Fatty Acid Composition of the Plasma Lipids of Newborn and Maternal Ruminants

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1. The fatty acid compositions of the plasma lipids of newborn unsuckled lambs, kids, calves and piglets have been determined and compared with those of maternal plasma lipids at parturition. 2. The predominating plasma fatty acids in the newborn of all species are palmitic acid, $C_{16:1}$ acid, stearic acid and $C_{18:1}$ acid. This finding is consistent with the synthesis of the major proportion of fatty acids from non-lipid sources within the foetus. 3. Very small amounts of $C_{18:2}$ acid and $C_{18:3}$ acid are present in the plasma lipids of newborn ruminants, although considerable amounts of these fatty acids are contained in maternal plasma. The plasma fatty acids of the newborn piglet contained 5.5% of $C_{18:2}$ acid, those of the calf 2.0%, and those of the lamb and kid less than 1.0%. This finding is discussed in relation to the higher content of $C_{18:2}$ acid in the plasma non-esterified fatty acid fraction of the sow (15%) compared with that of the ruminant (less than 4%). 4. In the lamb and kid, but not in the calf or piglet, a $C_{20:3}$ acid was detected in plasma lipids that was very similar to, if not identical with, the $C_{20:3}$ acid that accumulates in the plasma of animals given diets low in essential fatty acids. The possible significance of this finding is discussed. 5. The cholesteryl esters of cow plasma were found to contain a higher percentage (43%) of $C_{18:3}$ acid than those of goat and sheep plasma (5–10%). The possible reasons for this difference are discussed.

During an investigation of the plasma lipids of the neonatal lamb, it was noted that the total fatty acids of the plasma of newborn unsuckled lambs contained negligible amounts of octadecadienoic ($C_{18:2}$) acid. As the plasma of newborn monogastric animals contains appreciable amounts of $C_{18:2}$ acid (Satomura & Söderhjelm, 1962), it was decided to investigate the plasma fatty acids of other newborn ruminants to determine whether or not they were similar to the lamb. In addition, the plasma fatty acids of the sow and newborn piglet were examined as representatives of a non-ruminant farm animal. A preliminary account has been published (Leat, 1964).

EXPERIMENTAL

Animals. Blood samples were obtained from newborn unsuckled lambs, kids, calves and piglets, and from their mothers immediately after parturition. Samples were usually obtained from the external saphenous vein of newborn ruminants, from the jugular vein in adult ruminants, from the anterior vena cava of the piglet and from the ear vein of the sow. Blood was collected into chilled tubes containing a dry mixture (3:2, w/w) of ammonium oxalate and potassium oxalate (20 mg./10 ml. of blood) and stored

in crushed ice until centrifuged at 4° to obtain the plasma. Samples of heart and liver were obtained from a foetal lamb estimated to be within 14 days of birth.

Extraction of plasma lipids. Plasma (5 ml.) was poured into 50 ml. of methanol-chloroform (1:1, v/v) containing 10 mg. of quinol, and allowed to stand for 1 hr. with occasional shaking. After filtration, 10 ml. of the filtrate was evaporated almost to dryness in a rotary evaporator under reduced pressure; the residue was saponified and the methyl esters of the fatty acids were prepared as described previously (Leat, 1963). The remainder of the filtrate was similarly reduced to a small volume; the individual plasma lipids were separated by thin-layer chromatography on silica gel G and the methyl esters of each fraction were prepared as described by Bowyer, Leat, Howard & Gresham (1963).

Heart and liver samples. A 0.5 g. sample of tissue was cut into small pieces and digested by heating with 10 ml. of methanolic N-KOH containing 20 mg. of quinol to minimize oxidation. After refluxing for 1 hr. the fatty acids were extracted and the methyl esters prepared as above.

Gas-liquid chromatography. The methyl esters were separated at 180° on a 4 ft. column of 10% polyethylene glycol adipate on 100–120 mesh Gas-Chrom Z (Applied Science Laboratories Inc., Pennsylvania, U.S.A.). Peaks were identified and estimated as described previously (Leat, 1963).

