A STUDY OF LIPID ABSORPTION IN YOUNG MILK-FED CALVES WITH THE USE OF A LYMPHATICO-VENOUS SHUNT FOR THE COLLECTION OF THORACIC DUCT LYMPH

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Summary

A technique has been described for the collection of thoracic duct lymph from the young milk-fed calf. The establishment of a recirculating lymphatico-venous shunt permitted the repeated sampling of lymph from three calves for periods of 9–17 days, during which the animals remained in good health.

The pattern of absorption of lipid was studied by determining the changes in the output of lipid in lymph of the calves fed whole milk either twice or once daily. When the calves were fed twice daily it was found that the mean hourly flow of lymph and output of neutral lipid, free fatty acid, and phospholipid changed relatively little with time after feeding, compared with a definite pattern of change observed over the first 12 hr after feeding when the calves were fed once daily.

With once daily feeding the flow of lymph reached a peak 4–5 hr after feeding. The concentration of neutral lipid was at its lowest level at this time but subsequently increased sharply to reach its highest level at about 10 hr. The output of neutral lipid, free fatty acid, and phospholipid also reached their highest levels at about 10 hr after feeding. There was a sharp decrease in the output of the lipids between 10 and 12 hr with relatively constant values over the 12–24-hr period after feeding. The mean hourly output of lipid in the lymph over this latter period was found to be similar to that in the first 12-hr period, emphasizing the continuous nature of lipid absorption.

The ingestion of approximately 140–190 g/24 hr of longer-chain fatty acid ($> C_{12}$) in one or two feeds of milk resulted in the transport of an estimated 100–160 g/24 hr of neutral lipid fatty acid in the thoracic duct lymph. Since the concentrations of neutral lipid and triglyceride were found to be similar, the estimate for the output of neutral lipid would represent approximately the output of chylomicron triglyceride. The lipid composition of the lymph was found to be similar for samples collected before feeding and at the time of maximum lipid absorption.

I. INTRODUCTION

The development of techniques for the collection of thoracic and intestinal lymph over long periods of time has enabled the quantitative study of the absorption of long-chain fatty acid from the alimentary tract of a number of species of animals. The first detailed report on the absorption of lipid in ruminants was presented by Heath and Morris (1962). Their studies on milk-fed lambs were carried out by collecting all the lymph which flowed from the cannulated intestinal lymphatic duct. Lymphatico-venous shunts were not established in these experiments probably because it was considered that the flow of lymph was too small for continuing recirculation. More recently Hartmann and Lascelles (1966) described a technique for the collection of thoracic duct lymph from the cow over long periods of time and

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were able to determine the quantity of long-chain fatty acid transported in the lymph of cows at pasture. The flow of lymph was very high and the effects of continuing drainage of lymph were overcome by establishing a lymphatico-venous shunt from which lymph samples were obtained when required.

In the present study a similar technique has been used to determine the pattern of lipid absorption in the young calf fed milk once or twice daily. It is considered that the use of this technique has enabled us to carry out these studies in physiologically healthy animals.

II. Materials and Methods

(*a*) *Animals*

Lymphatico-venous shunts were established in three Friesian heifer calves. Calf 1 (34·2 kg) was cannulated at 4 days, calf 2 (36·6 kg) at 7 days, and calf 3 (30·8 kg) at 4 days of age. The calves were taken from their dams at 1 day of age and taught to drink from a bucket. Prior to the operation they were fed 2·27 litres (0·5 gal) of milk twice daily.

(*b*) *Anaesthesia*

The calves were not fed for a period of 15 hr prior to anaesthesia. Pentothal sodium (Abbott) as a 5% solution was injected intravenously to induce anaesthesia before intubation with a Magill endotracheal tube. Surgical anaesthesia was maintained with a mixture of cyclopropane and oxygen in closed circuit. Approximately 30 min before the end of the operation the cyclopropane was discontinued and the system flushed with oxygen. In calf 1, which received a dose of 22 mg/kg of pentothal, recovery from the anaesthetic was prolonged, with the animal still unsteady on its feet at 24 hr. In calves 2 and 3, doses of only 8 and 11 mg/kg of pentothal respectively were used to induce light anaesthesia. Intubation was carried out easily and recovery was rapid with the animals standing steadily within 6–12 hr of the operation.

