plasmalogen (Marinetti,

Temple & Stotz, 1961). In all other foetal tissues examined, including perirenal fat, which at this stage of development is largely brown fat (Hull, 1966), the concentration of ethanolamine plasmalogen exceeded that of choline plasmalogen, and this difference was still apparent in adult tissue. In most tissues, ethanolamine plasmalogen is present in higher concentrations than the choline plasmalogen (Rapport & Norton, 1962). However, ram spermatozoa (Hartree & Mann, 1959), in contrast with rat spermatozoa (Scott, Dawson & Rowlands, 1963), have almost as much choline plasmalogen as lecithin and virtually no ethanolamine plasmalogen. Thus significant differences in the ratio of choline to ethanolamine plasmalogen do exist in different animal tissues, but at this stage it is not possible to speculate on their biochemical significance.

The higher content of sphingomyelin in adult brain is consistent with myelination proceeding actively in the sheep after birth. The content of sphingomyelin in rat brain also increases after birth (Ansell & Spanner, 1961).

The very low proportion of linoleic acid (18 : 2) and linolenic acid (18 : 3) in foetal phospholipids and neutral lipids is in agreement with the results of Body & Shorland (1964), Shorland *et al.* (1966) and Leat (1966). The present studies reveal that the most significant differences in the proportions of unsaturated fatty acids between individual foetal and adult tissues occur in the phospholipid fraction. Such differences may be due to a rapid conversion of the C_{18} unsaturated acids into C_{20} or higher polyunsaturated acids by foetal tissues via the pathway outlined by Steinberg, Slaton, Howton & Mead (1957). Alternatively the placenta of the sheep may be impermeable to C_{18} di- and triunsaturated fatty acids, since these are present in appreciable amounts in adult plasma (Leat, 1966).

Histochemical observations by Fawcett (1952) and Menschik (1953) show that brown fat contains more phospholipid than neutral lipid. This large amount of phospholipid may well be explained by the inclusion of numerous subcellular organelles (e.g. mitochondria), which are involved in the thermoregulation of newborn animals (Hull, 1966). Foetal adipose tissue incorporates more ^{14}C from [^{14}C]glucose and [^{14}C]fructose into neutral lipid than any other foetal tissue and this further supports its potential biochemical importance as an energy source in newborn animals.

In previous studies when ^{32}P-labelled phospholipids in emulsions or serum were injected intravenously into the maternal circulation of rats or rabbits, no evidence was obtained for the direct transfer of intact phospholipids across the placenta (Nielson, 1941; Popják, 1954). These findings were substantiated by the results of Villee & Hagerman (1958) and Roux (1966), who showed that ^{14}C derived from [^{14}C]glucose or [^{14}C]acetate was incorporated into lipid by slices of liver from foetal rat and rabbit. The incorporation of ^{14}C derived from [U-^{14}C]glucose and [U-^{14}C]fructose into neutral lipid and phospholipid by ovine foetal tissues *in utero* in the present studies is further evidence for lipid synthesis. Further, if the foetal lipid were derived from the maternal circulation one would expect the specific activity of the maternal phospholipids to be greater than that of the foetal phospholipids under conditions of continuous infusion of ^{14}C-labelled precursor. Since the liver is the site of synthesis of most plasma phospholipids (Fishler, Entenman, Montgomery & Chaikoff, 1943; Harper, Neal & Hlavacek, 1953), the specific activity of maternal liver phospholipids was examined and the values obtained were below those of the corresponding foetal liver phospholipid. Hence it is most unlikely that the foetal liver phospholipids are derived from the mother. The concept of synthesis by ovine foetal tissues is further enhanced by the incorporation of [^{32}P]orthophosphate into phospholipids by slices of ovine foetal heart, liver and kidney (T. W. Scott & G. Alexander, unpublished work).

The labelling of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidic acid and phosphatidylinositol in foetal brain from [^{14}C]glucose can be contrasted with the results obtained by Dawson (1954) for adult guinea-pig brain (with [^{32}P]orthophosphate), where phosphatidic acid and phosphatidylinositol were the only two lipids significantly labelled. However, in young rat brain (3–13 days old) significant labelling of all the above phospholipids occurred (Sperry, 1962). The rate of incorporation of ^{14}C from [^{14}C]glucose into other foetal phospholipids clearly establishes that significant differences exist between tissues. The relative labelling patterns of the heart phospholipids, with phosphatidylinositol being the highest and phosphatidylethanolamine the lowest, is similar to that reported by Marinetti, Erbland, Albrecht & Stotz (1958) for adult rat heart. However, the higher specific activities of the phosphatidic acid in foetal liver and kidney can be contrasted with the results obtained by Marinetti *et al.* (1958), Tinker, Koch & Hanahan (1963) and Tinker & Hanahan (1966) for these adult rat and rabbit tissues.

The lack of significant incorporation of ^{14}C from [^{14}C]glucose or [^{14}C]fructose into foetal brain triglyceride, in comparison with other tissues, is not surprising as brain is characterized by having negligible amounts of this neutral lipid (Rossiter, 1962). The incorporation of ^{14}C-labelled precursor into liver neutral lipids is consistent with the data of Villee & Hagerman (1958). The fact that fructose infused into the foetal circulation is converted into

lipid in a manner analogous to glucose raises a number of questions with regard to the mechanism, as it is generally assumed that fructose is not utilized by the foetal lamb, despite its relatively high concentration in the plasma (Alexander, Britton & Nixon, 1966; Dawkins, 1966), nor is it converted back into glucose by the placenta (Britton, Huggett & Nixon, 1963).