RESULTS

Total fatty acids of plasma. Table 1 shows that the most notable difference between the fatty acid composition of the newborn of all species and their mothers was in the content of C_{18:2} acid, which was very much lower in the offspring. Among the newborn animals, piglets had the highest concentrations of C_{18:2} acid (5%) and lambs and kids the lowest concentrations (less than 1%). Octadecatrienoic (C_{18:3}) acid was virtually absent from the newborn of all species although present in the mother, this difference being particularly noticeable between the cow and her calf. Compared with the mother, the newborn had higher concentrations of palmitic acid, C_{16:1} acid and C_{18:1} acid. The C_{20:4} acid content of both maternal and newborn plasma was low in all animals studied, with the exception of the piglet. In all adult and infant animals there were trace amounts of an eicosatrienoic (C_{20:3}) acid having a carbon number of 21.2 (Woodford & Van Gent, 1960). In the lamb and kid, another eicosatrienoic acid of carbon number 21.0 was also present.

Plasma phospholipids (Table 2). The differences in fatty acid composition of the total lipids of newborn and maternal plasma were reflected in the phospholipid fatty acids. A lower content of stearic acid in the phospholipids of the newborn compared with the maternal plasma also became apparent. The high content of C_{20:4} acid in the piglet is of interest. Although the composition of the phospholipids of the newborn and maternal plasma differed considerably, the percentage of total unsaturated acids was rather constant in both mother and offspring at about 50%.

Plasma cholesteryl esters (Table 2). The small

amount of C_{18:2} acid present in the plasma of the newborn appeared to be mainly associated with the cholesteryl ester fraction, where the major unsaturated acids were C_{18:1} acid and C_{16:1} acid. In the maternal plasma cholesteryl esters, the major unsaturated fatty acid in the pig, sheep and goat was C_{18:2} acid, whereas in the cow C_{18:3} acid predominated. Although C_{18:3} acid accounted for nearly half of the fatty acids in cow plasma cholesteryl esters, only trace amounts were present in those of the calf. The fatty acids of the newborn of all species were more saturated than those of the mother, containing more palmitic acid and stearic acid.

Plasma triglycerides (Table 2). Adult ruminant plasma triglycerides were more saturated than those of the sow, containing considerably less C_{18:2} acid and more stearic acid. Newborn ruminant triglycerides were more unsaturated than maternal triglycerides, containing less stearic acid and C_{18:2} acid and more C_{18:1} acid.

Plasma non-esterified fatty acids (Table 2). The plasma non-esterified fatty acids of the sow were more saturated than the triglycerides. In all the ruminants, however, the reverse was the case, with the plasma triglycerides being more saturated than the non-esterified fatty acids. The C_{18:2} acid content of the sow plasma non-esterified fatty acids was much higher than that of ruminant plasma non-esterified fatty acids (15% compared with less than 4%). Of note is the high saturation of piglet plasma non-esterified fatty acids.

Total fatty acids of foetal liver and heart (Table 3). The low concentrations of C_{18:2} acid noted in the plasma fatty acids of the lamb were also apparent in the fatty acids of liver and heart, but, despite

Table 1. *Percentage composition of the total fatty acids of maternal and newborn blood plasma*

The carbon no. is as defined by Woodford & Van Gent (1960), and the fatty acid designation is given as *x:y*, where *x* is the no. of carbon atoms/mol. and *y* is the no. of double bonds/mol. Each result given is the average of those for two animals. Some minor fatty acids have been omitted for clarity.