(*c*) *Surgical Technique*

Calves were laid on the operating table with their left side uppermost. The head and neck were extended, with the head lowered slightly. In the young calf, unlike the adult cow, it was found that excellent access to the region of the first rib was obtained when the left forelimb was held back. A skin incision of 10–12 cm, extending from approximately midway along the neck to the point of the shoulder, was made immediately dorsal to the external jugular vein. The surgical technique employed in locating and cannulating the thoracic duct, and the cannulation of the jugular vein via the communicating branch of the cephalic vein, was similar to that described for the cow by Hartmann and Lascelles (1966). In calves 1 and 3 the cervical and brachial ducts, as well as numerous smaller ducts, were ligated to prevent lymph from these ducts entering the thoracic duct cannula. In calf 2, however, the thoracic duct was cannulated distal to the common ampulla and the other lymphatic ducts in the region allowed to flow freely into the blood stream. It is interesting to note that the flow and output of lipid in lymph was lower in this calf than in the other two calves. While it was considered that the bulk of the thoracic duct lymph was

being collected in this animal, the possibility of a smaller secondary thoracic duct entering the ampulla in this case cannot be ruled out.

Polyvinylchloride cannulas (1·5 mm int. diam., 2·5 mm ext. diam., Dural Plastics and Engineering Pty. Ltd., Sydney, N.S.W.) were used to cannulate the thoracic duct and the jugular vein. The lymphatico-venous shunt was completed as in the cow and functioned satisfactorily for a period of 12 days in calf 1, 9 days in calf 2, and 17 days in calf 3.

(*d*) *Post-operative Care*

The calves were fed milk soon after completion of the operation. Calf 1 was fed by stomach tube during the first 24 hr whereas calves 2 and 3 recovered quickly and were drinking normally within the first 5 hr. The calves were kept in 6 by 6 ft indoor pens on straw bedding during the experimental period. Each calf was given 1×10^6 i.u. procaine penicillin and 0·5 g streptomycin intramuscularly each day for 5 days after the operation, and the operative area dressed daily. There was no evidence of infection of the operative region in any of the calves.

(*e*) *Management and Feeding*

The calves were initially fed 2·27 litres (0·5 gal) of whole milk twice daily at 12-hr intervals, beginning on the second day following the operation. 2–3 days were allowed on this feeding regime before calves were sampled at regular intervals for two periods of 12 hr. Following these experiments the calves were fed 4.55 litres (1·0 gal) of milk once daily. 2 days were allowed for the calves to accustom to the new feeding regime before samples were collected at intervals over a single period of 24 hr. Calves were offered a little water to drink during the course of the day.

(*f*) *Collection of Samples*

The shunt was disconnected and sterile saline was flushed through the venous cannula and its end then blocked with a plug. Lymph was collected in a measuring cylinder for a measured period of time (2–3 min) with occasional blood samples being taken from the venous cannula. Heparin (Pularin, Evans) was the anticoagulant used throughout. After once again flushing the venous cannula with sterile saline the shunt was re-established. Calves quickly became accustomed to the sampling routine and could be sampled in either the standing or recumbant position without disturbance.

(*g*) *Analytical Technique*

Total esterified fatty acid was determined by the method of Stern and Shapiro (1953), assuming the average molecular weight of the fatty acid to be 280. Preliminary analyses of the fatty acid composition of the lipids in the lymph have indicated that this value is a reasonable estimate. The values of phospholipid were calculated from the amount of phosphorus as determined by the procedure of Zilversmit and Davis (1950) on alcohol–ether (3 : 1 v/v) extracts of the lymph samples. The weight of lipid phosphorus was divided by 31 and multiplied by 560 (2×280) to give the weight of fatty acid. The total esterified fatty acid minus the phospholipid fatty acid will

be referred to as the neutral lipid. Free fatty acid (F.F.A.) was determined by the titration method of Dole (1956). The extraction of lipid in the lymph samples with chloroform–methanol (2 : 1 v/v) and the analysis for triglyceride, cholesterol ester, and free cholesterol by thin-layer chromatography, was carried out as previously described (Hartmann and Lascelles 1965). Samples of the milk fed to the calves were analysed for total fat by the Babcock method as described by Davis and MacDonald (1953).