T. W. S. was in receipt of a Queen Elizabeth II Fellowship during this work. Miss S. Milner rendered very skilful technical assistance.

REFERENCES

Ahrens, E. H., Insull, W., Hirsch, J., Stoffel, W., Petersen, M. L., Farquhar, J. W., Miller, T. & Thomasson, H. J. (1959). *Lancet*, i, 115.

Alexander, D. P., Britton, H. G. & Nixon, D. A. (1966). *J. Physiol.* **185**, 382.

Alexander, G. (1962). *Aust. J. agric. Res.* **13**, 144.

Ansell, G. B. & Spanner, S. (1961). *Biochem. J.* **79**, 176.

Body, D. R. & Shorland, F. B. (1964). *Nature, Lond.*, **202**, 769.

Body, D. R., Shorland, F. B. & Gass, J. B. (1966). *Biochim. biophys. Acta*, **125**, 207.

Britton, H. G., Huggett, A. St G. & Nixon, D. A. (1963). *J. Physiol* **166**, 21.

Dawkins, M. J. R. (1966). *Brit. med. Bull.* **22**, 27.

Dawson, R. M. C. (1954). *Biochim. biophys. Acta*, **14**, 374.

Dawson, R. M. C. & Dittmer, J. C. (1961). *Biochem. J.* **81**, 540.

Dawson, R. M. C., Hemington, N. & Davenport, J. B. (1962). *Biochem. J.* **84**, 497.

Fawcett, H. D. (1952). *J. Morph.* **90**, 363.

Fishler, M. C., Entenman, C., Montgomery, M. L. & Chaikoff, C. L. (1943). *J. biol. Chem.* **150**, 47.

Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.

Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). *J. biol. Chem.* **226**, 497.

Goldrick, B. & Hirsch, J. (1963). *J. Lipid Res.* **4**, 483.

Gray, G. M. & Macfarlane, M. G. (1958). *Biochem. J.* **70**, 409.

Harper, P. V., Neal, W. B. & Hlavacek, S. R. (1953). *Metabolism*, **2**, 69.

Hartree, E. F. & Mann, T. (1959). *Biochem. J.* **71**, 423.

Hull, D. (1966). *Brit. med. Bull.* **22**, 92.

Leat, W. F. M. (1966). *Biochem. J.* **98**, 598.

Marinetti, G. V., Erbland, J., Albrecht, M. & Stotz, E. (1958). *Biochim. biophys. Acta*, **30**, 543.

Marinetti, G. V., Temple, K. & Stotz, E. (1961). *J. Lipid Res.* **2**, 188.

Menschik, Z. (1953). *Anat. Rec.* **116**, 439.

Nielson, P. E. (1941). *Amer. J. Physiol.* **135**, 670.

Popják, G. (1954). *Cold Spr. Harb. Symp. quant. Biol.* **19**, 200.

Rapport, M. R. & Norton, W. T. (1962). *Annu. Rev. Biochem.* **31**, 103.

Rossiter, R. J. (1962). In *Neurochemistry*, p. 27. Ed. by Elliott, K. A. C., Page, I. H. & Quastel, J. H. Springfield, Ill: Charles C. Thomas.

Roux, J. F. (1966). *Metabolism*, **15**, 856.

Schlenk, H. & Gellerman, J. L. (1960). *Analyt. Chem.* **32**, 1412.

Scott, T. W. & Beeston, J. W. U. (1966). *J. Lipid Res.* **7**, 456.

Scott, T. W., Dawson, R. M. C. & Rowlands, I. W. (1963). *Biochem. J.* **87**, 507.

Scott, T. W., Good, B. F. & Ferguson, K. A. (1966*b*). *Endocrinology*, **79**, 949.

Scott, T. W., Jay, S. & Freinkel, N. (1966*a*). *Endocrinology*, **79**, 591.

Shorland, F. B., Body, D. R. & Gass, J. B. (1966). *Biochim. biophys. Acta*, **125**, 217.

Sperry, W. M. (1962). In *Neurochemistry*, p. 75. Ed. by Elliott, K. A. C., Page, I. H. & Quastel, J. H. Springfield, Ill.: Charles C. Thomas.

Steinberg, G., Slaton, W. H., jun., Howton, D. R. & Mead, J. F. (1957). *J. biol. Chem.* **224**, 841.

Thiele, O. W., Schröder, H. & Berg, W. (1960). *Hoppe-Seyl. Z.* **322**, 147.

Tinker, D. O. & Hanahan, D. J. (1966). *Biochemistry*, **5**, 423.

Tinker, D. O., Koch, A. & Hanahan, D. J. (1963). *Arch. Biochem. Biophys.* **103**, 66.

Villee, C. A. & Hagerman, D. D. (1958). *Amer. J. Physiol.* **194**, 457.

Ward, P. F. V., Scott, T. W. & Dawson, R. M. C. (1964). *Biochem. J.* **92**, 60.