FACTORS AFFECTING LIPID OUTPUT AND FLOW OF THORACIC DUCT LYMPH IN NEWBORN CALVES

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Abstract

The factors affecting lipid absorption in unanaesthetized calves less than 24 hr old have been studied by comparing the output of lipid in the thoracic duct lymph of calves fed one of the following diets: (1) colostrum, in which the colostral fat was replaced with milk fat globules; (2) milk; (3) colostral whey containing milk fat globules; (4) isotonic saline containing milk fat globules. The animals were fed after they had recovered sufficiently from the operation to stand and suck from a nipple feeder.

In calves fed diets 1 and 2 the maximum output of lipid in lymph occurred 12 and 6 hr after feeding respectively. In contrast, lipid absorption in calves fed the casein-free diets 3 and 4 was rapid, the maximum output of lipid in lymph occurring at 2–3 hr. It is apparent from these results that newborn calves are capable of absorbing large quantities of lipid and that the delayed absorption associated with colostrum and milk feeding was a function of casein concentration.

The lipid in all diets was absorbed with equal efficiency. It was evident from a comparison of the rate and efficiency of lipid absorption in calves fed diets 3 and 4 that factors in colostrum known to be associated with enhanced protein absorption do not significantly influence lipid absorption.

Immediately prior to feeding, lymph flow had reached comparatively stable values of $536 \pm 5 \cdot 4$ ml/hr (mean $\pm$ S.E.) for 13 calves. Flow increased almost twofold within the first 2–3 hr after feeding colostrum and remained elevated for a further 3–4 hr. This occurred in the absence of significant lipid absorption but it was associated with an increase of up to threefold in globulin concentration of lymph. In contrast there was little change in flow over the first 4 hr after feeding milk, although lymph flow increased up to 1·5-fold at 5–6 hr, corresponding with the peak of lipid absorption.

I. Introduction

In a previous paper from this laboratory (Shannon and Lascelles 1969), attention was drawn to the extremely slow increase in lipid output in thoracic duct lymph of newborn calves fed colostrum. It was considered that this was probably associated with the formation of a very firm casein curd from which the entrapped fat was only slowly released into the duodenum. However, recent studies in lambs (Smeaton 1969) have shown that during the first 2 or 3 days of life there is a rapid replacement of the highly vacuolated, immature mucosa, capable of massive pinocytosis of dietary macromolecules, with mature non-vacuolated cells (see also Clark and Hardy 1970). The question arising from these observations is whether the delay in absorption of lipid in lymph in the colostrum-fed newborn calf is associated with a relative incapacity on the part of the immature vacuolated cell to absorb lipid.

The primary aim of the present study, therefore, has been to find out whether the newborn calf can efficiently absorb large quantities of lipid and to determine the

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influence of the casein curd on lipid absorption. This has been carried out by measuring lipid output in lymph of newborn calves fed colostrum, milk, and milk fat globules in casein-free media. The opportunity has been taken to examine the effect of colostral factors (Balfour and Comline 1962; Hardy 1969; Yamazaki and Moriya 1969) on lymph flow and lipid absorption in newborn calves.

II. Materials and Methods

(*a*) *Animals*

Newborn calves were taken from their dams immediately after birth and before suckling had occurred. Surgery commenced within 2–5 hr of birth and was usually completed within 2 hr.

(*b*) *Surgery and Post-operative Care*

Anaesthesia was induced and maintained using halothane (Fluothane, I.C.I., Melbourne) and oxygen. Lymphatico-venous shunts were established between the thoracic duct and the left common jugular vein by way of the communicating branch of the cephalic vein, according to the method described by Shannon and Lascelles (1967).

In three calves the intestinal lymph duct was cannulated as described for sheep (Lascelles and Morris 1961) and modified for calves by Shannon and Lascelles (1968). In these calves plastic tubes (1·2 mm int. diam., 2·0 mm ext. diam.) were also fixed in the lumen of the duodenum immediately distal to the entrance of the bile duct.

Recovery from anaesthesia was rapid; the animals stood and could suck from a nipple feeder within 2 hr of the operation. Daily injections of penicillin and streptomycin were given intramuscularly for 3 days following surgery.

