THE UPTAKE OF PLASMA LIPID AND SOME NON-LIPID CONSTITUENTS BY THE MAMMARY GLAND OF THE COW

By P. E. HARTMANN* and A. K. LASCELLES*

[*Manuscript received June* 1, 1964]

Summary

Arteriovenous difference studies have been carried out in four cows at various stages of lactation to determine the plasma lipid and non-lipid constituents which make a net contribution to the synthetic activities of the mammary gland.

There were large arteriovenous differences for glucose, triglycerides, and volatile fatty acids and a smaller but significant difference for free fatty acids and β-hydroxybutyrate in lactating cows. The arteriovenous difference measurements showed that glucose and triglycerides were not taken up by the non-lactating gland. Since there was no uptake of phospholipids, cholesterol esters, and cholesterol by the lactating or non-lactating gland it is clear that plasma triglycerides and free fatty acids must be the main sources of fatty acids for that portion of the milk fat which is derived from the plasma lipids.

I. INTRODUCTION

It is generally accepted that the fatty acids of ruminant milk fat are derived from two sources—the blood plasma lipids and lipogenesis in the mammary gland. Acetate and β-hydroxybutyrate on the one hand and glucose on the other are important precursors for the fatty acids and glycerol moieties, respectively, of ruminant milk fat (Folley 1961). Although it is known that large amounts of plasma triglycerides are taken up and incorporated into milk fat by the mammary gland of the lactating goat (Barry *et al.* 1963; Lascelles *et al.* 1964) the situation is not as clear for the cow. Arteriovenous studies have demonstrated an uptake of plasma neutral lipids (glycerides and possibly cholesterol esters) by the mammary gland of the cow (Lintzell 1934; Graham, Kay, and McIntosh 1936; Voris, Ellis, and Maynard 1940; McClymont and Vallance 1962) but have not shown any uptake of phospholipids. However, recent experiments with a lactating cow, which was given ^{14}C- and ^{32}P-labelled plasma lipids intravenously, have suggested that triglycerides, cholesterol esters, and phospholipids were involved in the transport of fatty acids to the mammary gland. Significant arteriovenous differences across the lactating mammary gland of ruminants have not been demonstrated for free fatty acids although Lauryssens, Verbeke, and Peeters (1961) found that with excised udders of lactating cows labelled free fatty acids in the perfusate were incorporated into the udder glycerides.

This paper describes arteriovenous difference studies carried out on lactating and dry cows to determine the plasma lipid and non-lipid constituents which made a net contribution to the synthetic activities of the mammary gland.

* Dairy Husbandry Research Foundation, Department of Animal Husbandry, University of Sydney, University Farms, Camden, N.S.W.

II. Methods

(a) *Blood Sampling*

Blood samples were collected simultaneously from the carotid artery and subcutaneous abdominal vein of two sets of identical twin cows on 16 different occasions. Two of the cows (T_1 and T_2) were $5\frac{1}{2}$-year-old crossbreds (Australian Illawarra Shorthorn × Ayrshire) in their third lactation and the other two cows (T_3 and T_4) were $3\frac{1}{2}$-year-old Jerseys in their second and first lactations respectively. A carotid artery in each cow was either exteriorized in a conventional skin loop or was held in a subcutaneous position over a specially shaped Perspex plate. Three of the cows (T_1, T_3, and T_4) were very docile and with a little training would stand quietly with no more than a loosely held halter during sampling. Cow T_2 was more difficult to handle and was only sampled on one occasion.

(b) *Management*

The cows grazed a mixed pasture of subterranean clover, white clover, and ryegrass and each cow was given an additional 12 lb of a concentrate mixture together with 8 lb of a medium-quality pasture hay per day. The cows were managed according to normal dairy farm practice.

