FATTY ACID COMPONENTS OF OVINE TISSUE LIPIDS DURING RUMEN DEVELOPMENT

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[*Manuscript received August* 28, 1963]

Summary

The lipids from the liver, depot fat, heart, kidney, and skeletal muscle of young lambs has been separated into cholesterol ester, triglyceride, free fatty acid, and phospholipid fractions. Weights of these fractions and the fatty acid components of these lipids have been determined in lambs of varying ages covering the period from birth to nutritional independence. Results have been compared to the changes occurring in ovine plasma under the same conditions, and the interrelationships between the changes in the levels of these fractions in the various tissues have been discussed in relation to lipid metabolism and the development of an active rumen.

I. Introduction

The development of an active rumen in the young lamb is associated with marked changes in lipid metabolism (Annison and Lewis 1959; Garton 1960). In the preceding paper (Masters 1964) the nature of these sequential changes was indicated by following changes in the content of the fatty acid components of plasma lipids throughout the period of rumen development.

As a complementary study, changes in the content of the fatty acid components of the main ovine tissues concerned in fat oxidation have been followed during the first months of the lamb's life. The lipids of liver, heart, kidney, skeletal muscle, and depot fat have been separated into cholesterol ester, triglyceride, free fatty acid, and phospholipid fractions, and the fatty acid components determined by gas chromatography.

II. Methods

Male Merino lambs from well-matched ewes and by the same ram were used in these experiments. They were kept with their mothers under the identical natural grazing conditions of the previous experiment (Masters 1964). Experimental animals were slaughtered at monthly intervals during this treatment, i.e. soon after birth, and at the age of 1, 2, 3, 4, and 5 months, respectively. The two oldest lambs had been grazing independently of the maternal ewes for periods of 2 and 6 weeks respectively.

From the freshly slaughtered lambs, heparinized blood samples were collected, and the plasma separated by centrifugation. Liver, heart, and kidneys were excised as well as samples of lean skeletal muscle and depot fat. Specimens of the last two tissues were taken from similar sites in the different animals. Perinephric and myocardial depot fat were removed, and the plasma and tissue samples were stored in closed vessels at −10°C in the dark until required.

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The lipids were then extracted from these samples, fractionated on silicic acid columns, and the fatty acid components determined by gas chromatography. These techniques have been fully described previously (Horgan and Masters 1963).

III. Results

The relationship between the age of the lambs and their plasma lipids are depicted in Figures 1, 2, and 3. The resultant curves are similar to those observed in sequential analyses from single animals under identical treatment (Masters 1964).

The total lipid concentration in the tissues of new-born lambs was: liver, 6·1% by weight of fresh tissue; heart, 3·1%; kidney, 3·4%; skeletal muscle, 2·3%. The highest values for lipid were obtained in 2-month-old lambs (liver 9·2%, heart 4·2%, kidney 4·1%, skeletal muscle 3·0%), with a return towards initial values in the older lambs.

(*a*) *Liver Lipids*

The main alterations in the weight of liver lipids during growth takes place in the triglyceride and phospholipid fractions (Fig. 1). Triglyceride rises to a peak at 2 months, corresponding to a negative peak or trough in the phospholipid fraction. Cholesterol ester and free fatty acid fractions show smaller variations, with peaks at 4 and 3 months, respectively.

