COMPOSITION AND OUTPUT OF LIPID IN THE THORACIC DUCT LYMPH OF THE NEWBORN CALF

By A. D. SHANNON* and A. K. LASCELLES*

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Summary

The composition and output of lipid in thoracic duct lymph has been studied in unanaesthetized calves during the first 6 days after birth. Recirculating lymphatico–venous shunts were established in a total of six calves within 3 hr of birth. The calves recovered quickly from the operation and were able to suck strongly within 1–2 hr of its completion. The concentration of lipid in lymph collected from calves before the first feeding was very high, ranging from 164 to 694 mg/100 ml. Triglyceride comprised 54% of this lipid with free fatty acid, phospholipid, free cholesterol, and esterified cholesterol comprising, respectively, 6·4, 25·6, 2·5, and 2·5%. Despite the very high output of triglyceride in lymph at this time (mean value 0·86 g/hr), there was no visual evidence of chylomicrons.

Although lymph flow in three calves increased from 380 ml/hr before, to peak values of 850 ml/hr 8 hr after first feeding, the outputs of total esterified fatty acid and phospholipid showed only gradual increases to reach maximum stable values at 36–48 hr after the first feeding. It is suggested that the delay in lipid absorption in newborn calves reflects a slow release of the casein curd from the abomasum.

I. INTRODUCTION

In a previously reported investigation, the pattern of lipid absorption in calves was studied by the periodic collection of thoracic duct lymph from lymphatico–venous shunts (Shannon and Lascelles 1967). These studies demonstrated that lipid absorption in milk-fed calves, 5–21 days old, was a relatively continuous process even when the animals were fed once daily. In order to complete our previous studies of lipid absorption in milk-fed calves, the present work was undertaken to determine the lipid composition of thoracic duct lymph before and following the first feeding, and also the pattern of absorption of lipid in calves from 0 to 6 days of age.

II. MATERIALS AND METHODS

(*a*) *Animals*

Lymphatico–venous shunts were established in six crossbred Friesian calves (one female and five males). The average weight of the calves at birth was 34·5 kg. The calves were taken from their dams within 5 min of birth and kept indoors on straw bedding.

(*b*) *Surgical Techniques*

The calves were anaesthetized in a similar manner to that described by Shannon and Lascelles (1967). Recovery from anaesthesia was rapid with the animals standing steadily within 6–12 hr after the operation.

* Dairy Research Foundation, University of Sydney, University Farms, Camden, N.S.W. 2570.

Cannulation of the thoracic duct and recirculation of lymph into the jugular vein were carried out as previously described by Shannon and Lascelles (1967). The lymphatico–venous shunts in calves 2, 3, and 4 functioned satisfactorily for periods of 14–21 days. Difficulty was encountered in the cannulation of calves 5 and 6. In calf 5, only one sample had been collected (before feeding) when the lymphatico–venous shunt suddenly failed. In calf 6, it was considered that not all of the thoracic duct lymph was being collected since the flow and output of lipid in this calf was considerably lower than that in the first four calves. The results presented for calves 5 and 6 relate only to the lipid composition of lymph.

(c) *Management and Feeding*

On completion of the operation the calves were placed on straw bedding in a 6 by 6 ft indoor pen. A recovery period of 1–2 hr was allowed after the operation before the calves were fed. The approximate volume of lymph taken at each collection was replaced in calf 1 with sterile 0·9% saline. This calf died suddenly 18 hr after the first feeding. An acute mineral imbalance was suspected and in the remaining calves a sterile, balanced electrolyte solution (McSherry and Grinyer 1954) was used to replace the fluid loss incurred by the collection of lymph. Each calf was given 500 mg chloromycetin intramuscularly each day for 3 days after the operation.

The calves were not fed after the operation until a strong suckling reflex had returned. Each calf was fed 1·14 litres (0·25 gal) of colostrum at the first feeding and a similar amount of colostrum at 5, 14, and 20 hr after the first feeding. The calves were fed utilizing a calf nipple and the calf's natural suckling reflex until they were strong enough to stand and drink from a bucket. Subsequently, the calves were fed 2·27 litres (0·5 gal) of whole milk at 12-hr intervals beginning at 26–30 hr after the first feeding. The colostrum and milk fed to each calf in the first 48 hr of life were obtained from the calf's own dam.

The collection and subsequent treatment of samples was similar to that described by Shannon and Lascelles (1967).