The collection and subsequent treatment of lymph samples was similar to that described by Shannon and Lascelles (1967). The calves were allowed 3–4 hr to recover from the operation before feeding, by which time lymph flow had increased to relatively stable levels.

(*c*) *Analytical Techniques*

Total esterified fatty acid in lymph was determined by the method of Stern and Shapiro (1953). Total protein and albumin were estimated in lymph samples by the procedure described by Gornall, Bardawill, and David (1949) as modified by Shannon and Lascelles (1968). Total fat content of the diets was measured by the Babcock method (Davis and MacDonald 1953).

Casein determinations were carried out in the following manner. Casein micelles were harvested from a fixed volume of colostrum or milk by centrifugation (50,000 *g*) in a Beckman L2 ultracentrifuge. To ensure complete separation of casein micelles a small amount of saturated $CaCl_2$ (von Hippel and Waugh 1955) was added prior to centrifugation. Whey and fat were removed from the tubes and the casein micelles were resuspended in distilled water and recentrifuged as above. After removal of the supernatant the casein pellet was dissolved in 0·1M NaOH and brought to the volume of the original sample. Protein concentration was estimated by the method of Gornall, Bardawill, and David (1949).

Globulin concentration in the diets (total immunoglobulin) was determined by radial immunodiffusion of the four immunoglobulins IgG_1, IgG_2, IgM, and IgA as described by Brandon, Watson, and Lascelles (1971).

(*d*) *Preparation of Experimental Diets*

Calves were fed one of the following diets: (1) colostrum in which the colostral fat was replaced with milk fat globules; (2) milk; (3) colostral whey containing milk fat globules; (4) isotonic saline containing milk fat globules. Colostral whey was prepared by rennet precipitation of casein after prior removal of fat by centrifugation. Fat globules were harvested from milk by centrifugation, washed once in phosphate-buffered saline (pH 7·2), and added with thorough mixing to colostrum (subjected to prior centrifugation to remove its fat), colostral whey, or isotonic saline. Diets 1, 2, 3, and 4 are hereafter referred to as colostrum, milk, fatty whey,

and fatty saline respectively. Each calf fitted with a thoracic duct cannula was fed 1 litre of one of the experimental diets, the composition of which is presented in the following tabulation, as means (g/100 ml diet) ± standard errors:

Diet	No. of Animals	Milk Fat	Casein	Globulin (total immunoglobulin)
1 (colostrum)	3	3·90±0·50*	6·65±0·20	6·87±1·62
2 (milk)	3	3·43±0·33	2·91±0·28	0·08±0·02
3 (fatty whey)	4	3·35±0·66	0	8·34±1·73
4 (fatty saline)	3	2·93±0·53	0	0

* Mean value for two calves only; one sample could not be analysed due to inadvertent freezing before assessment of percentage fat.

III. Results

(a) *Lipid Absorption in Newborn Calves Fed Colostrum, Milk, and Fatty Whey*

Three calves were fed colostrum, three milk, and four fatty whey and the output of total esterified fatty acids (T.E.F.A.) in thoracic duct lymph was measured. The