III. Results

During anaesthesia lymph flow varied between 60 and 120 ml/hr. The lymph at this time was opalescent in appearance and the output of neutral lipid in the lymph varied between 0·15 and 0·64 g/hr. The triglyceride fraction in the lymph collected during anaesthesia was less than 50% of the total lipid. Lymph flow increased during the first 24 hr after the operation, and by the second day had reached a mean level of 375 ml/hr which was maintained over the period in which the shunt functioned satisfactorily. As the flow increased the lymph became milky in appearance due to its increased content of chylomicrons. The estimated daily output of neutral lipid in the lymph did not vary greatly from 2 days after the operation by which time the calves were in excellent health and so remained during the entire period of lymph collection. Rates of weight gain over this period were 0·64 kg/day for calf 1, 0·45 kg/day for calf 2, and 0·73 kg/day for calf 3.

(*a*) *Lymph Flow*

Over relatively short periods of time lymph was observed to flow from the cannula at a variable rate. There were intermittent increases in flow every few seconds which may have been associated with rhythmic contractions of the thoracic duct (cf. Hall, Morris, and Woolley 1965). The minute to minute variation observed when consecutive samples were collected over 15-min periods was very much smaller than that found when samples were collected at 6-sec intervals (coefficients of variation 14·2 and 41·5%, respectively). Thus the estimates for the average flow and output of lipid in lymph were derived from samples collected over periods of 2–3 min. The flow of lymph was found to be similar when animals were standing quietly or resting in a sternal recumbant position. However, a small pooling effect was evident in the recumbant position since the flow was observed to increase sharply for a period of 15–30 sec immediately the animal stood up. For this reason, if the calves were resting in a recumbant position they were sampled either while recumbant, or if they stood, a period of 5 min was allowed to elapse before an estimate of the lymph flow was made.

(*b*) *Lymph and Lipid Output*

The results presented in Figure 1 were derived from samples collected over the one 24-hr period of sampling in each of the three calves when they were being fed once daily, whereas the results in Figure 2 were from the two 12-hr periods on each calf when fed twice daily. A comparison of the data in these two figures reveals that the change in the flow of lymph, output of neutral lipid, phospholipid, and F.F.A., and in the concentration of neutral lipid, with time after feeding are much more striking

for once daily feeding than for twice daily feeding. The greatest changes over the 24-hr period may be seen to occur during the first 12 hr after feeding, with relatively constant values over the subsequent 12 hr (Fig. 1). A summary of an analysis of

