The metabolism of linoleic acid by the young lamb

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1. Groups of lambs were killed immediately after birth; others were reared on a diet of cow's milk and were killed at 10, 20 and 30 d after birth. The concentrations of 18:2ω6 (linoleic acid) were determined in cow's milk and the concentrations of 18:2ω6, 20:4ω6 (arachidonic acid) and 20:3ω9 ($\Delta^{5,8,11}$ eicosatrienoic acid) were determined in the whole carcasses of the lambs.

2. The carcass of the newborn lamb contained only about 300 mg 18:2ω6. During the first 20 d after birth, there were large increases in the 18:2ω6 contents of the carcasses, and on the 10th and 20th days, their contents of 18:2ω6 were similar to the dietary intakes of 18:2ω6 at the corresponding time-intervals after birth.

3. The 20:3ω9 to 20:4ω6 ratio in the carcass of the newborn lamb was about 1·6, but after 10 d the ratio had decreased to 0·38 in spite of the fact that 18:2ω6 contributed only about 1 % of the total calories in the diet.

4. The minimum dietary requirements for essential fatty acids for young ruminants and for non-ruminent animals are compared.

Although the concentrations of 18:2ω6 (linoleic acid) in the various tissues of the newborn lamb are very small, it has been shown that there are pronounced increases in the concentrations of this essential fatty acid in the plasma and liver during the first 3–4 d after birth (Leat, 1966; Noble, Steele & Moore, 1971*a*, *b*). During this period the diet consists entirely of ewe's milk and it has been suggested that one of the functions of ewe's colostrum is to provide the newborn lamb with essential fatty acids (Leat, 1964). However, determinations of the fatty acid compositions of ewe's colostrum and milk secreted during early lactation have shown that the concentration of 18:2ω6 never exceeded 1 % of the total fatty acids present (Noble, Steele & Moore, 1970). Moreover, it may be calculated that 18:2ω6 provides only about 0·4 and 0·5 % respectively of the total calories available to the suckling lamb on the day of parturition and on the 3rd day after parturition (Noble *et al.* 1970). According to Holman (1960), the minimum requirement for essential fatty acids by the young rat and human infant is met when 1 % of the dietary calories is provided by 18:2ω6. Thus, it seems possible that the dietary requirement of the lamb for 18:2ω6 is considerably less than that of the young rat and human infant. It is also possible that, in the newborn lamb, there is some store of 18:2ω6 in certain tissues other than the plasma and liver and that immediately after birth there is a redistribution of this store. This latter possibility has been investigated in an experiment in which the 18:2ω6 intakes of lambs have been compared with the 18:2ω6 contents of the whole carcasses of the animals up to 30 d after birth. A preliminary account of this work has been given previously (Noble & Moore, 1971).

Table 1. *Live weights (kg) of the lambs from birth to slaughter*

	Days after birth			
Animal no.	0	10	20	30
1	5·17	—	—	—
2	3·58	—	—	—
Mean	4·38	—	—	—
3	3·47	5·70	—	—
4	3·70	5·83	—	—
Mean	3·59	5·76	—	—
5	4·81	6·54	9·03	—
6	5·90	7·66	9·86	—
Mean	5·36	7·10	9·44	—
7	5·79	7·42	9·43	10·8
8	4·60	6·55	8·97	10·7
Mean	5·20	6·99	9·20	10·8

EXPERIMENTAL

Lambs were obtained from a flock of pure bred Cheviot ewes. The ewes had been given a diet of good hay and concentrates; water was available *ad lib.* All the lambs were segregated from their mothers at birth and received a diet of cow's milk. One pair of lambs was killed immediately after birth, before access to food; the remaining lambs were killed at intervals of 10, 20 and 30 d after birth, two animals being killed at each time-interval. The lambs were weighed daily and Table 1 gives the live weight of each lamb immediately before slaughter. The lambs were killed by an intravenous injection of Euthatol (May and Baker Ltd, Dagenham, Essex). Lambs nos. 3–8 received an intramuscular injection of a multivitamin preparation (Crooks Laboratories Ltd, London) on the 2nd day after birth. A uniform homogenate of the carcass of each lamb was obtained by passing the carcass several times through a mincer (Model B200LF; Wolf King Mincers Ltd, Denmark). The intake of 18:2ω6 by each lamb was calculated from the analyses of daily milk samples and a record of the total milk intake at each feeding; replicate analyses of the homogenates of the whole carcasses gave results for the total amounts of fatty acids in all the tissues of each lamb. The concentrations of the fatty acids in the carcass homogenates were determined by the gas–chromatographic procedure described by Christie, Noble & Moore (1970) using an internal standard. The identities of 18:2ω6, 20:4ω6 (arachidonic acid) and 20:3ω9 ($\Delta^{5,8,11}$ eicosatrienoic acid) were checked by the gas–chromatographic procedures of Ackman & Burgher (1963) and Kepler, Hirons, McNeil & Tove (1966). The properties of the isomers were also compared with authentic standard acids (obtained from the Hormel Institute, Austin, Minnesota, USA) on a gas–chromatograph fitted with a single-flame ionization detector and a support-coated open capillary column (1525 cm × 0·05 cm) with a stationary phase of diethylene glycol succinate (Perkin-Elmer Ltd, Beaconsfield, Bucks.). The identity of each isomer was checked further by oxidation of the methyl ester fractions (Chang & Sweeley, 1962) and analysis of the resulting mono- and di-carboxylic acids by gas–chromatography as described by Moore & Williams (1966). On average, 18:2ω6 accounted for about 80% of the