(d) *Analytical Techniques*

Methods used in the determination of total esterified fatty acid (T.E.F.A.), phospholipid, and free fatty acid (F.F.A.) have been described previously (Shannon and Lascelles 1967). The extraction of lipid in lymph and the determination of triglyceride and esterified and free cholesterol by thin-layer chromatography were carried out according to the methods of Hartmann and Lascelles (1965). Samples of the colostrum and 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 90 and 145 ml/hr. The lymph at this time was a clear yellow colour after centrifugation, with no evidence of chylomicrons on dark-ground examination under the microscope. The output of T.E.F.A. during anaesthesia varied between 0·10 and 0·45 g/hr. After an average recovery period of 90 min, the flow had risen to a mean value of 380 ml/hr and the output of T.E.F.A. to a mean value of 0·91 g/hr.

A sharp increase in lymph flow occurred following the first feeding and this was accompanied by a much slower rise in output of T.E.F.A. The lymph did not become milky in appearance until 12–24 hr after the first feeding, although a transitory opalescence was evident 1 hr after first feeding.

(a) *Lymph Flow and Lipid Output*

The results presented in Figure 1 were derived from lymph samples collected from calves 2, 3, and 4 from the time the thoracic duct was cannulated during anaesthesia, until 28 hr after the first feeding. It may be seen that while lymph flow

increased rapidly to reach a peak 4–8 hr after feeding, the output of T.E.F.A. and phospholipid showed only a gradual increase over the 28-hr period. There was, however, a transitory rise 1 hr after first feeding. Similar trends were also evident in calf 1 up until the time of its sudden death.

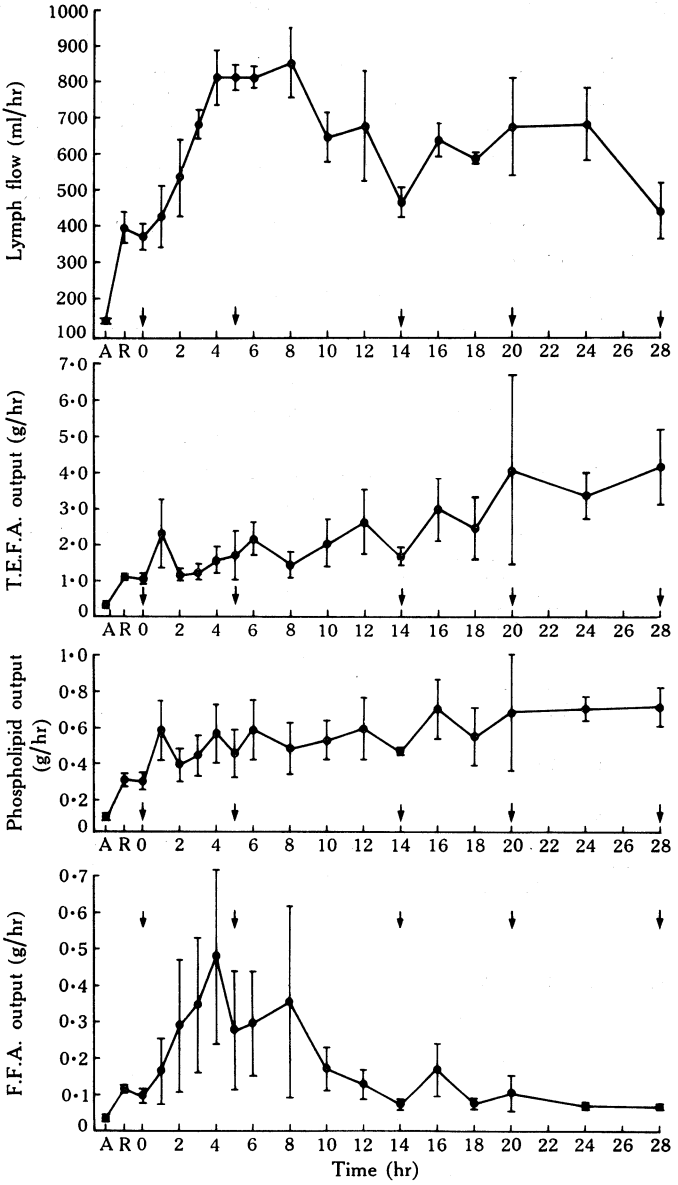

Fig. 1.—Lymph flow and output of total esterified fatty acid, phospholipid, and free fatty acid in thoracic duct lymph of calves 2, 3, and 4 immediately before, and during the 0–28-hr period after, the first feeding. The values plotted are means ± standard errors for the results of three calves. The arrows indicate the times at which the calves were fed.