Liver C_{18} fatty acids (Fig. 2) exhibit wide variations in most fractions. Cholesterol esters show a marked increase in stearic acid content from 2 to 4 months, whereas oleic acid percentages rise for 2 months, then decrease sharply from 2 to 4 months. Both linoleic and linolenic acid percentages decrease in the initial stages but rise terminally. In the triglyceride fraction, there is a considerable decrease in stearic acid content over the first 2 weeks, along with a marked increase in oleic acid content. Both these fatty acids return towards starting percentages by the age of 5 months. Linoleic acid decreases in the initial stages. In the first 2 months stearic acid content of the free fatty acid fraction also decreases, then overcompensates for this fall by rising sharply from 2 to 3 months. Oleic acid gradually declines to a minimum value at 4 months, while linoleic and linolenic acids both demonstrate maximum values at 2 months. The C_{18} fatty acids in the liver phospholipid fraction exhibit smaller variations in percentage content than the other fractions. The stearic acid curve dips in the early stages before returning to a high value at 3 months, that for oleic acid shows a negative peak at 4 months, while that for linoleic acid shows two maxima, at 2 and 4 months respectively. Palmitic acid content of the cholesterol ester, triglyceride, and free fatty acid fractions of liver also shows appreciable variation (Fig. 3), with maximum values at 1 and 3 months, at 2 months, and at 2 and 5 months, respectively, for these three fractions.

(*b*) *Depot Fat Lipids*

The variations in the weights of the depot fat fractions follow a different pattern (Fig. 1). Triglyceride, the predominant fraction, falls to a minimum value at 3 months, followed by a sharp increase. The other fractions show smaller variations, in general rising to a peak and falling. The peak position is at 3 months

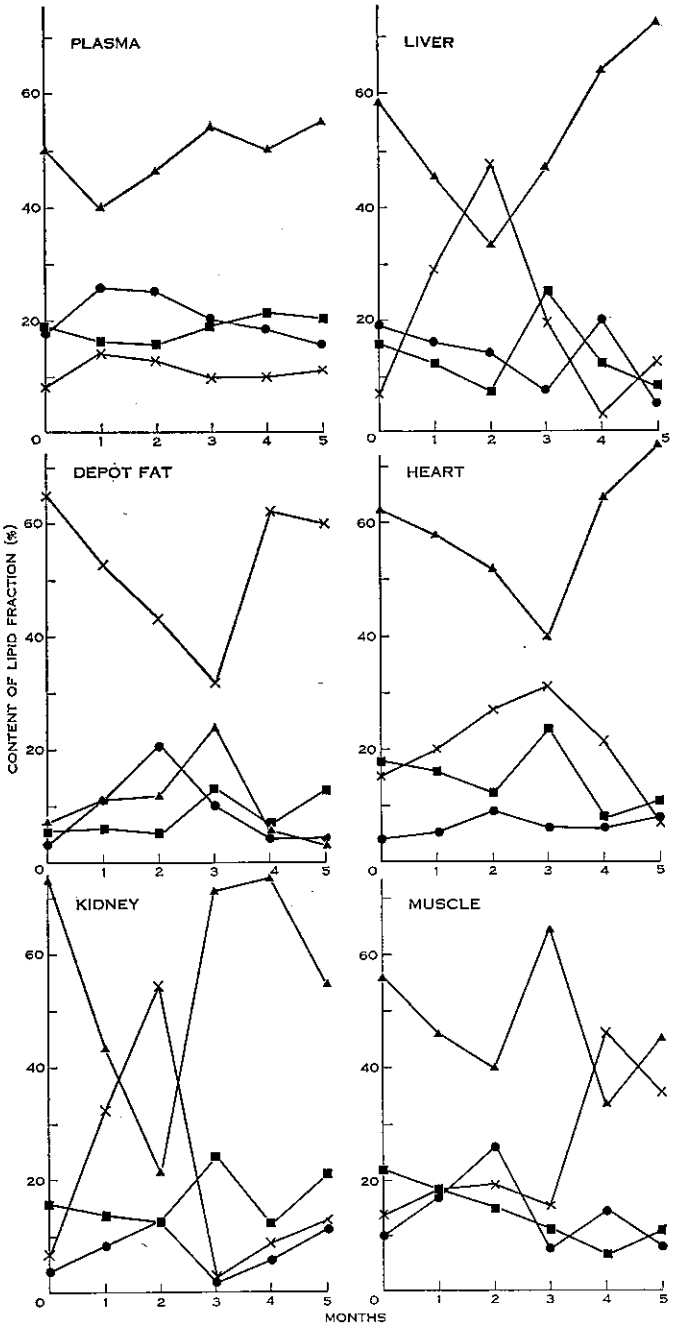

Fig. 1.—Alterations in the percentage content of the main lipid fractions of lamb tissues during growth. × Triglyceride fraction. ■ Free fatty acid fraction. ● Cholesterol fraction. ▲ Phospholipid fraction.

for the free fatty acid and phospholipid fractions, and at 2 months for the cholesterol ester fraction.