Table 2. *Total intakes of* 18:2ω6 *in the diet and total amounts of* 18:2ω6, 20:4ω6 *and* 20:3ω9 *in the carcass homogenates of each lamb at slaughter*

Animal no.	Time of slaughter (d after birth)	Total intake of 18:2ω6 (g)	Total carcass content (g)		
			18:2ω6	20:4ω6	20:3ω9
1	0	—	0·324	1·28	2·06
2	0	—	0·266	0·863	1·36
Mean	—	—	0·295	1·07	1·71
3	10	11·1	10·6	2·83	1·10
4	10	10·7	10·5	3·52	1·37
Mean	—	10·9	10·6	3·18	1·23
5	20	23·9	26·9	6·14	1·29
6	20	24·9	24·4	5·02	1·10
Mean	—	24·4	25·6	5·58	1·19
7	30	35·2	26·4	7·54	1·28
8	30	36·5	25·5	8·04	1·05
Mean	—	35·8	25·9	7·79	1·16

total 18:2 present in the cows milk given to the lambs; 18:2ω6 accounted for 90% or more of the total 18:2 present in the carcasses of the lambs.

RESULTS

Table 2 shows the total intake of 18:2ω6 for each lamb up to the time of slaughter and the total contents of 18:2ω6, 20:4ω6 and 20:3ω9 in the carcass homogenates of each of the lambs. Since the polyunsaturated acid content of the lambs slaughtered immediately after birth was extremely small, the determination of the 18:2ω6 content was subject to some error. For instance, replicate determination of the 18:2ω6 content of the carcass homogenate of animals nos. 1 and 2 ranged from 269 to 360 mg and 237 to 303 mg respectively, resulting in a general mean of 300 mg. By the 10th and 20th days the amounts of 18:2ω6 present in the carcasses had increased considerably and were similar to the dietary intakes of 18:2ω6 during these periods. However, on the 30th day after birth the total amount of 18:2ω6 was similar to that in the lambs killed on the 20th day after birth, in spite of the fact that the total dietary intake of 18:2ω6 had increased by about 10 g during the period between the 20th and 30th days. On the day of birth, the total contents of 20:4ω6 and 20:3ω9 in the carcass were each greater than the total carcass content of 18:2ω6. During the first 20 d after birth, the 20:4ω6 content of the carcass increased considerably, but this increase was not as marked as that observed for the 18:2ω6 content during the same period. However, unlike the 18:2ω6 content, the 20:4ω6 content of the carcass continued to increase between the 20th and 30th days after birth. There were no pronounced changes in the total content of 20:3ω9 in the carcasses of the lambs during the experiment.

DISCUSSION

To be strictly comparable with previous work (Noble *et al.* 1971 *a*, *b*), it would have been better if the experiments now reported had been carried out with lambs suckling

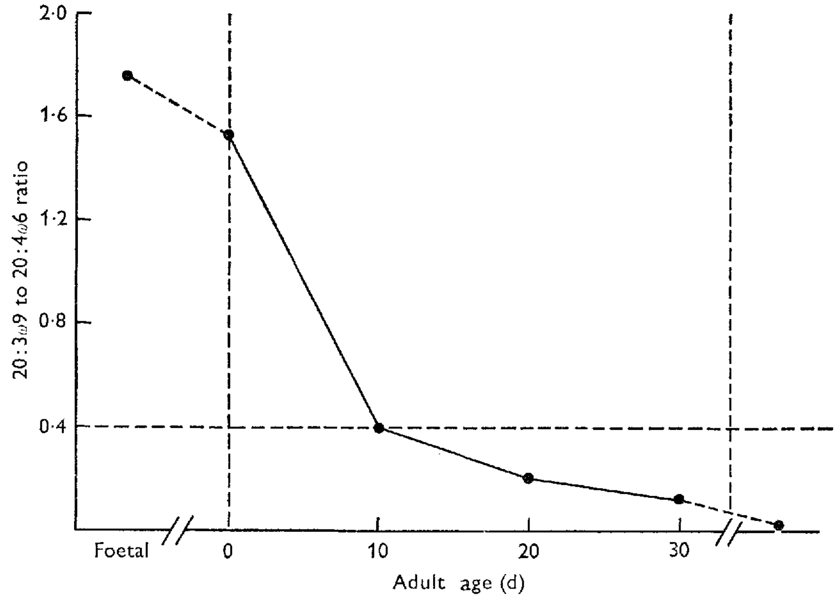

Fig. 1. Effect of age on the 20:3ω9 to 20:4ω6 ratio in the tissues of sheep. Values for foetal and adult tissues are taken from Shorland *et al.* (1966).

the ewe. However, the difficulties of measuring with any degree of accuracy the 18:2ω6 intake of suckling lambs are considerable, and hence the lambs were hand-reared on cow's milk. The results given in Table 2 must be viewed in the knowledge that any comparative slaughter techniques are inevitably subject to relatively large errors.