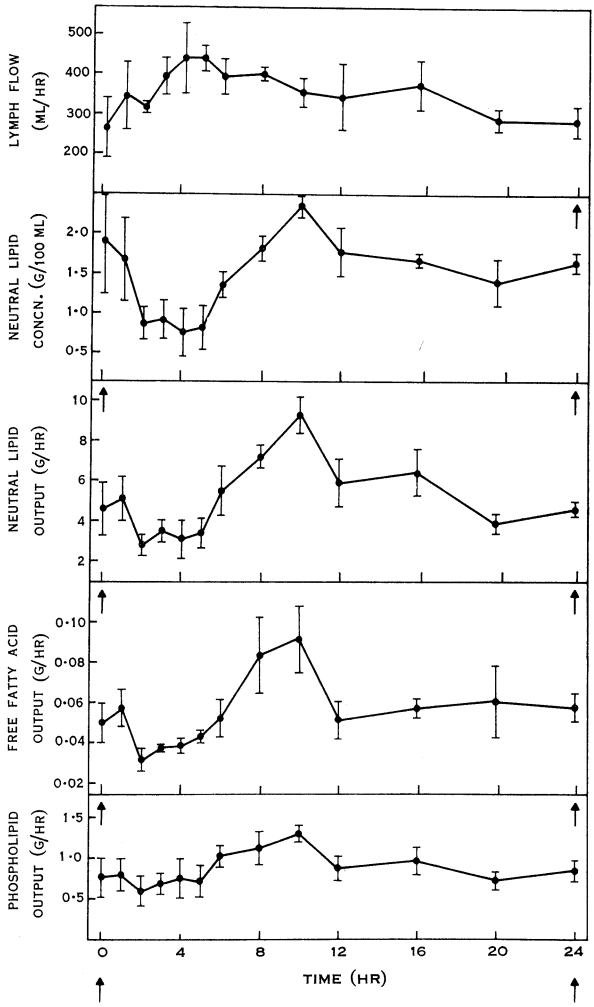

Fig. 1.—Lymph flow, concentration of neutral lipid, and output of neutral lipid, free fatty acid, and phospholipid in thoracic duct lymph of calves over a 24-hr period after once daily feeding. The values plotted are means ± standard errors. These were derived from the results of a total of three experiments in the three calves. Arrows indicate the time at which the calves were fed 4·55 litres of milk.

variance of the results derived from samples collected at 0, 1, 2, 3, 4, 5, 6, 8, 10, and 12 hr after feeding for both regimes of feeding is presented in Table 1. Thus the results for the one period of once daily feeding have been compared with those for the two periods of twice daily feeding and are referred to as treatments in the analysis.

It is noteworthy that the variation due to differences between treatments (once daily *v.* twice daily feeding) is very small for all variates with the exception of phospholipid output which indicates that the mean level of the variates over the 12-hr period was

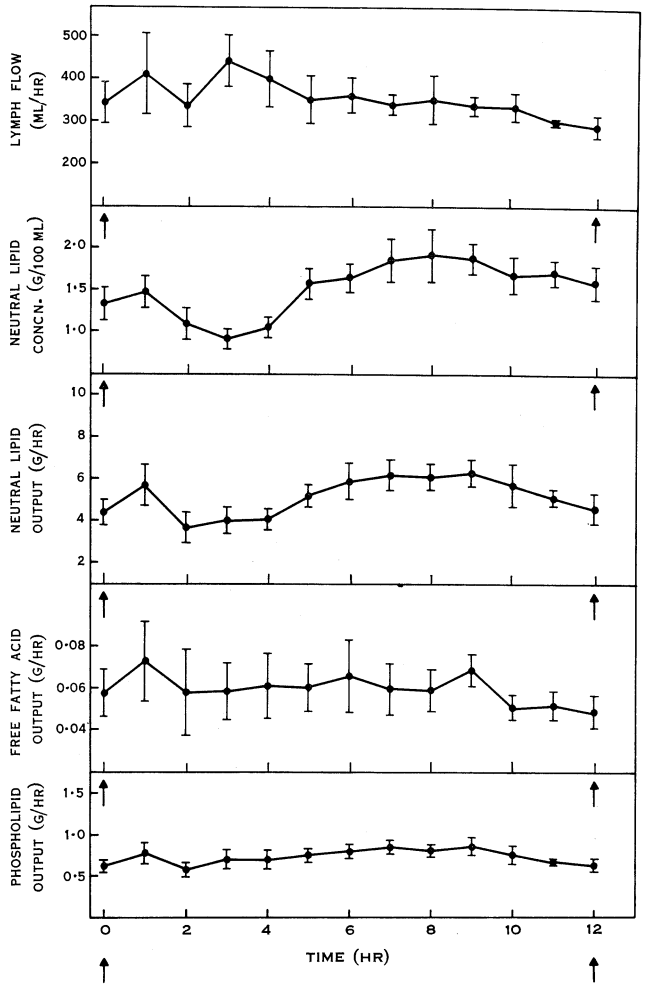

Fig. 2.—Lymph flow, concentration of neutral lipid, and output of neutral lipid, free fatty acid, and phospholipid in the thoracic duct lymph of calves over a 12-hr period after twice daily feeding. The values plotted are means ± standard errors. These were derived from the results of a total of six experiments in the three calves. Arrows indicate the time at which the calves were fed 2·27 litres of milk.

similar whether the milk was fed once or twice daily. However, the significant treatment × linear or treatment × quadratic component of the treatment × times interaction was due to the different pattern of change of each variate with time, for the two feeding regimes. Thus with twice daily feeding there was little change in the

flow and output of lipid in lymph with time after feeding compared with the definite pattern of change observed with once daily feeding (cf. Figs. 1 and 2).