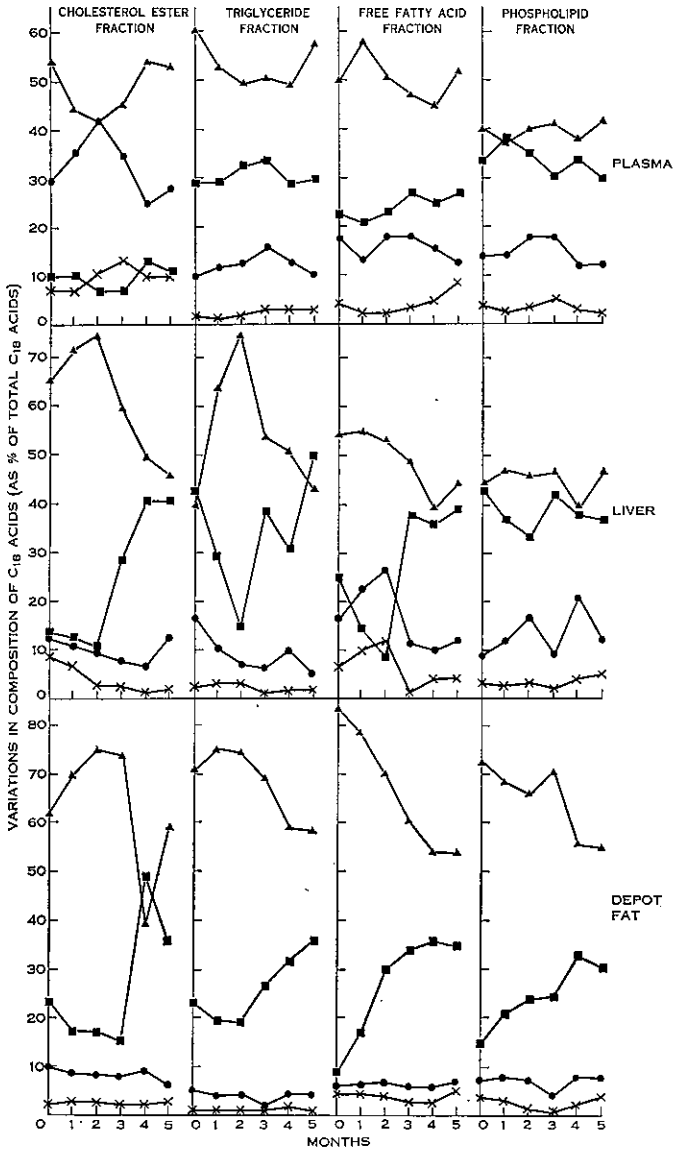

Fig. 2.—Variations in the percentage composition of individual C_{18} acids in the four main lipid fractions of plasma, liver, and depot fat during growth. ■ Stearic acid. ▲ Oleic acid. ● Linoleic acid. × Linolenic acid.

Percentage composition patterns of the C_{18} acids of depot fat, however, are not greatly different to those for liver. With the cholesterol esters the pattern is similar, with the extremes of stearic and oleic acids in depot fat occurring about

1 month after those in liver. With the triglyceride fraction, the main components move in the same direction as for liver fractions but not to the same extent. The sequence for the free fatty acid fraction is characterized by an increase in stearic acid percentages over the experimental period at the expense of oleic acid, while a similar but less marked tendency occurs with the phospholipid fraction.