(c) *Collection of Samples*

Blood (200 ml) was obtained simultaneously from the subcutaneous abdominal vein and carotid artery by venipuncture. The puncture area was anaesthetized prior to sampling. Cow T_1 was sampled 12 days before and 1 day after calving and then at monthly intervals for the next 5 months. One sample only was obtained from cow T_2 four months after calving. Cow T_3 was sampled 13 days before and 23 and 42 days after calving. Samples were collected from cow T_4 at 27 and 18 days before calving and 1, 19, and 60 days after calving. All samples were collected at about 5 p.m. which was just before the afternoon milking.

(d) *Analytical Determinations*

The following biochemical analyses were carried out: free fatty acids (Dole 1956); phospholipids (Zilversmit and Davis 1950); total protein (Gornall, Bardawill, and David 1949); glucose, glucose oxidase method of Huggett and Nixon (1957) in which Boehringer blood-sugar kits were used; volatile fatty acids (Annison 1954); β-hydroxybutyrate (Peden 1964); and milk fat by the Babcock method as described by Davis and MacDonald (1953). The extraction of plasma lipids with chloroform–methanol (2 : 1 v/v) and the analysis for cholesterol, triglycerides, and cholesterol esters by thin-layer chromatography was carried out as described by Hartmann and Lascelles (1965).

Free fatty acids were determined in quadruplicate while all other determinations were carried out in duplicate.

The results were analysed by conventional analysis of variance and least significant difference techniques. The sample from cow T_2 was not included in these analyses.

III. Results

(a) *Average Milk and Milk Fat Production*

The average milk and milk fat production over the sampling period of each of the cows computed from individual measurements made on the days (after calving) when blood samples were collected are presented in the following tabulation:

	Cow T_1	Cow T_2	Cow T_3	Cow T_4
Milk (kg/24 hr):	16·6	13·4	10·7	10·4
Milk fat (g/24 hr):	694·3	682·2	407·8	522·2

The arterial concentrations and the arteriovenous differences of the plasma constituents which were significantly decreased in concentration after passage through the mammary gland are shown in Table 1. The arterial and venous concentrations of the above constituents for all samples collected from cow T_1 are depicted in Figure 1.

Table 1

Mean concentrations of glucose, triglycerides, volatile fatty acids, and free fatty acids in the plasma samples collected from the carotid artery of the various cows before calving (non-lactating) and at monthly intervals after calving (lactating), together with mean arteriovenous differences

Standard deviations of duplicate determinations for each constituent are also presented. Results are expressed as mg/100 ml blood plasma. The negative sign indicates that the concentration in the venous plasma was higher than that in the arterial plasma

	Cow T_1		Cow T_2	Cow T_3		Cow T_4		Standard Deviation
	Non-lactating	Lactating	Lactating	Non-lactating	Lactating	Non-lactating	Lactating	
No. of samples:	1	6	1	1	2	2	3	
Glucose								
From carotid artery	56·7	61·9	62·76	67·9	71·0	68·9	65·2	1·2
Arteriovenous difference	−0·6	15·4	13·78	3·1	17·9	0·6	11·5	
Triglycerides								
From carotid artery	18·3	17·0	11·0	27·0	12·2	27·2	11·3	1·4
Arteriovenous difference	0·8	7·7	5·5	0·5	7·1	−0·2	6·4	
Volatile fatty acids								
From carotid artery	12·42	11·12	11·9	14·54	12·36	11·67	12·34	0·9
Arteriovenous difference	4·90	6·98	5·92	7·81	7·05	6·66	7·22	
Free fatty acids								
From carotid artery	8·79	12·75	18·23	9·98	11·42	11·32	12·59	0·5
Arteriovenous difference	1·09	2·53	3·90	1·05	0·54	0·76	1·0	

(b) *Volatile Fatty Acids and Free Fatty Acids*

The finding of a large arteriovenous difference ($P < 0·01$) for volatile fatty acids (V.F.A.) across the lactating and non-lactating gland is in accordance with that

of McClymont (1951). There was a smaller but consistent arteriovenous difference for free fatty acids (F.F.A.) in cow T_1 ($P < 0{\cdot}01$) and a similar difference was observed for the arteriovenous samples collected from cow T_2. The concentration of F.F.A.