An analysis of variance of the results for the entire 24-hr period after feeding (once daily feeding) was then carried out. This analysis showed that there were significant differences between times for all variates. This component of variance

TABLE 1

SUMMARY OF THE ANALYSIS OF VARIANCE OF FLOW, CONCENTRATION OF NEUTRAL LIPID, AND OUTPUT OF NEUTRAL LIPID, FREE FATTY ACID, AND PHOSPHOLIPID IN THORACIC DUCT LYMPH COLLECTED AT 0, 1, 2, 3, 4, 5, 6, 8, 10, AND 12 HR AFTER FEEDING, FOR TWO PERIODS OF TWICE DAILY AND ONE PERIOD OF ONCE DAILY FEEDING IN EACH OF THE THREE CALVES

The variance attributable to differences between the two periods of twice daily feeding (between "replicated treatments") and "replicated treatments × calves" comprises the "error (*a*)" term, and the "treatments (including replicated treatments) × calves × times" (36 degrees of freedom) together with the "times × replicated treatments" (9 degrees of freedom) the "error (*b*)" term. To calculate the variance ratios for between "treatments", "calves", and "treatment × calves", the mean square for "error (*a*)" has been used as the denominator while the mean square for "error (*b*)" has been used as the denominator for the calculation of the variance ratio of the remaining sources of variation

Source of Variation	Degrees of Freedom	Mean Squares				
		Flow	Neutral Lipid Concentration	Neutral Lipid Output	Free Fatty Acid Output	Phospho-lipid Output
Treatments†	1	971	0·0037	0·188	0·00070	0·5092**
Calves	2	351,310*	3·1449	25·095	0·01153	1·0655**
Treatments† × calves	2	20,909	0·0110	4·586	0·00585	0·2576*
Error (*a*)	3	31,862	0·6827	7·502	0·00311	0·0173
Times	9	14,334*	1·2478***	12·652***	0·00045	0·1154**
Treatments† × times	9	6,391	0·0469	4·721	0·00095**	0·0605
Linear	1	35,094*	0·0036	14·446*	0·00345**	0·2416**
Quadratic	1	8,037	2·2438**	12·383	0·00119	0·0795
Remainder	7	2,055	0·3288	2·236	0·00065	0·0319
Calves × times	18	6,299	0·1872	2·486	0·00040	0·0192
Error (*b*)	45	5,078	0·2144	2·307	0·00029	0·0306

* $P < 0·05$. ** $P < 0·01$. *** $P < 0·001$.

† The "treatments" in these sources of variation are comparisons between once and twice daily feeding and therefore carry only one degree of freedom.

was subdivided by the use of orthogonal coefficients. It was found that there was a significant increase ($P<0·05$) in the lymph flow between 3 and 4 hr and then a decrease ($P<0·05$) between 16 and 20 hr after feeding. A significant decrease ($P<0·05$) in the concentration of neutral lipid occurred between 1 and 2 hr after feeding, followed by an increase ($P<0·01$) between 6 and 8 hr and another decrease ($P<0·05$) between 10 and 12 hr after feeding. On the other hand, the output of neutral lipid showed no early decrease but there was a similar increase ($P<0·01$) between 6 and 8 hr and a decrease ($P<0·05$) between 10 and 12 hr after feeding. It is interesting to note that the increase in lymph flow occurred several hours before

the increase in the concentration and output of neutral lipid, which is in agreement with findings in other animals (Borgström and Laurell 1953; Morris 1954; Simmonds 1955), and with more recent observations in young lambs by Heath and Morris (1962). The pattern of output of F.F.A. in lymph was similar to that of neutral lipid with a significant increase ($P<0\cdot01$) between 6 and 8 hr and a decrease ($P<0\cdot01$) between 10 and 12 hr after feeding. The output of phospholipid increased ($P<0\cdot05$) 5–6 hr after feeding and decreased ($P<0\cdot05$) between 10 and 12 hr. Thus the peak output of neutral lipid, F.F.A., and phospholipid all occurred at about 10 hr after feeding. The values of all variates were relatively constant between 12 and 24 hr after feeding.