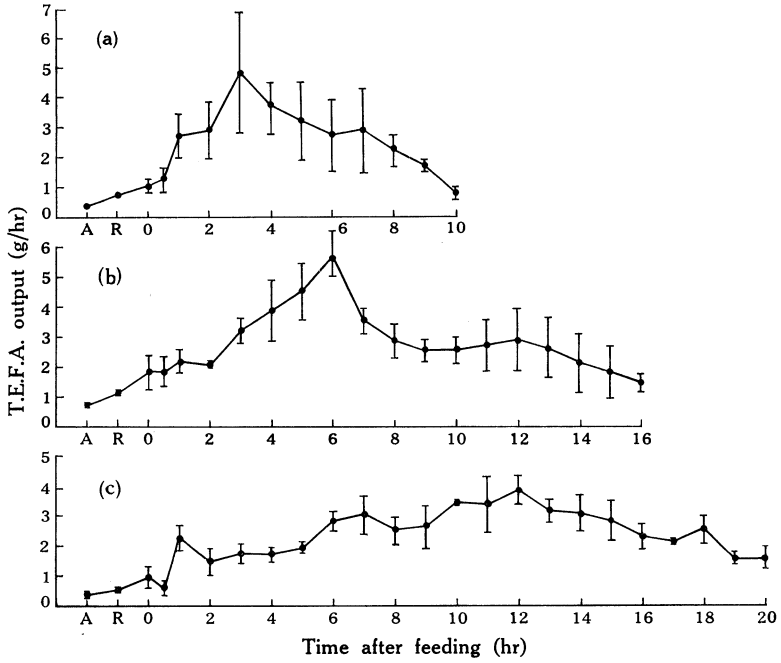

Fig. 1.—Output of total esterified fatty acid in thoracic duct lymph of 10 newborn calves following surgery and during the 0–20-hr period after first feeding: (*a*) four calves fed fatty whey; (*b*) three calves fed milk; (*c*) three calves fed colostrum. Values plotted are means ± standard errors. *A*, during anaesthesia. *R*, during recovery period.

change in output of T.E.F.A. with time after feeding for each treatment is illustrated in Figure 1. The peak output of T.E.F.A. in lymph of calves fed fatty whey was reached 2–3 hr after feeding and values decreased to pre-feeding levels by 10 hr.

In milk-fed calves peak values were observed at 5–6 hr after feeding and lipid output decreased slowly to basal levels 13–15 hr later. In contrast the absorption curve for each calf fed colostrum was flat in form, the increase in output of T.E.F.A. in lymph being extremely slow and reaching a maximum around 12 hr after feeding.

In the three additional calves fitted with cannulae in the intestinal lymphatic duct and fed by intraduodenal infusion, recovery from the effects of surgery was considerably delayed and peak lymph flow was only about 25% of that in calves with a cannulated thoracic duct. Nevertheless, it is worth reporting that the pattern of lipid absorption following intraduodenal infusion of 300 ml of colostrum or milk was similar to that observed in calves fed fatty whey (Fig. 1).

(*b*) *Lipid Absorption of Newborn Calves fed Fatty Saline*

Attention has been drawn to factors in colostrum which accelerate the absorption of protein by newborn calves (Balfour and Comline 1962; Hardy 1968, 1969). It was of interest, therefore, to determine whether colostral factors increase the absorption of fat. This was carried out by comparing the output of lipid in lymph of calves fed fatty saline with those fed fatty whey (see Figs. 1 and 2).

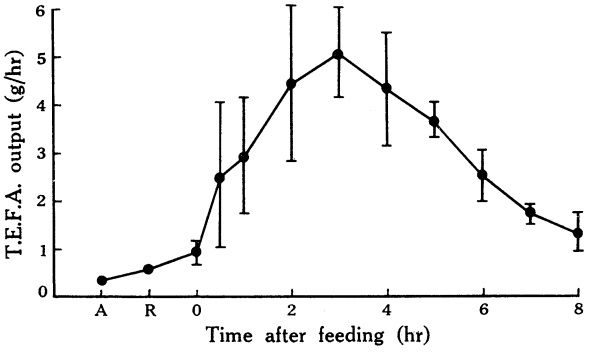

Fig. 2.—Output of total esterified fatty acid in thoracic duct lymph of three newborn calves following surgery and during the 0–8 hr period after feeding of fatty saline. The values plotted are means ± standard errors. *A*, during anaesthesia. *R*, during recovery period.

The pattern of absorption of lipid in the three calves fed fatty saline (Fig. 2) was similar to that shown for fatty whey in Figure 1. The maximum output of lipid in lymph ranged from 4·1 to 6·9 g/hr in the calves fed fatty saline and was attained 2–3 hr after feeding.

(*c*) *Efficiency of Lipid Absorption on the Various Diets*

Estimates of the quantity of long-chain fatty acid fed in the diet, the output of dietary fatty acid in lymph, and the efficiency of absorption of lipid in calves fed colostrum, milk, fatty whey, and fatty saline are presented in Table 1. The quantity of long-chain fatty acid in the diet was computed by multiplying the value of total fat, as determined by the Babcock test, by the factor 0·8 representing the proportion of long-chain fatty acid ($> 10:0$) in the triglyceride molecule (cf. Shannon and Lascelles 1967). An estimate of the dietary fatty acid in lymph was obtained by correcting the output of T.E.F.A. for fatty acids derived from non-dietary sources.