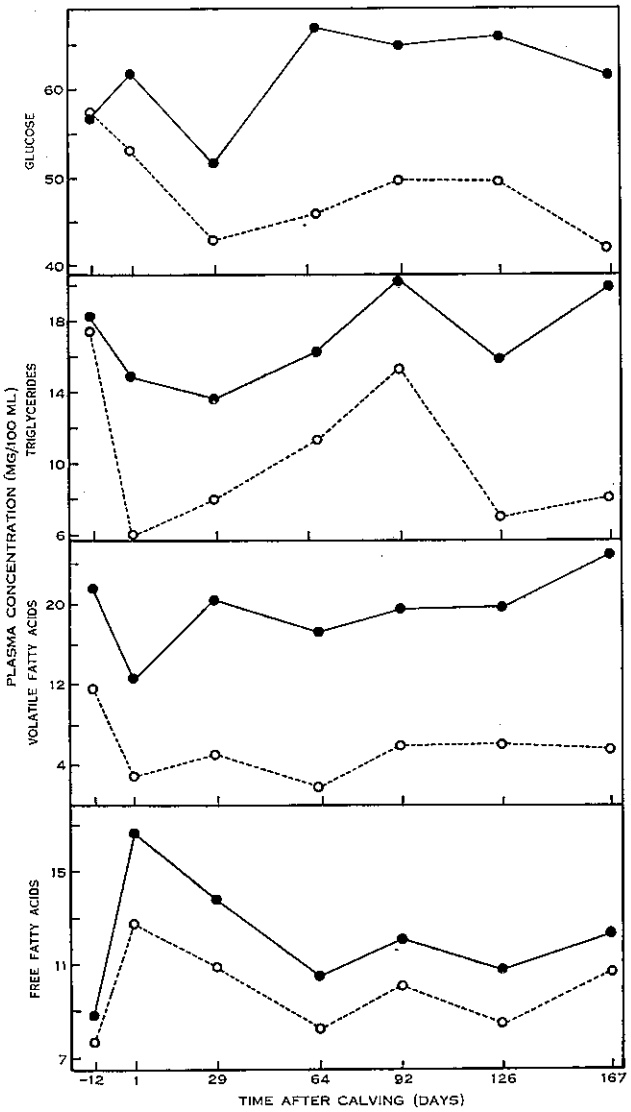

Fig. 1.—Concentrations of glucose, triglycerides, volatile fatty acids, and free fatty acids, in the blood plasma samples collected simultaneously from the carotid artery (●) and subcutaneous abdominal vein (○) of cow T_1 on seven occasions. One of the collections was made 12 days before calving, and is indicated by −12 on the abscissa.

in the venous plasma of these two cows varied between 80 and 90% of the arterial concentration. There was a similar arteriovenous difference ($P < 0{\cdot}01$) for cows T_3 and T_4 in all but two of the samples in which the difference was not significant.

(c) *Glucose and Triglycerides*

During lactation, there was a marked arteriovenous difference for glucose and triglycerides ($P < 0{\cdot}01$). The venous concentrations were 73–83% and 40–60% of the arterial concentrations, respectively; there was no uptake of these substances by the non-lactating gland. A sharp rise in the concentration of F.F.A., a small

TABLE 2

MEAN CONCENTRATIONS OF TOTAL PROTEIN, TOTAL FAT, PHOSPHOLIPIDS, CHOLESTEROL ESTERS, AND CHOLESTEROL IN THE PLASMA SAMPLES COLLECTED FROM THE CAROTID ARTERY OF THE VARIOUS COWS BEFORE CALVING (NON-LACTATING) AND AT MONTHLY INTERVALS AFTER CALVING (LACTATING), TOGETHER WITH MEAN ARTERIOVENOUS DIFFERENCES