TABLE 2

ESTIMATES OF THE TOTAL OUTPUT OF NEUTRAL LIPID IN THE THORACIC DUCT LYMPH OF EACH OF THREE CALVES

Estimates were made over the 12- and 24-hr periods for the two twice daily and the one once daily feeding experiments, respectively. Estimates of the quantity of longer-chain fatty acid ($>C_{12}$) in the milk ingested prior to each of the experimental periods are also shown

Feeding Experiments	Feeding Period (hr)	Calf 1		Calf 2		Calf 3	
		Longer-chain Fatty Acid Ingested in Milk (g)	Neutral Lipid Output in Lymph (g)	Longer-chain Fatty Acid Ingested in Milk (g)	Neutral Lipid Output in Lymph (g)	Longer-chain Fatty Acid Ingested in Milk (g)	Neutral Lipid Output in Lymph (g)
Twice daily feeding	12	82·2	57·3	67·9	48·3	87·6	82·1
	12	83·5	76·2	89·3	48·7	85·0	62·9
Once daily feeding	24	164·7	142·5	187·5	123·7	171·0	122·9

The average hourly lymph flow was 369·9 and 323·4 ml respectively, during the first and second 12-hr periods after feeding when the three calves were fed once daily. The corresponding average hourly outputs for neutral lipid were 5·033 and 5·192 g, for phospholipid 0·873 and 0·854 g, and for F.F.A. 54 and 56 mg. The similarity of the values of each variate for both periods emphasizes the continuous nature of lipid absorption in milk-fed calves even when fed once daily.

Correlation coefficients were calculated from the results for the three 24-hr periods of once daily feeding. The between-calves and between-times partial correlations were calculated from the respective between-calves and between-times variances and covariances for the outputs of neutral lipid, F.F.A., and phospholipid. There were significant between-times positive correlations between the output of neutral lipid and F.F.A. ($r=0\cdot91$, $P<0\cdot001$) and between the output of neutral lipid and phospholipid ($r=0\cdot97$, $P<0\cdot001$). The correlation between the output of neutral lipid and F.F.A. may reflect incomplete esterification of the absorbed fatty acid within the cells of the intestinal mucosa following the hydrolysis of triglyceride in the intestinal lumen (Borgström and Tryding 1956). The correlation between the output of neutral lipid and phospholipid in the calf indicates that phospholipid is associated

with neutral lipid in the structure of chylomicrons. This finding is similar to that reported for the adult cow and other animals (cf. Hartmann and Lascelles 1966).

Estimates of the output of neutral lipid in lymph collected over the 12- and 24-hr periods for the once and twice daily feedings respectively, together with the quantity of the longer-chain fatty acid ($> C_{12}$) in the milk ingested are presented in Table 2. In computing the latter values the fatty acid of the milk diet was assumed to be similar to that reported by Hilditch and Jasperson (1943). It was considered that the shorter-chain fatty acid in the milk fat would be absorbed by way of the portal vein as has been described for monogastric animals (cf. Senior 1964) and for lambs by Heath, Adams, and Morris (1964) who found only small amounts of C_{10} and C_{12} fatty acid in the intestinal lymph following the ingestion of cow's milk. Thus an estimate of the quantity of fatty acid in milk fat which would be expected to be available for absorption by the lymphatic system has been derived. It may be seen (Table 2) that the ingestion of 140–190 g per 24 hr of longer-chain fatty acid in one or two feeds resulted in the transport of 100–160 g per 24 hr of neutral lipid fatty acid in the lymph.

(c) *Lipid Composition of Lymph*

The mean composition of lymph collected from the three calves just before feeding, and again at the peak of absorption, is given in the following tabulation. Values for triglyceride, F.F.A., phospholipid, cholesterol ester, and free cholesterol are expressed as a percentage of the total lipid concentration:

Lymph	No. of Samples	Total Lipid (g/100 ml)	Triglyceride (%)	F.F.A. (%)	Phospho-lipid (%)	Cholesterol Ester (%)	Free Cholesterol (%)
Collected before feeding	20	2·171	80·8	0·86	12·0	1·84	0·73
Collected after feeding	11	2·966	83·6	0·82	10·1	1·57	0·73