The latter value was derived from the hourly output of fatty acid in lymph immediately prior to first feeding and was considered to represent the contribution

TABLE 1

EFFICIENCY OF LIPID ABSORPTION FOR NEWBORN CALVES FED LIQUID DIETS

Values are means ± standard errors. Methods of estimating the quantity of long-chain fatty acid fed and the quantity of dietary fatty acid absorbed in lymph are described in the text. Efficiency of lipid absorption determined by expressing the estimated quantity of dietary fatty acid absorbed in lymph as a percentage of the long-chain fatty acid fed

	Colostrum	Milk	Fatty Whey	Fatty Saline
Number of calves	2	3	4	3
Long-chain fatty acids fed (g)	31·2±4·0	27·7±1·9	27·3±5·6	23·5±4·3
Dietary fatty acid absorbed (g)	24·3±1·5	21·4±0·7	19·9±5·2	19·2±5·2
Efficiency (%)	78·6±5·3	78·2±8·4	71·5±4·9	79·8±8·2

from endogenous sources. On this basis the output of endogenous lipid was 1·29±0·15 g/hr for the 13 calves under study.

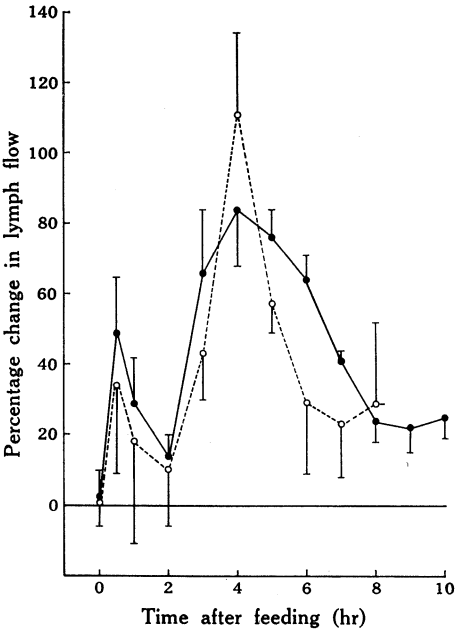

Fig. 3.—Flow of thoracic duct lymph of newborn calves during the 0–10-hr period after feeding fatty whey or fatty saline. The values plotted are means ± standard errors, expressed as a percentage change of values immediately prior to feeding. ●——● Four calves were fed fatty whey. ○– – –○ Three calves fed fatty saline.

It may be seen from the table that the efficiencies of lipid absorption were similar for the four diets; there were no significant differences. It is important to emphasize that the efficiency of absorption in calves fed fatty whey certainly was

not greater (indeed the mean value obtained was somewhat less) than for calves on the fatty saline diet. It is reasonable to conclude, therefore, that colostral factors did not increase the quantity of fat absorbed in newborn calves.

(*d*) *Lymph Flow Response to Feeding*

Immediately prior to feeding, lymph flow from the thoracic duct was 536±5·4 ml/hr (mean ± S.E.) for the 13 calves. This value is considerably higher than that reported by Shannon and Lascelles (1969) who used pentothal sodium–cyclopropane anaesthesia. Recovery of animals from Fluothane anaesthesia (used in the present experiments) certainly was rapid and it is considered that the higher flow recorded here was associated with the rapid recovery. Because of the wide variation in flow between calves prior to feeding, the changes in flow after feeding are expressed as a percentage of pre-feeding values.