A, During anaesthesia. *R*, During the recovery period.

Although there was considerable between-calf variation, the concentration and output of F.F.A. was strikingly higher 2–8 hr after the first feeding than at birth. The F.F.A. concentration in lymph fell rapidly after 8–10 hr and relatively constant values were attained 14–28 hr after first feeding (Fig. 1).

A comparison of the flow of lymph and output of T.E.F.A. over the 0–12-hr and 12–24-hr periods after the first feeding, with values obtained over a 12-hr period when the calves were 6 days of age, is shown in Table 1. The results were derived from samples collected from calves 2, 3, and 4. It may be seen that lymph flow was very high over the first 24 hr after the first feeding compared with the flow at 6 days of age. On the other hand, the output of T.E.F.A. was very low in the 0–12-hr period after the first feeding. Although there was a substantial increase in the output of T.E.F.A. in the 12–24-hr period, the average hourly output was still only 55% of the corresponding output at 6 days of age. The output of T.E.F.A. continued to rise gradually, reaching a stable value 36–48 hr after the first feeding. The values obtained at this time were comparable to those at 6 days of age.

TABLE 1

LYMPH FLOW AND OUTPUT OF TOTAL ESTERIFIED FATTY ACIDS IN THORACIC DUCT LYMPH OF CALVES 2, 3, AND 4

Collections were made in the 0–12- and 12–24-hr periods after first feeding and again in a 12-hr period when the calves were 6 days old. Thirteen samples were collected in each period. Values are means ± standard errors

Period of Collection*	Lymph Flow (ml/hr)	T.E.F.A. Output (g/hr)
Newborn calves		
0–12 hr	660 ± 47	1·742 ± 0·171
12–24 hr	618 ± 38	2·827 ± 0·472
6-day-old calves		
0–12 hr	402 ± 20	5·189 ± 0·380

* After feeding.

It is of interest to note that calves 2, 3, and 4 ingested an average of 206 g of lipid containing an estimated 160 g of longer-chain fatty acid ($>C_{12}$) in the 24-hr period following the first feeding (cf. Shannon and Lascelles 1967). In a comparable 24-hr period when the calves were 6 days of age, they ingested an average of 195 g of lipid containing an estimated 155 g of longer-chain fatty acid.

(*b*) *Lipid Composition of Lymph*

The lipid composition of thoracic duct lymph before first feeding, at 8 hr after the first feeding when lymph flow and protein output were maximal (Shannon and Lascelles 1968), and again at 24–32 hr after the first feeding, when flow was declining and lipid absorption was approaching that observed in older calves, is presented in Table 2. The results for lipid composition of lymph collected

TABLE 2

LIPID COMPOSITION OF THORACIC DUCT LYMPH COLLECTED FROM THE CALVES AT VARIOUS TIMES

Values (expressed as g/100 ml) are means ± standard errors, with the number of samples (number of calves sampled) shown in parentheses

Time of Collection of Lymph Samples	Total Lipid	Triglyceride	Phospholipid	Free Fatty Acid	Esterified Cholesterol	Free Cholesterol
Before first feeding (0 hr) (6)	0·359 ± 0·078	0·194 ± 0·054	0·092 ± 0·017	0·023 ± 0·003	0·009 ± 0·001	0·009 ± 0·002
6–8 hr after first feeding (5)	0·215 ± 0·044	0·088 ± 0·021	0·067 ± 0·011	0·026 ± 0·010	0·007 ± 0·001	0·005 ± 0·001
24–32 hr after first feeding (4)	1·063 ± 0·177	0·794 ± 0·143	0·150 ± 0·014	0·018 ± 0·002	0·014 ± 0·002	0·008 ± 0·001

from calves 5 and 6 were in agreement with those obtained in the first four calves and have thus been included in Table 2. The concentration of lipid in lymph before first feeding was much higher than in plasma of newborn calves (Shannon and Lascelles 1966). The lymph:plasma ratio for triglyceride at birth was estimated to be 29:1 whereas the comparable ratios for phospholipid, F.F.A., esterified cholesterol, and free cholesterol were 1·8:1, 1·1:1, 0·2:1, and 1·1:1 respectively.