Standard deviations of duplicate determinations for each constituent are also presented. Results are expressed as mg/100 ml blood plasma. The negative sign indicates that the concentration in the venous plasma was higher than that in the arterial plasma

	Cow T_1		Cow T_2	Cow T_3		Cow T_4		
	Non-lactating	Lactating	Lactating	Non-lactating	Lactating	Non-lactating	Lactating	Standard Deviation
No. of samples:	1	6	1	1	2	2	3	
Total protein								
From carotid artery	7·19	7·45	8·35	7·85	8·13	6·95	7·56	0·06
Arteriovenous difference	−0·09	−0·06	−0·01	−0·1	−0·07	−0·08	−0·05	
Total fat								
From carotid artery	290·3	546·9	517·0	279·0	384·2	305·5	293·2	7·8
Arteriovenous difference	−3·7	−3·5	4·0	6·0	9·3	−3·0	7·9	
Phospholipids								
From carotid artery	123·2	192·2	173·13	112·1	142·2	107·6	110·04	2·0
Arteriovenous difference	−1·4	−4·6	0	3·6	2·1	−1·0	−0·86	
Cholesterol esters								
From carotid artery	130·3	253·6	249·0	128·8	173·0	135·8	133·5	5·0
Arteriovenous difference	−3·5	−4·7	1·0	9·5	−0·8	0·2	1·2	
Cholesterol								
From carotid artery	16·92	31·84	33·54	15·2	23·1	14·36	14·16	0·4
Arteriovenous difference	−0·18	−0·69	0	0	0·4	−1·59	0·26	

rise in glucose, and a fall in V.F.A. was observed in arterial plasma collected just after parturition (cows T_1 and T_4) (see Fig. 1). These changes may have been due to stress factors and some degree of starvation associated with parturition. The concentration of the triglycerides in arterial plasma decreased at parturition and remained at a lower concentration for the next 2 or 3 months. This decrease was much more marked in the twin Jersey cows (T_3 and T_4) than in cow T_1.

The arterial concentrations and the arteriovenous differences of the remaining plasma constituents, the concentrations of which did not alter significantly during