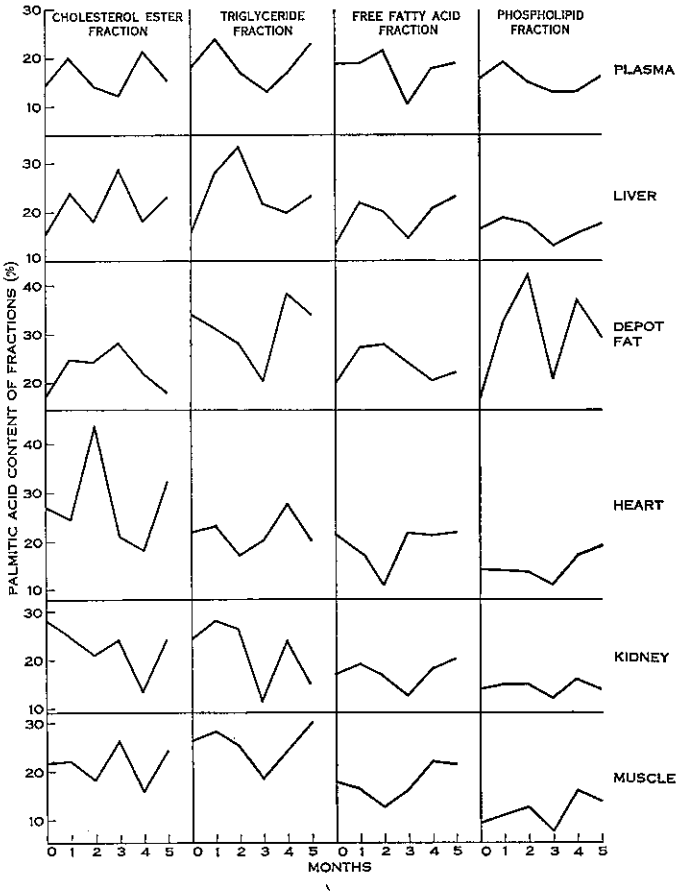

Fig. 3.—Variations in the percentage of palmitic acid of the four main lipid fractions of the tissues investigated during growth.

The palmitic acid content of the cholesterol ester fraction of depot fat reaches a maximum at 3 months whereas there is a minimum at this age for the triglyceride fraction, rising to a maximum at 4 months. It also reaches maximum values at 2 months and at 2 and 4 months, respectively, for the free fatty acid and phospholipid fractions.

(c) *Lipids of Heart, Kidney, and Skeletal Muscle*

The percentage phospholipid of heart, skeletal muscle, and kidney at first falls but subsequently increases (Fig. 1), but whereas heart phospholipid is still

rising at 5 months, both skeletal muscle and kidney phospholipid show a terminal decline. Peak triglyceride and free fatty acid percentages of heart tissue coincide