Carbon no.	Fatty acid designation	Fatty acid composition (% w/w)							
		Sow	Piglet	Cow	Calf	Goat	Kid	Sheep	Lamb
14.0	14:0	1.3	2.1	1.5	1.5	1.0	2.2	1.2	1.8
16.0	16:0	19.4	23.1	11.5	32.3	17.5	24.1	16.7	22.5
16.3	16:1	4.2	10.5	3.1	11.0	2.9	13.0	3.3	8.4
18.0	18:0	14.0	11.8	9.5	10.2	12.7	6.8	12.2	9.3
18.3	18:1	18.7	36.9	15.4	39.5	27.6	49.5	28.3	50.0
18.9	18:2	34.2	5.5	24.9	2.0	29.5	<0.5	26.1	0.9
19.6	18:3	1.3	—	30.3	<0.5	3.4	—	4.6	<0.5
21.0	20:3	—	—	—	—	—	1.4	—	2.0
21.2	20:3	<0.5	1.1	0.6	0.8	<0.5	<0.5	1.0	0.8
21.5	20:4	1.8	6.3	1.0	1.2	3.0	0.5	2.1	1.2
Total unsaturated acids		60.2	60.3	75.3	54.9	66.7	64.7	65.4	63.6

Table 2. *Percentage composition of the major fatty acids of the component lipids of maternal and newborn blood plasma*

The fatty acid designation is defined in Table 1. Each result given is the average of those for two animals. Some minor fatty acids have been omitted for clarity.

Fatty acid designation	Fatty acid composition (% w/w)							
	Sow	Piglet	Cow	Calf	Goat	Kid	Sheep	Lamb
(a) Cholesteryl esters								
14:0	1.5	2.9	2.1	2.8	1.6	2.6	0.9	0.9
16:0	12.3	24.6	7.4	29.0	17.0	23.5	14.6	32.8
16:1	7.0	16.5	4.3	16.2	5.6	24.5	2.6	13.8
18:0	5.5	7.3	1.9	7.7	6.7	8.2	5.9	11.0
18:1	16.7	34.0	6.4	28.0	24.3	28.3	27.1	35.7
18:2	48.2	5.8	29.8	5.5	34.1	1.5	37.0	1.5
18:3	0.6	0.5	43.7	0.8	5.0	0.9	10.2	<0.5
20:3	—	—	—	—	—	—	—	2.5
20:3?	—	—	0.6	1.0	—	—	<0.5	—
20:4	1.5	2.5	1.1	2.4	1.1	<0.5	0.7	—
Total unsaturated acids	74.0	59.3	85.9	53.9	70.1	55.2	76.9	52.1
(b) Phospholipids								
14:0	<0.5	0.7	0.9	0.5	<0.5	<0.5	<0.5	1.0
16:0	18.6	23.7	20.5	30.5	21.0	36.0	23.9	35.8
16:1	1.1	4.0	1.7	4.3	0.9	9.6	<0.5	6.6
18:0	34.6	25.7	25.2	21.4	28.8	10.8	26.4	15.1
18:1	16.3	26.2	16.9	34.0	14.5	36.6	20.3	33.7
18:2	23.8	4.4	15.8	1.7	22.6	<0.5	19.5	0.6
18:3	<0.5	0.7	11.0	<0.5	1.6	—	3.0	<0.5
20:3	—	—	—	—	—	3.2	—	3.2
20:3?	<0.5	0.5	1.3	1.9	<0.5	<0.5	<0.5	<0.5
20:4	4.4	11.8	1.8	3.5	4.1	2.2	2.5	2.2
Total unsaturated acids	46.0	47.6	48.5	45.4	43.7	51.6	45.7	46.3
(c) Triglycerides								
14:0	1.5	3.0	2.2	2.2	1.9	1.0	<0.5	<0.5
16:0	28.8	30.4	34.4	43.3	29.1	26.7	29.7	37.0
16:1	4.7	11.9	13.3	13.3	6.3	8.4	3.2	7.5
18:0	5.5	9.7	17.9	6.9	18.7	8.2	25.5	9.5
18:1	36.8	38.5	24.2	30.2	34.5	51.2	33.9	45.0
18:2	21.0	2.2	1.5	0.9	4.2	0.7	4.8	0.5
18:3	0.5	<0.5	1.0	—	0.9	0.6	—	—
Total unsaturated acids	63.0	52.8	40.0	45.4	45.9	60.9	41.9	53.0
(d) Non-esterified fatty acids								
14:0	0.8	3.9	1.6	1.3	1.3	0.8	<0.5	<0.5
16:0	34.9	43.2	21.2	31.3	17.9	17.5	17.8	21.8
16:1	5.3	5.2	4.3	8.1	3.5	3.9	0.9	4.1
18:0	17.6	21.1	22.6	11.3	23.1	14.5	34.9	16.2
18:1	20.8	15.2	45.8	43.8	46.8	62.0	41.8	57.6
18:2	15.2	4.0	1.5	1.3	3.6	<0.5	3.0	<0.5
18:3	1.3	—	<0.5	0.7	0.7	—	0.5	—
Total unsaturated acids	42.6	24.4	52.0	52.9	54.6	65.9	46.2	61.7