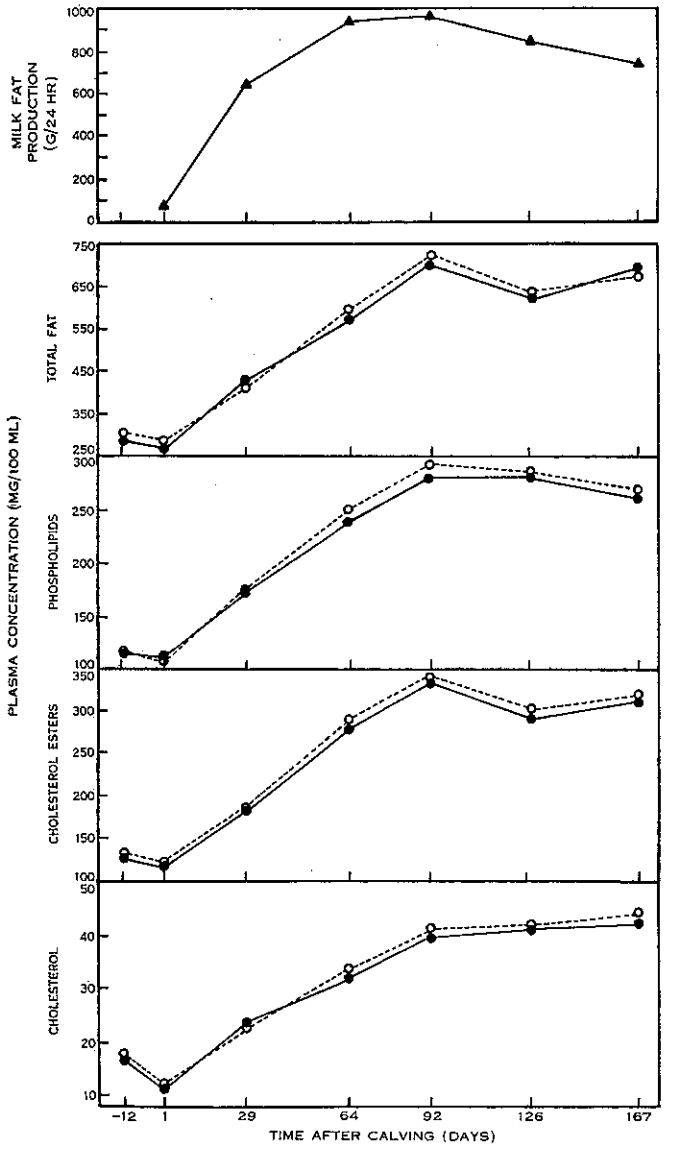

Fig. 2.—Concentrations of total fat, phospholipids, cholesterol esters, and cholesterol in the blood plasma samples collected simultaneously from the carotid artery (●) and subcutaneous abdominal vein (○) of cow T_1 on seven occasions. One of the collections was made 12 days before calving, indicated by −12 on the abscissa. The daily milk fat production is also presented.

passage through the mammary gland, are shown in Table 2. The arterial and venous concentrations of the above constituents from all samples collected from cow T_1 are shown in Figure 2. There was no suggestion of an uptake of cholesterol esters,

cholesterol, phospholipids, and protein by the mammary gland. If anything, there was a tendency for the concentration of these constituents to be slightly higher in the venous than in the arterial plasma. There was a marked and sustained increase in the plasma concentration of total fat, phospholipids, cholesterol esters, and cholesterol after parturition (see Fig. 2). Maximum concentrations were observed about 3 months after calving which coincided with the peak daily milk fat production. The change in concentration of the plasma lipids during lactation in the other cows was similar but smaller in magnitude than those of cow T_1. The various plasma lipids from identical twin cows were similar in concentration as were the changes in concentration during late pregnancy and early lactation. This is in contrast to the large variation which has been observed between unrelated cows at the same stage of lactation (Hartmann and Lascelles 1965).

IV. Discussion

Any disturbance of cows during the collection of arteriovenous samples may seriously alter blood composition and reduce blood flow through the mammary gland (Folley 1949). In the experiments described in this paper the use of local anaesthesia and the careful handling of the cows permitted rapid collection of blood samples without noticeable disturbance of the cows. The animals stood quietly and their pulse rate did not increase significantly during the period of collection.

The subcutaneous abdominal vein of the cow and goat may not always contain purely mammary venous blood due to the variable extent of valvular incompetence (Linzell 1960*a*). In the present experiments the possibility of obtaining venous blood contaminated with blood from tissues other than the udder was reduced by collecting the blood samples with the animals in the standing position.

The arteriovenous difference technique has been criticized on the grounds that an unknown portion of the fluid and solutes which leave the blood stream during their passage through the mammary gland are returned to the general circulation not in the venous effluent but by way of the lymphatics. Recent studies have shown that lymph flows at rates up to 1 litre per hour from the udder half of cows just before calving and in early lactation (Lascelles *et al.* 1964). The concentration of protein, total cholesterol, and phospholipids in lymph was 30–55% of the plasma concentrations and the loss of mammary lymph would be expected to increase the concentration of these constituents in the venous plasma at the most 0·4%. In lactating animals the loss of fluid relatively free of plasma protein and lipoproteins to the milk would tend to accentuate this effect. These factors probably were responsible for the slight negative arteriovenous difference for protein and the slightly higher haematocrit values of the venous blood observed in these studies (see Table 2).

Barry (1961) considers that if a compound has a consistent arteriovenous difference of over 20% in the lactating animal and there is no difference in the dry animal the compound is almost certainly a metabolite of the mammary gland. Statistical analyses of our data indicate that arteriovenous differences of over 10% for glucose, triglycerides, V.F.A., and F.F.A. and over 5% for total fat, phospholipids, cholesterol esters, and cholesterol were significant. This order of accuracy is supported by the consistency of the results.