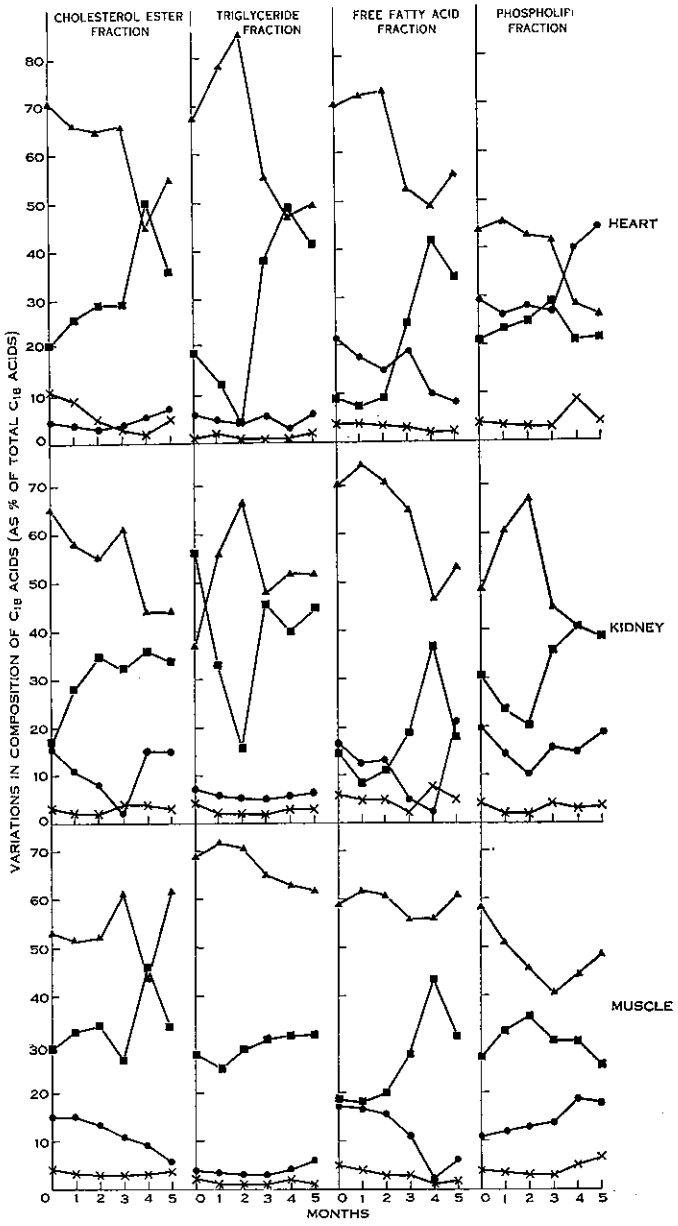

Fig. 4.—Variations in the percentage composition of individual C_{18} acids in the four main lipid fractions of heart, kidney, and skeletal muscle during growth. ■ Stearic acid. ▲ Oleic acid. ● Linoleic acid. × Linolenic acid.

with this initial phospholipid trough. The percentage triglyceride of kidney also reaches a maximum at the same time whilst, with skeletal muscle, the first phospho-

lipid trough (2 months) coincides with an increase in the percentage of cholesterol ester and the second (4 months) to an increase in triglyceride.

While the behaviour of the C_{18} acids of the cholesterol ester and free fatty acid fractions of heart, kidney, and skeletal muscle is not dissimilar, a different picture is presented with regard to the C_{18} acids of the triglyceride fraction of muscle. On the other hand, no two of the patterns for the C_{18} acids of the phospholipid fraction of the three tissues are closely similar (Fig. 4). In the cholesterol ester fractions of these tissues, stearic acid rises to a maximum value at 2 and 4 months, while oleic acid shows an opposite trend. The stearic acid content of heart and kidney triglycerides decreases sharply in the first 2 months, then rises to a maximum value at 3–4 months. Oleic acid content meanwhile rises sharply initially but decreases in the final stages of the experimental period. The C_{18} acids of the muscle triglyceride fraction show similar trends though of considerably smaller magnitude. In all three tissues stearic acid content of the free fatty acid fraction rises to a peak at 4 months, corresponding to a decrease to a minimum value for oleic acid. In addition, linoleic acid decreases quite markedly over the experimental period. On the other hand, stearic and oleic acid content of the heart phospholipid fraction decline, while linoleic acid increases. There is also an initial decrease in stearic and linoleic acid content of the kidney phospholipid fraction, followed by a subsequent increase, whilst oleic acid content rises to a maximum value at 2 months followed by a decline. The stearic acid content of muscle phospholipid rises initially, and then declines, while oleic acid content decreases to a minimum at 3 months and then rises; linoleic acid increases throughout the experimental period.

The sequential behaviour of the palmitic acid percentages in heart, kidney, and skeletal muscle is also similar. With the cholesterol ester fraction, palmitic acid content decreases initially, rises to a peak at 2–3 months, falls, and again increases terminally. Triglyceride palmitic acid declines to a minimum at 2–3 months then increases—a similar behaviour to that shown in the free fatty acid fraction. Phospholipid palmitic acid shows consistent but less marked changes with peaks at 2 and 4 months.