The extremely small amount of 18:2ω6 in the carcass of the newborn lamb precludes the possibility of there being any large store of this essential fatty acid in tissues other than the liver and plasma and, since ewe's milk is very low in this acid (Noble *et al.* 1970), it must be concluded that the minimum dietary requirement for 18:2ω6 of the young lamb is quite different from that of many other animal species. The essential fatty acid requirement of man and a wide variety of animals is met when 18:2ω6 constitutes 1–2 % of the total dietary calories (Holman, 1968). In animals given diets deficient in 18:2ω6, the concentrations of 18:2ω6 and 20:4ω6 in the tissues decrease and the concentration of 20:3ω9 increases; these biochemical effects are accompanied by the appearance of the characteristic external signs of essential fatty acid deficiency. The 20:3ω9 to 20:4ω6 ratio in the tissues has been used by Holman (1960) to determine the essential fatty acid status of man and several non-ruminant animals. In general, it may be concluded that the minimum dietary requirement for 18:2ω6 has been met when the 20:3ω9 to 20:4ω6 ratio in the tissues is less than about 0·4. For instance, the work of Holman (1960) has shown that, when rats were given a diet in which 18:2ω6 supplied only 0·14 % of the total calories, dermal lesions developed and the 20:3ω9 to 20:4ω6 ratio in the tissues was about 1·0. When rats were given a diet in which 18:2ω6 supplied 5·1 % of the total calories, there were no external signs of essential fatty acid deficiency and the 20:3ω9 to 20:4ω6 ratio in the tissues was about

0·06. Fig. 1 shows the mean 20:3ω9 to 20:4ω6 ratios in the carcasses of the experimental lambs, together with corresponding values for foetal lambs and adult sheep calculated from the results of Shorland, Body & Gass (1966). The high values for the foetal and newborn lambs (1·75 and 1·55 respectively) are of particular interest since ratios of this magnitude in the tissues of the rat and many other non-ruminant species would be associated with external signs of essential fatty acid deficiency. After 10 d on a diet of cow's milk, the 20:3ω9:20:4ω6 ratio in the carcasses of the lambs had decreased to 0·38, a value that is slightly less than the upper limit (0·4), indicating an adequate essential fatty acid status. Again, this is of interest for, in the cow's milk given to the lambs, 18:2ω6 contributed, on average, only about 1% of the total calories.

The 18:2ω6 content of ewe's milk fat is even less than that of cow's milk fat, contributing only about 0·5% of the total calories in ewe's milk (Noble *et al.* 1970). Nevertheless, even with lambs reared solely on ewe's milk, the 20:3ω9 to 20:4ω6 ratio in the liver, for instance, decreased from about 1·2 on the day of birth to about 0·2 on the 8th day after birth (Noble *et al.* 1971*b*). It appears that, under normal conditions of rearing, just as in the present experiments, the young lamb is able to improve its essential fatty acid status even on a diet that would presumably result in the appearance of external signs of essential fatty acid deficiency in the young rat.

The similarities between the intakes of 18:2ω6 from the diet and the increases in the total amounts of 18:2ω6 in the carcasses of the lambs during the first and second 10 d periods after birth (Table 2) indicate that the absorption and retention of 18:2ω6 by the lamb during the first 20 d after birth is a highly efficient process. This retention of 18:2ω6 by the tissues also implies that there is a high initial requirement for this fatty acid during this period. The results in Table 2 show that there was a marked decrease in the efficiency of retention of 18:2ω6 between the 20th and 30th days after birth. It seems that the deficit of 18:2ω6 in the tissues of the newborn lamb is made good after about 20 d on a diet of cow's milk; presumably a somewhat longer period would be required to make good this deficit on a diet of ewe's milk.

It must be remembered that large proportions of the polyunsaturated fatty acids in the diet of the adult ruminant are hydrogenated by the micro-organisms in the rumen (Dawson & Kemp, 1970). In the tissues of the young or adult ruminant, 18:2ω6 and other polyunsaturated fatty acids tend to be confined to the phospholipid and cholesteryl ester fractions (Moore & Steele, 1968; Moore, Noble & Steele, 1968; Moore, Steele & Noble, 1969; Noble *et al.* 1971*a*, *b*), and it is in these two lipid fractions that the essential fatty acids are believed to play an important part in the structure of biomembranes (Aaes-Jørgensen, 1961; Van Deenen, 1966). In the tissues of the non-ruminant animal, polyunsaturated fatty acids are more widely distributed among all lipid classes, including the triglycerides (Moore & Williams, 1963; Swell & Treadwell, 1963) which serve mainly as a store of energy. The highly efficient utilization of polyunsaturated fatty acids for cholesteryl ester and phospholipid synthesis may be the evolutionary mechanism whereby the ruminant animal has become adapted to limited supplies of essential fatty acids from the diet.

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