To make quantitative estimates of uptake from arteriovenous difference studies it is necessary to measure blood flow through the udder at the same time as the arteriovenous samples are taken. Blood flow was not determined in these experiments; however, the results do permit a comparison of the relative net uptakes of the various constituents for each 100 ml of blood plasma passing through the udder at the time of sampling. Blood flow measurements through the udder have been determined by Rasmussen (1963) for the cow and by Linzell (1960*b*) for the lactating goat and expressed in terms of flow per minute per 100 g of mammary tissue: similar values were obtained for both animals. Linzell calculated that about 375 volumes of plasma flows through the lactating mammary gland of the goat for each volume of milk secreted, and this value is of a similar order to that reported by other workers for cows and goats using indirect methods based on the arteriovenous uptake of substances appearing in the milk (Folley 1949). Thus on the basis of 375 volumes of plasma for each volume of milk secreted it can be calculated that roughly 5·5 g of glucose, 2·5 g of triglyceride-esterified fatty acids, 2·4 g of V.F.A. (expressed as acetate), and 0·7 g of F.F.A. were removed from the blood during the formation of 100 ml of milk. Arteriovenous differences of β-hydroxybutyrate were carried out on a few of the samples and an average value of 1·8 mg per 100 ml of plasma (equivalent to 0·7 g taken up for each 100 ml of milk secreted) was found. This is of similar order to that reported for the cow by Shaw (1941). Thus the total quantity of triglyceride, V.F.A., F.F.A., and β-hydroxybutyrate taken up for each 100 ml of milk secreted was about 6·3 g which is considerably in excess of the requirements for the synthesis of 4 g of milk fat. The excess is not doubt utilized for energy purposes. However, the possibility must not be overlooked that the uptake of these compounds may vary during the day although in the goat it has been shown that the blood flow and uptake of milk precursors do not fluctuate widely during a 24-hr period (Linzell 1960*b*).

The arteriovenous difference for triglycerides in lactating cows is very much less than that reported for the goat by Barry *et al.* (1963). This may be related to the apparent low concentration of chylomicrons in the arterial plasma of cows compared with that of the goat (Hartmann and Lascelles 1965). It is noteworthy that the mean arteriovenous difference of esterified fatty acids in the low-density lipoprotein fraction of two lactating goats is comparable with our results.

Peeters (1963, cited by Sloane-Stanley 1963) has concluded from *in vivo* and isolated perfused udder experiments that there is an exchange of F.F.A. between the blood and the fatty acids of the cow's udder but no net uptake. Likewise arteriovenous difference studies by McClymont (personal communication) have indicated that plasma F.F.A. are not a significant fatty acid source for milk fat synthesis in the bovine. However, our results have shown that there is a significant uptake of F.F.A. The fact that the uptake was not significant in two out of the 16 arteriovenous difference studies may indicate that plasma F.F.A. is a supplementary source of fatty acids for the mammary gland and its uptake can be adjusted according to the requirements of the gland and the availability of other substrates.

The results indicate that the arteriovenous difference for glucose can account for most if not all that observed for total reducing substances by other workers

(Graham *et al.* 1936; Shaw 1943). In the goat, much of the glucose taken up by the mammary gland is utilized for the production of lactose and glycerol as has been demonstrated in isolated perfused goat udders (Hardwick, Linzell, and Mepham 1963). In the present studies even assuming that all the lactose and milk fat glycerol is derived from the glucose there is still an excess of glucose taken up by the mammary gland which would serve as an additional energy source.

The arteriovenous differences for glucose, triglycerides, V.F.A., and F.F.A. across the non-lactating udder are comparable with those for the head and neck (Hartmann and Lascelles 1965). This serves to emphasize the specific role of glucose and triglycerides as metabolites for the lactating mammary gland.