this, appreciable amounts of C_{20:4} acid were present. Both eicosatrienoic acids were present, the one of carbon number 21:0 predominating. Analyses of adult sheep liver and heart are included for comparison.

DISCUSSION

Although adult ruminant plasma contains large amounts of C_{18:2} acid and, in the cow, considerable amounts of C_{18:3} acid also, very small amounts of

these acids are present in the plasma of the newborn ruminant. In the foetal lamb these low concentrations of C_{18:2} acid and C_{18:3} acid are not confined to the plasma but are also apparent in the total fatty acids of liver and heart. Body & Shorland (1964) have also shown that the foetal lamb has very little fat in its carcass and that this fat contains very little C_{18:2} acid. The newborn piglet, on the other hand, contains much more C_{18:2} acid in its plasma than does the newborn ruminant. The

Table 3. *Fatty acid compositions of the liver and heart of a foetal lamb (estimated to be 14 days from birth) compared with those of an adult sheep*

The fatty acid designation is defined in Table 1.

Fatty acid designation	Fatty acid composition (% w/w)			
	Sheep		Foetal lamb	
	Liver	Heart	Liver	Heart
14:0	0.5	1.1	<0.5	<0.5
15:0	0.2	<0.5	<0.5	<0.5
16:0	15.2	16.3	30.8	24.1
16:1	2.0	2.7	6.0	5.3
17:0	1.9	0.8	<0.5	<0.5
17:1	—	—	<0.5	<0.5
18:0	27.4	19.1	19.8	12.0
18:1	25.7	36.7	26.0	48.5
18:2	11.9	18.2	0.8	2.0
18:3	1.1	1.8	—	—
20:0	0.5	—	—	—
20:3	—	—	1.4	2.0
20:3?	0.4	<0.5	<0.5	<0.5
20:4	3.9	3.0	5.9	5.9
Higher acids	8.8	0.6	9.3	—

piglet therefore resembles other non-ruminants, e.g. rats, rabbits, guinea pigs and humans, where it has been shown that $C_{18:2}$ acid passes the placental membrane into the foetus (Söderhjelm, 1953; Satomura & Söderhjelm, 1962; Hansen *et al.* 1964). At first sight therefore it might appear that, in contrast with the non-ruminant, the ruminant placenta is relatively impermeable to $C_{18:2}$ acid and $C_{18:3}$ acid; but there may be another explanation. It has been shown that in rats, rabbits and guinea pigs the placenta is relatively impermeable to plasma cholesterol, phospholipids and chylomicron triglycerides (Goldwater & Stetten, 1947; Popják, 1954; McBride & Korn, 1964). However, in the sheep and the rabbit, plasma non-esterified fatty acids can pass the placental barrier into the foetus (Van Duyne, Parker, Havel & Holm, 1960; Van Duyne & Havel, 1960). In the present experiment, although the plasma phospholipids and cholesteryl esters of the sow and the ruminant contained high concentrations of $C_{18:2}$ acid, there was a striking difference in the $C_{18:2}$ acid content of the non-esterified fatty acid fraction. Whereas sow plasma non-esterified fatty acids contained 15% of $C_{18:2}$ acid, that in ruminant non-esterified fatty acids ranged from 1.5 to 3.6% (Table 2 and Fig. 1). If, as seems likely, the only plasma lipid to pass the placental membrane to any appreciable extent is the non-esterified fatty acid fraction, it might be expected that less $C_{18:2}$ acid would pass into the ruminant foetus than into the non-ruminant foetus. The same argument would apply to $C_{18:3}$ acid in the cow and calf.

