FATTY ACID COMPONENTS OF OVINE PLASMA LIPIDS DURING RUMEN DEVELOPMENT

By C. J. Masters*

[*Manuscript received August* 28, 1963]

Summary

The plasma lipids of young lambs have been separated by chromatography on silicic acid into cholesterol ester, triglyceride, free fatty acid, and phospholipid fractions. Alterations in the fraction weights and fatty acid components of these lipid fractions have been followed from birth to the stage of dietary independence. During this period, a number of changes were evident, and these results have been discussed in relation to the alterations in lipid metabolism with growth and rumen development.

I. Introduction

Lipid metabolism in ruminants has long been recognized as differing from that in monogastric herbivores, ruminant depot fats being characterized by their highly saturated nature, the presence of unusual fatty acids, and the lack of marked response to unsaturated dietary fats (Garton 1961). These features are now known to be caused by the extensive modification of dietary lipids by rumen microorganisms, causing the hydrolysis of glycerides and phospholipids, the hydrogenation of unsaturated fatty acids, and the production of considerable quantities of volatile fatty acids (Shorland *et al.* 1957; Garton, Hobson, and Lough 1958; Annison and Lewis 1959).

At birth, however, the suckling lamb does not possess an active rumen and a period of 3 months is usually required before this organ develops sufficiently in size and microflora to allow the animal to graze self-sufficiently (Barnett and Reid 1961). Associated with rumen development, then, is a considerable modification of lipid metabolic processes from those possessed by the simple-stomached infant to those characteristic of the adult ruminant (Cunningham and Loosh 1954; Garton 1960). Whereas many facets of adult ovine biochemistry have been contrasted to those of the young lamb (Jarret and Filsell 1958; Lindsay 1959; Caiger *et al.* 1962), detailed data on the transitional changes of serum fatty acids are not available in the literature.

In order to rectify this lack of data, the plasma lipids of lambs have been separated into cholesterol ester, triglyceride, free fatty acid, and phospholipid fractions, and alterations in the fatty acid components of these fractions have been followed during the period from birth to dietary independence.

II. Methods

The two animals used in these experiments were male Merino lambs, born to well-matched ewes by the same ram. They were kept with their mothers under natural grazing conditions for 5 months following birth. During this time green

* Department of Biochemistry, University of Queensland, St. Lucia, Brisbane.

pasture feed was plentiful and over the final 6 weeks of this period, no contribution was made to the lamb's diet by the maternal ewes.

Blood specimens were collected from the lambs at fortnightly intervals, heparinized, and the plasma separated by centrifuging soon after withdrawal of the blood. These plasma specimens were stored in a closed vessel in the dark at −10°C until required.

Lipids were then extracted from the plasma, and divided into four fractions (cholesterol esters; triglycerides; mono- and diglycerides and free fatty acids; phospholipids) by chromatography on silicic acid. Columns were run in duplicate—the fractions collected from one column were assayed for lipid content by weighing, and the fractions from the other column were interesterified and the component fatty acids in each fraction identified and estimated by means of gas chromatography. These analytical procedures have been described in detail previously (Horgan and Masters 1963).

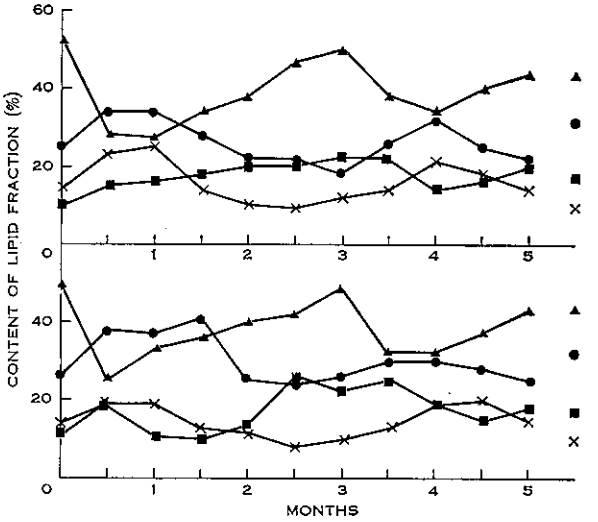

Fig. 1.—Alterations in the percentage content of the main lipid fractions of lamb plasma during growth. × Triglyceride fraction. ■ Free fatty acid fraction (including mono- and diglycerides). ● Cholesterol ester fraction. ▲ Phospholipid fraction. Normal adult mean values are indicated by the isolated symbols at the right-hand side of each figure.

III. Results

The percentages by weight of the lipid fractions which were separated on silicic acid columns are plotted against the age of the lambs in Figure 1. Individual graphs (rather than mean values) have been drawn to emphasize the similarity in the shape of the curves derived from separate animals.