The general tendency observed in the case of arachidonic acid components was an initial gradual decline in percentage followed by a rise between 2–4 months, and a terminal decline. This behaviour was followed, also, by two unsaturated moieties present as major components in the phospholipid fraction of heart, kidney, and muscle, but not yet positively identified.

Palmitoleic, behenic, and the minor fatty acid components of these tissues and also of liver and depot fat did not exhibit considerable alterations during the experimental treatment. The detailed distribution of the fatty acid components will be detailed elsewhere.

IV. Discussion

In studying the sequence of fatty acid changes occurring during the first months of a lamb's life, the use of different animals as tissue sources suffers from the disadvantage of a possible individual variation, which might tend to cloud the true sequence of alterations. In these experiments, however, it was not feasible to obtain sequential tissue samples from a single experimental animal. Furthermore,

this type of procedure would probably introduce additional errors because of the necessity for different sampling sites in individual tissues. As an alternative, the possibility of considerable abnormalities due to individual variations has been obviated as far as possible by comparing the sequence of values obtained from the plasma of the individual animals with those curves given by the sequential studies of the plasma from single lambs which had been observed under identical experimental conditions. The agreement between these two cases is good, and this would seem to mitigate against the possibility of gross distortion due to individual aberrations. Nevertheless, in the following discussion, only variations of considerable magnitude have been considered as suitable bases for interpretation.

In the case of liver lipids, the initial drop in phospholipid content is probably in response to the transition from the foetal state to that of the infant lamb. Whereas the foetal blood stream is in direct equilibrium with the maternal blood supply, and consequently reflects the lipid composition of ewe plasma, the responsibility for the supply of plasma phospholipid shifts, at birth, to the infant liver. This is exemplified by the decrease observed in this plasma fraction after birth. At the same time, phospholipid percentages decrease in liver, heart, kidney, and skeletal muscle, which feature would seem to imply that the tissue phospholipids in the foetus are also derived in part from the maternal blood circulation.

The subsequent rises in phospholipid percentage (both of liver and plasma) are probably mainly due to adaptation of the hepatic synthetic processes to the increased load. A further relevant factor, however, may be the increased liver phospholipid turnover displayed by fat-fed animals (Olson and Vester 1960). Since the adult ruminant exists on a diet which is largely composed of fatty acids (Barnett and Reid 1961), this regimen might contribute to the subsequent rise in hepatic phospholipid to higher levels than those observed initially.

The increase in the triglyceride content of the liver lipid which occurs after birth is of interest in relation to the increased energy requirements of the animal at this stage. Further to this point, C_{18} acids of milk fat are chiefly present as the monoene, and this acid increases in all the liver lipid fractions, but particularly the triglyceride fraction, during this stage. All of these facts support the interpretation (Masters 1964) of active mobilization of depot fat, abstraction of the free fatty acids by the liver, and utilization for oxidation and for phospholipid synthesis.

At least 40% of the fasting energy requirements of the adult ruminant are derived from the volatile fatty acids produced by ruminal fermentation (Annison and Lewis 1959), and it is noticeable that in the period following rumen development (2–5 months) the hepatic triglycerides decrease, while depot fat triglyceride increases. This would correspond to a reduced necessity to mobilize depot fat, and oxidize it in the tissues for energy purposes.

Since liver is the main source of plasma cholesterol esters (Kritchevsky 1958), the marked increase in stearic acid content of the liver cholesterol ester fraction, which occurs in the 2–4-month period (corresponding to the development of the rumen), would seem to be related to the similar increase which occurs in the plasma cholesterol ester fraction at 3–4 months. Hepatic triglyceride and free fatty acids also show marked increases in stearic acid percentages over this period, but in this

instance the change is not observable in plasma. This points to an extraction of dietary lipids by the tissues (mainly the liver) and replacement of the fatty acid moieties with the more highly unsaturated fatty acids characteristic of liver and tissue lipids.