Fig. 1. Percentage of $C_{18:2}$ acid in the fatty acids of plasma (a) cholesteryl esters, (b) phospholipids, (c) triglycerides and (d) non-esterified fatty acids from the pig (▨), cow (■), goat (□) and sheep (■).

This striking dissimilarity between the fatty acid composition of the plasma of the newborn lamb compared with its mother is at variance with the observations of Masters (1964), who showed that the fatty acid composition of the plasma of the newborn lamb resembled that of the adult sheep. However, in Masters's (1964) experiment 'the initial plasma samples were obtained a day or so after birth', during which time it is assumed that the lamb was allowed access to its mother's milk.

It is known that during the first 2 days of life considerable changes occur in the fatty acid composition of the plasma lipids of the lamb allowed to suck (Leat, 1964). At 48 hr. after birth the fatty acid composition of lamb plasma is similar to that of the adult sheep, although completely different at birth.

In contrast with newborn ruminants, the plasma of the newborn piglet contains appreciable amounts of $C_{20:4}$ acid, which is associated mainly with the phospholipid fraction. In this respect the newborn piglet is similar to the newborn human infant, which has also been reported to contain large amounts of $C_{20:4}$ acid in its plasma (Hansen *et al.* 1964). The $C_{20:3}$ acid, noted in the plasma of lambs and kids, and in the heart and liver of the foetal lamb, has the same carbon number (21.0) as and similar properties to the fatty acid noted in the tissues of essential fatty acid-deficient rats and in pigs given diets low in linoleic acid (Leat, 1963). This fatty acid is probably the $\Delta^{5,8,11}$ -eicosatrienoic acid characteristic of essential fatty acid deficiency (Mead, 1961). Holman (1960) has suggested that, in rats, a triene/tetraene ratio greater than 0.4 was indicative of a dietary insufficiency of essential fatty acids. If the same criterion is applicable to newborn lambs and to kids, it would appear that in these animals insufficient linoleic acid is passing the placental barrier to prevent the appearance of one of the characteristic metabolites in essential fatty acid deficiency in adult animals, i.e. eicosatrienoic acid. Whether or not the lamb and the kid can be said to be suffering from essential fatty acid deficiency is debatable, since information on the requirement of the young and mature ruminant is sparse. Further, the requirement of the foetus for linoleic acid is unknown and need not be the same as that of the adult animal. In the growing pig, for example, it has been shown that the requirement for linoleic acid is not constant but varies with age (Leat, 1962). When pigs are raised on low-fat diets varying degrees of centrilobular fatty changes occur in the liver (Gresham, Leat, Howard & Jennings, 1964). At this stage the plasma of these pigs contained about 1% of linoleic acid. Although lambs at birth have a similar concentration of plasma $C_{18:2}$ acid there is no evidence of any fatty change in the liver (W. M. F. Leat & T. Gillman, unpublished work).

In the calf it appears that the slightly higher concentration of $C_{18:2}$ acid in the plasma lipids is sufficient to prevent the appearance of the $C_{20:3}$ acid associated with essential fatty acid deficiency.

In samples of plasma lipids from all species examined here, small amounts of another fatty acid, having carbon number 21.2, were located between $C_{20:3}$ acid and $C_{20:4}$ acid on separation by gas-liquid chromatography. It is possible that this

acid is the isomeric $\Delta^{8,11,14}$ -eicosatrienoic acid, which is an intermediate in the formation of arachidonic acid from linoleic acid (Morin, Bernick, Mead & Alfin-Slater, 1962).

In all the newborn animals the major fatty acids of plasma were $C_{18:1}$ acid, palmitic acid, stearic acid and $C_{16:1}$ acid, which are acids that can be synthesized from non-fat sources. This agrees with the view that most of the foetal lipids are derived by synthesis within the foetus (Popják, 1954).