The average concentration of serum lipid in the new-born lambs was 270 mg/100 ml. This value rose to 380 mg/100 ml between 1 and 2 months before decreasing to a final value of 280 mg/100 ml.

The curve for the cholesterol ester fraction shows two peaks in both instances (at 1–1½ and 3½–4 months) as does the curve for the triglyceride fraction (at 1–2 and

4 months). The percentage of mono- and diglyceride and free fatty acid fraction gradually rises to a broad peak at 3–4 months, while that of the phospholipid fraction decreases initially, rises to a peak value at 2–3 months, falls, then rises again in the terminal stages.

In Figure 2, the fatty acid percentages of the main C_{18} acids are plotted against the age of the lamb concerned. It is evident that the sequence of alterations in the fatty acid percentages is very similar in both series of analyses. In the cholesterol

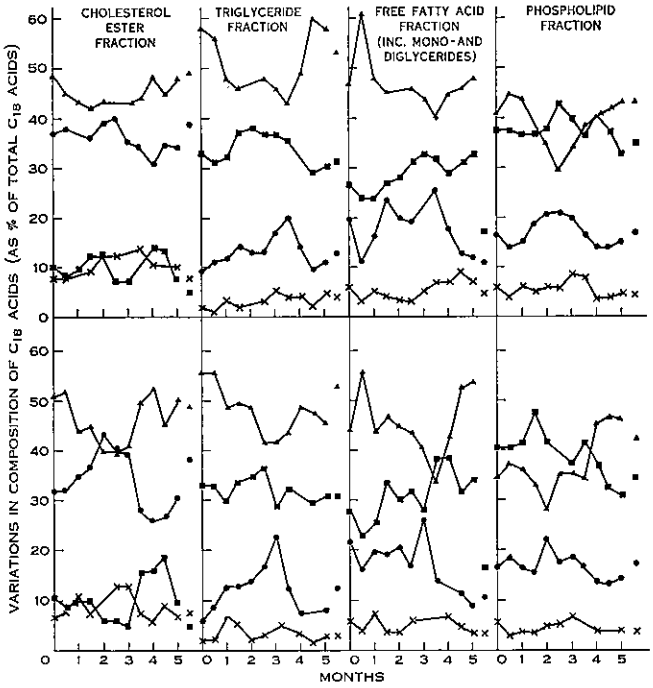

Fig. 2.—Variations in the percentage composition of individual C_{18} acids in the four main lipid fractions of lamb plasma during growth. ■ Stearic acid. ▲ Oleic acid. ● Linoleic acid. × Linolenic acid. Normal adult mean values are indicated by the isolated symbols at the right-hand side of each figure. Adult oleic acid values for the free fatty acid fraction are not given, but may be obtained by difference.

ester fraction, stearic acid percentages increase in the 3–4-month period with a subsequent terminal decrease. The oleic acid curve may be described in similar terms except that it rises in the final fortnight. In contrast, percentage of linoleic acid increases in the initial stages, decreases sharply during the 3–4-month period, then shows a terminal increase, as also does the linolenic acid percentage.

In the triglyceride fraction, stearic acid increases to a peak at about 2 months, and then gradually declines. The linoleic and linolenic curves have two maxima (1–1½ and 3–3½ months) and these correspond to the two negative peaks for oleic acid.

The stearic acid content of the mono- and diglyceride and free fatty acid fraction falls initially, rises to a peak at 3½ months, with a subsequent dip at 4 months. Oleic acid content rises initially and subsequently decreases until the 4-month sample

when a sharp increase occurs. Linoleic acid content usually varies inversely to oleic acid while there is little significant alteration in linolenic acid content.

The stearic acid curve for the phospholipid fraction is characterized by peaks near 2 and 4 months. Oleic acid content falls to a minimum at 2–2½ months and rises thereafter, while linoleic acid rises to a peak at 3 months and then decreases. Linolenic acid has a maximum at 3–3½ months and decreases thereafter.

Figure 3 shows the variations with age of plasma levels of palmitic acid. The percentage of palmitic acid esterified to cholesterol increases to a peak at 1–2 months, with a consequent fall, and rise to a further peak at 4 months. Triglyceride palmitic acid shows three peaks (at 1, 2½, and 4 months) in both instances, while the free fatty acid fraction is characterized by a broad maximum at 1–2 months. Phospholipid palmitic has peaks at 1–2 months, and then decreases gradually, with a slight terminal rise.