Depot fat exhibits a different sequence of changes to that in liver, and this is not surprising in view of its position as the opposite pole of the "liver–depot fat axis" along which fatty acid or fat is transported in response to the physiological needs of the animals (Jeanrenaud 1961). As regards fraction weight percentages, the marked decrease in triglyceride occurring during the first 3 months supports the previous suggestion that this represents an active mobilizing period. The mammalian foetus has little depot fat, and while depot fat as a whole is being built up in the body, the depot fat triglyceride is being actively mobilized at the same time as free fatty acid and transported to other tissues.

Although the cholesterol ester and triglyceride fractions show small initial increases in oleic acid content at the expense of stearic acid, this phenomenon is not as marked as in the case of liver. This, again, is consistent with the interpretation of active mobilization of oleic acid (depot fat→liver) during this period. Of all the ruminant tissues studied, depot fat demonstrates the most steady transition of its fatty acids towards saturation during rumen development.

With heart tissue, the fraction weights and C_{18} acids behave in an analogous manner to liver, a fact which would seem to indicate parallel changes in metabolism in these two tissues. It is known that the heart obtains its supply of energy largely from non-carbohydrate sources, and that this is particularly so at low levels of blood glucose, such as are characteristic of the adult ruminant (Thompson and King 1959; White *et al.* 1959). The similarity between these two tissues in this instance may be emphasized by the unusual pattern of metabolism in sheep liver which is very largely dependent on the oxidation of fatty acids for the maintenance of tricarboxylic acid cycle activity (Gallagher and Buttery 1959). Renal tissue, also, displays an overall sequence much like liver. Kidney phospholipid decreases in the terminal stages, however, and the C_{18} acids in this fraction show a much greater decrease of oleic acid than liver does. Feeding of fats to ruminants has been reported to cause reduced phospholipid synthesis in kidney (Olson and Vester 1960). This observation is consistent with lipid digestion and oleic acid utilization characteristic of the adult sheep (Annison and Lewis 1959; Masters 1964).

The sequential lipid patterns displayed by skeletal muscle seem to be somewhat unique with regard both to terminal fraction weight and C_{18} acid content. Little oleic acid accumulation is apparent in the triglyceride fraction of the young (2-month) lamb and the behaviour of the C_{18} acids in the phospholipid fraction differ markedly from the other tissues. Possibly these differences relate to a different emphasis on metabolic requirements and the fluctuating energy requirements of voluntary muscle. It is known that ketone body formation is a normal metabolic pathway in the ruminant (Barnett and Reid 1961) and the energy requirements of resting muscle could be largely met by oxidation of these compounds.

As regards palmitic acid levels, there is a broad similarity between the tissues in the case of the cholesterol ester and phospholipid fractions. It is recognized that

considerable equilibration occurs between the cholesterol esters of plasma and tissues (Cantarow and Schepartz 1962). However, the palmitic acid levels of the free fatty acid fractions of depot fat and liver show the closest similarity, with the extrahepatic tissues showing different patterns. This would be expected if most of the free palmitic acid mobilized from depot fat was abstracted by the liver.

Arachidonic behaviour during the experimental period would suggest a response to the increased supply of dietary precursors which follows pasture feeding (Shorland, Weenink, and Johns 1955; Wakil 1961). Although the young ruminant can incorporate polyunsaturated fatty acids from the diet into its tissues in the same way as monogastric animals (Hoflund, Holmberg, and Sellmann, 1961) there is little diethenoid C_{18} acid in ewe milk. Although the unidentified major fatty acid moieties have a similar response, their metabolic significance and interrelationships remain uncertain at present.

In conclusion, then, these results complement and amplify a previous description of the behaviour of plasma fatty acid moieties during rumen development. Changes in tissues are generally of a greater magnitude than those occurring in plasma, and the timing and location of these alterations provide additional insight into the relationship between the rumen and lipid metabolism.

V. Acknowledgments

The author is indebted to Professor E. C. Webb for his interest and encouragement, and to Professor O. E. Budtz-Olsen for the provision of experimental facilities.

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