On fractionating the plasma lipids the differences in the fatty acid composition between newborn and maternal plasma became more apparent in some fractions than in others. Not obvious in the analysis of total fatty acids was the lower percentage of stearic acid in the phospholipids, triglycerides and non-esterified fatty acids of the plasma of newborn ruminants compared with their mothers. This lower percentage of stearic acid has also been noted in the glycerides of the organs and carcass of newborn and foetal lambs (Downing, 1964; Body & Shorland, 1964).

The cholesteryl esters of newborn ruminant plasma contained a higher percentage of $C_{18:2}$ acid than the other lipid fractions. The high concentration of $C_{16:1}$ acid is also of note since the acid is known to increase in amount in the cholesteryl esters of rats and pigs given insufficient linoleic acid (Howton & Hashimoto, 1960; Leat 1963). With the exception of the high concentration of $C_{18:3}$ acid in the cow, the fatty acid composition of the plasma cholesteryl esters and phospholipids of the sow and ruminants did not differ appreciably. The major difference between the ruminant and the non-ruminant lay in the fatty acid composition of the plasma triglycerides and non-esterified fatty acids.

The fatty acid composition of the plasma triglycerides of the cow, goat and sheep were similar and differed from that of the sow in being more saturated, containing less $C_{18:2}$ acid and more stearic acid. The high content of stearic acid in the plasma triglycerides of ruminants is probably a reflection of the large amounts of this acid produced by microbial action in the rumen. In agreement with previous work (Leat, 1963) the plasma non-esterified fatty acid fraction of the sow was found to be more saturated than the triglyceride fraction. In ruminants, however, the plasma non-esterified fatty acids were less saturated than the triglycerides, although containing more stearic acid. The high content of stearic acid in the plasma non-esterified fatty acids of ruminants might be expected since this fraction is derived mainly by mobilization from adipose tissue, which in ruminants has a high content of stearic acid (Hilditch, 1956).

The present results on the fatty acid composition of the plasma lipids of the sheep are in broad agree-

ment with previous work (Horgan & Masters, 1963; Garton & Duncan, 1964). The highest concentrations of C_{18:2} acid and C_{18:3} acid were in the cholesteryl esters, and the highest concentrations of stearic acid and palmitic acid were in the phospholipids and triglycerides. The fatty acid compositions of goat plasma lipids were very similar to those of the sheep. The C_{18:1} acid contents of the cholesteryl esters and phospholipids of goat plasma reported in the present work are much lower than the values recorded by McCarthy (1962).

The fatty acids of cow plasma are characterized by the high content of C_{18:3} acid, which is associated mainly with the cholesteryl esters and, to a smaller extent, with the phospholipids. These results are in good agreement with those reported by Duncan & Garton (1962), who suggested that the difference in the content of C_{18:3} acid between the cow and other ruminants might be related to diet or to the preferential esterification of C_{18:3} acid with cholesterol in the cow. The cows studied in the present work had access to fresh grass, which is a rich source of linolenic acid, whereas the goats did not. However, the plasma cholesteryl esters of goats feeding on fresh pasture contained similar amounts of C_{18:3} acid to those of goats that did not have access to fresh grass. In sheep feeding on fresh pasture the C_{18:3} acid contents of plasma cholesteryl esters were somewhat higher than the values from stall-fed sheep. Also, the infusion of linseed oil, containing 67% of linolenic acid, directly into the duodenum of a sheep, thereby by-passing the hydrogenating mechanisms of the rumen, did increase the C_{18:3} acid content of the plasma cholesteryl esters from 7 to 22% (W. M. F. Leat, unpublished work). However, this maximum value for sheep was only half the value noted in cows. It is therefore unlikely that possible differences between the cow and the sheep in the extent of microbial hydrogenation of linolenic acid in the rumen would entirely account for the considerable difference in the C_{18:3} acid contents of their respective plasma cholesteryl esters. The results suggest that there is a greater tendency for C_{18:3} acid to be esterified with cholesterol in the cow than in other ruminants.

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