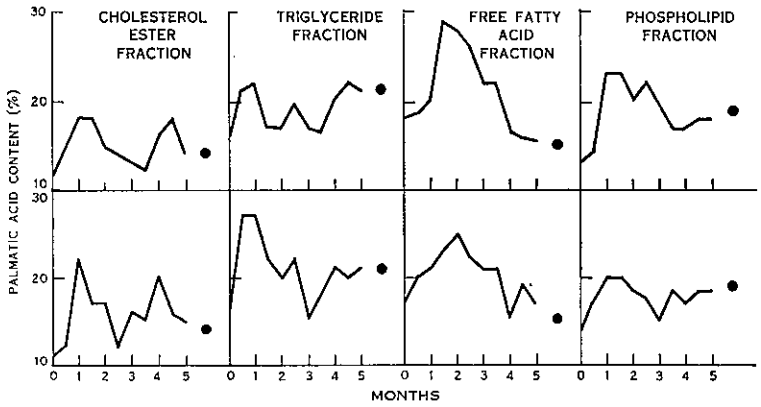

Fig. 3.—Variation in the percentage of palmitic acid in the four main lipid fractions of lamb plasma during growth.

Palmitoleic, arachidonic, and behenic acids are the remaining major fatty acid components of plasma, but no appreciable alteration occurs in the concentrations of any of these acids. With regard to the minor fatty acid components, percentage values are in general agreement with those reported for adult ovine plasma (Horgan and Masters 1963) and show no considerable alterations during the experimental treatment.

The mean values for adult ovine plasma are indicated in Figures 1, 2, and 3. These values are approximated, in general, by the initial and final lamb values. The fatty acid components of the mono- and diglyceride and free fatty acid fraction exhibit the widest divergence from the adult values.

IV. Discussion

Since the level of lipid in the blood is the resultant of a number of factors (e.g. diet, synthesis, mobilization, and oxidation), the strong similarity in behaviour of the sequential curves from the separate animals (Figures 1, 2, and 3) verifies that

these results represent the general nature of the alterations of plasma lipids in the young lamb under these conditions. This similarity of response also strengthens the validity of using these curves as a basis for interpretative discussion.

The sequence of changes in the plasma is obviously not a smooth transition from the fatty acid picture typical of a monogastric animal to the characteristic distribution of the adult ruminant. Rather a number of sequential changes appear to have taken place during this period. In general, these alterations are compatible with an interpretation based on a transition in status from foetal lamb to monogastric infant to self-sufficient ruminant.

As the foetus derives its nutrition across the placenta from the maternal circulation, it would be expected that plasma lipid values in the new-born lamb would approximate those of the adult ewe. This is found to be so with both the fraction percentages, and the percentages of individual fatty acid components within those fractions falling close to the mean values for adult plasma (Figs. 1–3; Horgan and Masters 1963). The fraction containing free fatty acids shows the widest divergence from the adult levels, but this is not surprising in view of the rapid turnover of this fraction and the fact that the initial plasma samples were obtained a day or so after birth.

The weeks following birth would seem to be conducive to changes in the lipid patterns of the plasma. Nutrient is no longer supplied direct from the maternal blood supply, but is now derived from the ewe milk, and has to be digested and absorbed from the alimentary tract. The fatty acid composition of these sources (i.e. adult plasma, milk) differ to a marked extent (Hilditch and Jasperson 1944; Horgan and Masters 1963), and a further factor is the considerable increase in the depot fat of the lamb, which is noticeable during this period.

An examination of the results reveals a number of changes during this time. With the fraction weights, the most significant change is the decrease in phospholipid content; a change which is consistent with an increased burden on hepatic phospholipid synthesis occurring at parturition. In the foetus the plasma lipid is in equilibrium with the lipids of the maternal blood stream, but after birth this source is removed and the infant liver has to assume responsibility for the supply of plasma phospholipid.

With the C_{18} acids, the characteristic change during this period is an increase of oleic acid in the free fatty acid and phospholipid fractions at the expense of linoleic acid. At the same time, oleic acid levels in the triglyceride fraction decline. Unesterified fatty acids are the form in which stored lipid is mobilized from the fat depots, and carried via the blood to the liver and other tissues for oxidation (Jeanrenaud 1961). Also, it is known that milk has a high content of oleic acid (Hilditch and Jasperson 1944), and that the young lamb builds up its depot fat during this period. Hence, the results are in agreement with an interpretation on the basis of the deposition of milk fat in storage sites, followed by an active mobilization of oleic acid, transport to the liver, and utilization in that tissue for phospholipid synthesis and the supply of energy. Additional evidence for this interpretation has been obtained in the analysis of tissue fatty acids of young lambs (Masters 1964) and by other indications of preferential mobilization and utilization of oleic acid by ovine tissues (Horgan and Masters 1963).

It is noticeable that the palmitic acid content of plasma lipid fractions increased during this initial period. This probably relates to the corresponding high content of palmitic acid in ovine milk fat, where it forms a much larger percentage of the long-chain fatty acids than it does in ovine plasma.