Although the lipid output in lymph had increased by 8 hr after first feeding, there was actually a fall in the concentration of lipid at this time (Table 2). It is suggested that the lipid in the lymph was diluted by the water absorbed in association with protein; protein absorption reached peak levels 8 hr after first feeding (Shannon and Lascelles 1968).

The concentration of the various lipid fractions had undergone major changes by 24–32 hr after the first feeding (Table 2). While the concentration of total lipid in the lymph at this time was still substantially less than that found in calves at 7–14 days of age, the relative composition of the lymph was approaching that seen in the older calves. This is further illustrated in the following tabulation in which the various lipid fractions have been expressed as a percentage of the total lipid concentration:

Time	No. of Calves	Triglyceride (%)	Phospholipid (%)	F.F.A. (%)	C.E.* (%)	C † (%)
Before feeding	6	54·0	25·6	6·4	2·5	2·5
8 hr after feeding	5	40·9	31·2	12·1	3·3	2·3
24–32 hr after feeding	4	74·7	14·1	1·7	1·3	0·7
7–14 days of age	3	82·2	11·0	0·8	1·7	0·7

* Esterified cholesterol. † Free cholesterol.

For comparison, the mean values for calves 7–14 days of age (Shannon and Lascelles 1967) have been included in the above tabulation. It may be seen that marked changes in the percentage composition of the lipid had occurred by 24–32 hr after first feeding.

IV. Discussion

The calves recovered rapidly from the anaesthetic and, by recirculating the lymph and collecting samples for only short periods of time, it was possible to maintain the calves in a healthy state throughout the entire period of the experiment.

A most striking feature of the results was the high concentration of lipid in lymph collected before feeding. Approximately 54% of this lipid was triglyceride. Since chylomicrons were not detected in the lymph in significant numbers, it is reasonable to assume that the considerable quantity of triglyceride present was being carried in a smaller lipoprotein complex. The concentration of lipid in blood plasma of calves at this age is very low (Shannon and Lascelles 1966), and it is clear that most of the lipid in the lymph must have been derived from sources other than the capillary filtrate.

It would appear that the lipid intake of preparturient calves must be very low because analysis of samples of amniotic fluid collected during parturition revealed the presence of only very small quantities of lipid. With regard to this low intake of lipid, the situation in the newborn calves might be compared with that in 7–14-day-old

calves fed skim milk (Shannon and Lascelles 1969). The lipid intake of these calves did not exceed 8 g in a 24-hr period. It is interesting to note, therefore, that although the concentration and output of triglyceride in lymph of skim-milk-fed calves was approximately 50% of the comparable values in newborn calves, significant numbers of chylomicrons were present in the lymph of the skim-milk-fed animals. It was considered that the fatty acids in the bile phospholipids were probably making a substantial contribution to the lymph triglyceride in the calves fed skim milk. It is apparent, however, that the composition and physical form of the lipid in the lymph of newborn calves is not comparable with that in the older calves fed skim milk. Thus it is suggested that stored lipid in the intestines (or other regions drained by the thoracic duct or both) was being released into the lymph of the newborn calves. Presumably this lipid was formed prior to birth and may represent a readily mobilized fat store in the young animal. The high F.F.A. levels in lymph during the 0–10-hr period (Fig. 1) may have resulted from the extensive lipolysis of the stored triglyceride, and the release of F.F.A. into the lymph.

Another particularly interesting feature of the data was the delay in lipid absorption during the 24-hr period after first feeding, despite the fact that comparable amounts of longer-chain fatty acid were ingested during this period and at 6 days of age. That the intestinal mucosa was capable of absorbing lipid during this period was indicated by the transitory appearance of chylomicrons and by the increase in concentration of T.E.F.A. and phospholipid in lymph during the first hour after first feeding. It was apparent that some of the milk fed to the calf at this time had escaped clotting in the abomasum and promptly passed into the small intestine. It would appear that the major factor involved in the delayed lipid absorption was the slow release of the casein curd, in which the fat was entrapped, from the abomasum. In this connection, it has been suggested by Hill (1956) that maximum proteolysis in the lamb may not occur until some 36 hr after birth. Hill further suggested that this delay in the onset of maximum proteolysis, which corresponded with the period when the whey proteins in the colostrum were being absorbed, may represent a mechanism whereby the colostral antibodies are protected from digestion as they pass through the abomasum. Certainly, in the calf the period of delayed lipid absorption corresponds with the period of maximum absorption of immunoglobulin (Shannon and Lascelles 1968).

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