Although the concentration of total lipid in lymph differed considerably at these times, the composition of the lipid fractions expressed as a percentage of the total lipid was found to be very similar. Triglyceride comprised, by weight, 81 and 84% of the total lipid just before feeding and at the peak of absorption, respectively. A comparison was made between the neutral lipid and triglyceride content of 66 samples of lymph collected from the three calves during the course of the experiments. The values for neutral lipid and triglyceride, expressed as a percentage of the total lipid, were found to be similar. Thus the estimate for the output of neutral lipid (Figs. 1 and 2) would represent approximately the output of chylomicron triglyceride. A total of nine simultaneous blood and lymph samples were collected from two of the calves. It was found that the concentration of total esterified fatty acid was about 10 times greater in lymph than in plasma. Both phospholipid and F.F.A. were in all cases higher in lymph than in plasma, the ratio of lymph to plasma concentrations being approximately 2 : 1 for both constituents. A comparison of the concentrations of cholesterol ester and free cholesterol in lymph from these three calves with the concentrations of these constituents in the plasma of calves 6–15 days old (Shannon and Lascelles 1966) showed that the values for cholesterol ester were considerably

lower in lymph than in plasma (approximate ratio 1 : 3) whereas the values for free cholesterol in lymph and plasma were of the same order. Similar observations were reported by Hartmann and Lascelles (1966) for the adult cow.

(*d*) *Cell Content*

The red cell content of the lymph varied between 5000 and 50,000 cells/mm^3, and sometimes sufficient numbers were present to impart a faint pink tinge to the lymph. Thus the red cell content of the lymph of calves is of the same order as that found in cows (Hartmann and Lascelles 1966) but considerably lower than that in sheep (Heath, Lascelles, and Morris 1962). The white cell content of the lymph varied between 8000 and 26,000 cells/mm^3; the majority of these cells (98%) were medium lymphocytes. The remainder comprised mainly large lymphocytes and a few blast cells. Occasional neutrophils, eosinophils, and monocytes were also seen. The output of white cells per kilogram body weight is considerably higher in the calf than in the cow. However, the distribution of cell types is similar, although large lymphocytes were more common in the calves.

IV. Discussion

Care was taken at operation to prevent lymph from the cervical and brachial ducts passing through the cannula and it was considered that the lymph collected in these experiments was purely thoracic duct lymph. The tendency for clots to form in the shunt was not marked, probably because the lymph flow in the calves was high and the lymph contained very few red blood cells. It was possible to maintain an unobstructed flow of lymph through the shunt for periods of 9–17 days. In these circumstances, anastomoses between the thoracic duct and either the vena azygos or right lymphatic duct, which have been described in a number of species, would not be likely to function to any significant extent (cf. Hartmann and Lascelles 1966). At the end of the period of unobstructed flow the venous cannula gradually became blocked and lymph flow decreased progressively over a period of 24–48 hr. When the shunt was disconnected at this time it was noticed that the lymph flow was constant, in contrast to the intermittent flow observed earlier. It was also noticed that the red cell content of the lymph had increased markedly. The calves showed little signs of discomfort following blockage, suggesting that direct communications between the lymph and blood started to function as soon as pressure in the thoracic duct began to increase.

The recirculation of the lymph and the collection of samples for only short periods of time was considered necessary to prevent drastic loss of protein and other solutes which would have occurred had all the lymph been collected. The calves recovered quickly from the effects of the operation and appeared clinically normal throughout the experiments. Indeed all calves gained weight in excess of 0·45 kg/day over the period that lymph flowed. We believe that this technique of sampling has given a reasonable estimate of the flow and output of lipids in lymph.

It was calculated that at least 8–14 litres of lymph and 100–160 g of chylomicron triglyceride passed through the thoracic duct in the young calf in a 24-hr period.

Output of lymph and output of chylomicron triglyceride per kilogram body weight in the calf were approximately three and six times higher respectively than in the non-lactating cow (Hartmann and Lascelles 1966). Chanana and Cronkite (1966) reported rates of lymph flow 500–3000 ml/hr in the thoracic duct of calves and young cattle in which a recirculating technique was used. The ages of these animals were not given but it would appear that the lymph flow in their youngest animals was of a similar order to that in our calves.