The dependence of the infant lamb on a milk diet gradually decreases during the first few months of life, coinciding with the development of an active rumen, and the ability to digest pasture grasses. At the stage of self-sufficiency, the dietary lipid is again considerably different to that of the simple-stomached suckling. Whereas milk lipids possess a high proportion of short-chain fatty acids, the fatty acids of pasture lipids are predominantly long-chain—more than 60% linolenic (Shorland 1944; Hilditch 1956). Short-chain acids (< 10 carbon atoms) are absorbed via the portal circulation, while long-chain acids enter the blood stream via the lymph (Cantarow and Schepartz 1962), and this difference may well be a contributing factor to the differences observable in the fraction weights during this period.

Further, ruminal hydrogenation developing during this period would be expected to result in a more saturated character of the fatty acid spectrum (Shorland *et al.* 1957). In the C_{18} acids, the cholesterol ester fraction does demonstrate a marked increase of stearic and oleic acid percentages during this period. Other fractions, however, do not follow this pattern, there being an actual decline of the stearic acid percentage in some fractions during the 3–4-month interval. This is generally accompanied, though, by an increase in oleic and a decrease in linoleic acid, so that the nature of the C_{18} acids as a whole is to become more saturated, even though stearic acid itself might not show an increased percentage. Another factor which would tend to disguise the increased saturation of dietary fatty acids, is the preference shown by the liver for exchanging unsaturated fatty acids from the plasma with more saturated components from hepatic lipids.

The significance of the increase in the palmitic acid percentages occurring in the plasma cholesterol ester, triglyceride, and phospholipid fractions at the age of 4 months is not readily apparent, although presumably the alterations in the relative contributions of the dietary C_{18} acids which occur during rumination would result in an altered status with regard to metabolic requirements and the maintenance of the physical characteristics of tissues (Okey *et al.* 1961). In this respect it is noteworthy that the main ovine tissues demonstrate an increased requirement for phospholipid palmitic acid at this stage, and a general decrease in cholesterol ester palmitic acid.

By the age of 5 months, fraction weights and component fatty acid levels approach the normal levels of adult animals (Horgan and Masters 1963). The main exception is in the fraction containing the free fatty acids, but as this is a very dynamic group of acids it would probably vary quite considerably in the growing animal.

Although plasma alterations over the experimental period do not indicate considerable changes in palmitoleic, arachidonic, and behenic acids, nor in the minor fatty acid components, moderate but significant alterations would be difficult to establish by the present technique at the levels of concentration of these compounds.

In conclusion, then, the data which have been presented represent information of a more exact nature than has been previously available on this subject and should shed increased light on a biochemical transformation of considerable interest and economic importance.

V. Acknowledgments

The author wishes to thank Professor E. C. Webb for his interest in this work and Professor O. E. Budtz-Olsen for the provision of experimental facilities.

VI. References

Annison, E. F., and Lewis, D. (1959).—"Metabolism in the Rumen." (Methuen and Co., Ltd.: London.)

Barnett, M. J. F., and Reid, R. L. (1961).—"Reactions in the Rumen." (Edward Arnold, Ltd.: London.)

Caiger, P., Morton, R. K., Filsell, O. H., and Jarret, I. G. (1962).—*Biochem. J.* **85**: 351.

Cantarow, A., and Schepartz, B. (1962).—"Biochemistry." 3rd Ed. (W. B. Saunders Company: London.)

Cunningham, H. M., and Loosh, J. K. (1954).—*J. Anim. Sci.* **13**: 265.

Garton, G. A. (1960).—*Nutrit. Abstr. Rev.* **30**: 1.

Garton, G. A. (1961).—In "Digestive Physiology and Nutrition of the Ruminant". (Ed. D. Lewis.) (Butterworths Scientific Publications: London.)

Garton, G. A., Hobson, P. N., and Lough, A. K. (1958).—*Nature* **182**: 1511.

Hilditch, T. P. (1956).—"The Chemical Constitution of Natural Fats." 3rd Ed. (Chapman and Hall, Ltd.: London.)

Hilditch, T. P., and Jasperson, H. (1944).—*Biochem. J.* **38**: 443.

Horgan, D. J., and Masters, C. J. (1963).—*Aust. J. Biol. Sci.* **16**: 905.

Jarret, I. G., and Filsell, O. H. (1958).—*Aust. J. Exp. Biol. Med. Sci.* **36**: 433.

Jeanrenaud, B. (1961).—*Metabolism* **10**: 535.

Lindsay, D. B. (1959).—*Vet. Rev. Annot.* **5**: 103.

Masters, C. J. (1964).—*Aust. J. Biol. Sci.* **17**: 190.

Okey, R., Shannon, A., Tinoco, T., Ostwald, R., and Miljanick, P. (1961).—*J. Nutrit.* **75**: 51.

Shorland, F. B. (1944).—*Nature* **153**: 168.

Shorland, F. B., Weenink, R. O., Johns, A. T., and McDonald, I. R. C. (1957).—*Biochem. J.* **67**: 328.