Although Riis, Luick, and Kleiber (1960) suggested that plasma cholesterol esters are a source of fatty acids for milk fat synthesis our results show no uptake of cholesterol esters. There was no evidence that fatty acids from the cholesterol ester fraction were taken up by the gland because the weights of cholesterol esters in the arterial and venous plasma were not significantly different and there was no significant increase in the free cholesterol content of the venous plasma. Thus the results indicate that plasma triglyceride and F.F.A. are the main sources of fatty acids for that portion of the milk fat which is derived from the plasma lipids.

V. Acknowledgments

The authors would like to thank Misses R. Pell, J. Rock, and other members of the Experimental Dairy Unit, Camden, N.S.W., for invaluable technical assistance. They are indebted to Mr. E. Best for statistical advice.

VI. References

Annison, E. F. (1954).—*Biochem. J.* **58**: 670.

Barry, J. M. (1961).—"Milk: The Mammary Gland and its Secretion." 1st Ed. (Academic Press Inc.: New York.)

Barry, J. M., Bartley, W., Linzell, J. L., and Robinson, D. S. (1963).—*Biochem. J.* **89**: 6.

Davis, J. G., and MacDonald, F. J. (1953).—Richmond's "Dairy Chemistry". 5th Ed. p. 358. (Charles Griffin & Co. Ltd.: London.)

Dole, V. P. (1956).—*J. Clin. Invest.* **35**: 150.

Folley, S. J. (1949).—*Biol. Rev.* **24**: 316.

Folley, S. J. (1961).—*Dairy Sci. Abstr.* **23**: 511.

Gornall, A. G., Bardawill, G. J., and David, M. M. (1949).—*J. Biol. Chem.* **177**: 751.

Graham, W. R. Jr., Kay, H. D., and McIntosh, R. A. (1936).—*Proc. Roy. Soc.* B **120**: 319.

Hardwick, D. C., Linzell, J. L., and Mepham, T. B. (1963).—*Biochem. J.* **88**: 213.

Hartmann, P. E., and Lascelles, A. K. (1965).—*Aust. J. Biol. Sci.* **18**: (in press).

Huggett, A. St. G., and Nixon, D. H. (1957).—*Biochem. J.* **66**: 12P.

Lascelles, A. K., Cowie, A. T., Hartmann, P. E., and Edwards, M. J. (1964).—*Res. Vet. Sci.* **5**: 190.

Lascelles, A. K., Hardwick, D. C., Linzell, J. L., and Mepham, T. B. (1964).—*Biochem. J.* **92**: 36.

Lauryssens, M., Verbeke, R., and Peeters, G. (1961).—*J. Lipid Res.* **2**: 383.

Lintzell, W. (1934).—*Z. Zücht.* B **29**: 219.

Linzell, J. L. (1960*a*).—*J. Physiol.* **153**: 481.

Linzell, J. L. (1960*b*).—*J. Physiol.* **153**: 492.

McClymont, G. L. (1951).—*Aust. J. Agric. Res.* **2**: 158.

McClymont, G. L., and Vallance, S. (1962).—*Proc. Nutr. Soc.* **21**: xli.
Peden, V. J. (1964).—*J. Lab. Clin. Med.* **63**: 332.
Rasmussen, F. (1963).—*Acta Vet. Scand.* **4**: 271.
Riis, P. M., Luick, J. R., and Kleiber, M. (1960).—*Amer. J. Physiol.* **195**: 45.
Shaw, J. C. (1941).—*J. Dairy Sci.* **24**: 500.
Shaw, J. C. (1943).—*J. Dairy Sci.* **26**: 1079.
Sloane-Stanley, G. H. (1963).—*Nature* **200**: 1158.
Voris, L., Ellis, G., and Maynard, L. A. (1940).—*J. Biol. Chem.* **133**: 491.
Zilversmit, D. B., and Davis, A. K. (1950).—*J. Lab. Clin. Med.* **35**: 155.