It was estimated that the neutral lipid transported in the thoracic duct lymph each day represented, on average, 75% of the longer-chain fatty acid ($>C_{12}$) in the milk-fat fed to the calves. The estimated output in the three experiments carried out on one of the calves in which it was suspected that some of the thoracic duct lymph may not have passed through the cannula was notably lower than that of the other two calves (Table 3). The high level of phospholipid in lymph, relative to that in plasma, indicated that substantial amounts of this lipid were added during fat absorption to that derived from the capillary filtrate. This additional source of lipid was estimated to contribute of the order of 10 g of fatty acid per 24 hr, but was not included in the estimates of the output of long-chain fatty acid in the lymph (Table 2). On the other hand, some of the fatty acid in the lymph was probably derived from the bile. In this connection, Adams and Heath (1963) reported that about 10–15 g of phospholipid entered the gut of the sheep by way of the bile and that the phospholipid content of the bile of cattle was similar to that of sheep. For the above reasons it was difficult to determine precisely the recovery in the lymph of the long-chain fatty acid ingested. The results indicate, however, that the recovery was high at least in two of the calves.

The results presented in this paper have demonstrated that lipid is absorbed over a long period in the milk-fed calf. It is probable that the prolonged absorption is associated with the formation of a firm casein curd (with entrapped milk fat) in the abomasum and its slow release into the small intestine. The finding that significant absorption of lipid was occurring 24 hr after feeding (Fig. 1) suggests that some of the casein curd would still be present in the abomasum at this time. It is noteworthy that there was an apparent decrease in the output of neutral lipid in the lymph 2–5 hr after feeding (Fig. 1) which may have been due to the incorporation of the remaining clots from the previous meal into the newly formed clot as freshly drunk milk entered the abomasum (Ash 1964). Indeed, Smith (1962) reported that substantial amounts of material do not begin to leave the abomasum until 30–60 min after feeding. It has been suggested also that the high fat content of milk in the diet of the calf may slow gastric emptying through the enterogastrone mechanism (Hill 1961). However, this hypothesis is not supported by the results obtained in calves by Ash (1964), who found no appreciable differences in either the pattern or cumulative rates of flow from the abomasum when the calves were fed skim or whole milk.

When calves were fed once daily a definite pattern of lipid absorption was evident (Fig. 1). The pattern obtained was different to that described by Simmonds (1955) for rats fed full-cream milk powder, and by Heath and Morris (1962) for lambs fed diluted cow's milk and olive oil. The peak absorption of lipid in the rats and

lambs occurred 2–4 hr after feeding, whereas in the calves used in the present study, the peak occurred 8–10 hr after feeding (Fig. 1).

In relating the pattern of absorption in monogastric animals and calves, it is pertinent that both milk clotting and proteolytic activity of the gastric juice in the monogastric is attributable to pepsin (Holter and Andersen 1934). This is not so in the young calf in which there is a very high ratio of milk clotting to proteolytic activity due to the action of rennin in the abomasum (Hill 1961). It would be expected, therefore, that the release of cow's milk from the abomasum in the calf would be slower than its release from the stomach of the monogastric animal.

While the differences in the pattern of absorption of fat observed in lambs (Heath and Morris 1962) and in our calves may have been due to a species difference, it is considered that valid comparisons cannot be made because of the marked dissimilarities in the volume and composition of the respective diets, and in the experimental procedures adopted. Thus the lambs were fed only 250 ml of diluted cow's milk together with olive oil and all the lymph (as in the rats) was collected from a lymphatic fistula, whereas a recirculating technique was used in the calves. Furthermore, the feeding regime of the lambs prior to the experimental period, of which no details were given, could have influenced the results.

The infrequent feeding of large amounts of milk to calves has been considered undesirable since it was thought to lead to the accumulation of large amounts of cheese-like clot in the abomasum with consequent digestive disturbances (Sheehy 1934). However, Mylrea (1966) reported that there was no evidence of digestive disturbance when young calves were fed up to 5 litres of milk in a single feed. Our results confirm those of Mylrea (1966) and, in addition, we have shown that the absorption of fat, which comprises over 50% of the total energy in the milk diet, occurs in a reasonably continuous manner over a 24-hr period. It is also likely that the absorption of casein, the major protein of the abomasal curd, follows a similar pattern.

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