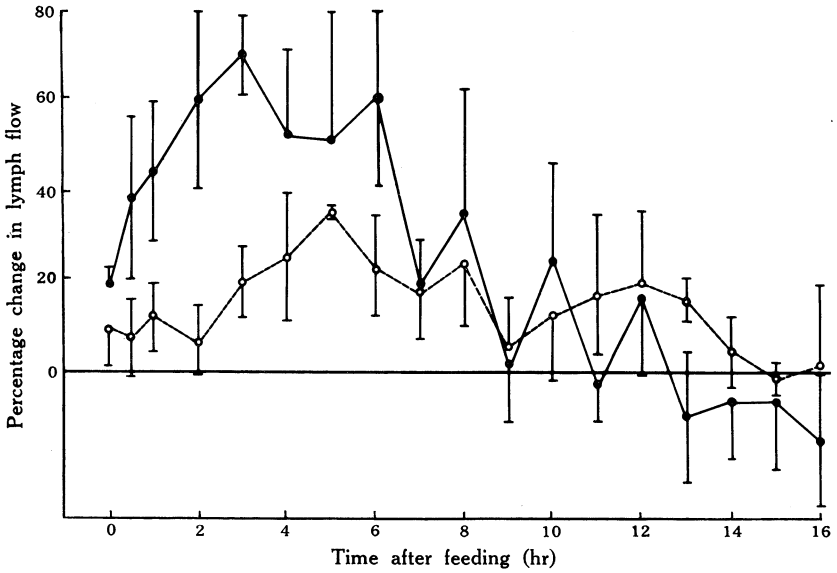

Fig. 4.—Flow of thoracic duct lymph of newborn calves during the 0–16-hr period after feeding colostrum or milk. The values plotted are means ± standard errors, expressed as a percentage change of values immediately prior to feeding. ●——● Three calves fed colostrum. O– – –O Three calves fed milk.

There was usually an increase in lymph flow during the first half-hour after feeding the liquid diets but its duration was so short that in most cases it was not evident 0·5–1 hr after feeding. This early flow response was particularly evident in calves fed fatty whey or fatty saline (Fig. 3). The change in flow for calves fed colostrum and milk is illustrated in Figure 4. It may be seen that lymph flow increased substantially after feeding colostrum, reaching a peak after 2–3 hr. Flow remained elevated for a further 3 or 4 hr and then declined rapidly to pre-feeding values. In calves fed milk there was a major increase in lymph flow after 4–6 hr but the magnitude and duration of the increase were not as great as was seen following colostrum feeding. A summary of the analysis of variance of the results for lymph flow during the period 1–3 hr after feeding calves either colostrum or milk

is shown in Table 2. It may be seen that during this period, lymph flow was significantly higher ($P < 0{\cdot}05$) in calves fed colostrum. The later-occurring flow peak following milk feeding corresponded with the peak of lipid absorption (Figs. 1 and 4).

TABLE 2

SUMMARY OF ANALYSIS OF VARIANCE OF PERCENTAGE CHANGE IN LYMPH FLOW DURING THE PERIOD 1–3 HR AFTER FEEDING CALVES COLOSTRUM OR MILK DIETS

To calculate the variance ratio for the "diets" source of variation the mean square for calves within diets has been used as the denominator

Source of Variation	Degrees of Freedom	Mean Squares (flow 1–3 hr)
Diets	1	9244*
Times	2	441
Diets × times	2	196
Calves within diets	4	644
Times × calves within diets	8	620

* $P < 0{\cdot}05$.

In calves fed fatty whey and fatty saline the major flow increase corresponded with the maximum lipid output in lymph (compare Figs. 1, 2, and 3). It is evident from Figure 3 that the increase in lymph flow was more prolonged following feeding of fatty whey than fatty saline.

TABLE 3

ALBUMIN AND GLOBULIN CONCENTRATIONS IN THORACIC DUCT LYMPH OF NEWBORN CALVES FOLLOWING FEEDING OF COLOSTRUM, FATTY WHEY, AND MILK

Values are means ± standard errors for three calves

Diet	Protein	Protein Concentration (g/100 ml) after:						
		0 hr	0·5 hr	1 hr	2 hr	3 hr	4 hr	6 hr
Colostrum	Albumin	1·76± 0·07	1·69± 0·12	1·54± 0·25	1·39± 0·18	1·39± 0·13	1·41± 0·11	1·61± 0·23
	Globulin	0·99± 0·11	0·86± 0·03	1·15± 0·17	1·90± 0·11	2·11± 0·44	2·52± 0·19	1·67± 0·32
Fatty whey	Albumin	1·01± 0·16	0·60± 0·13	0·41± 0·06	0·52± 0·11	0·43± 0·05	0·73± 0·14	0·59± 0·17
	Globulin	0·54± 0·21	0·52± 0·04	0·86± 0·07	1·26± 0·31	1·65± 0·31	1·46± 0·38	1·56± 0·14
Milk	Albumin	1·41± 0·06	1·48± 0·01	1·43± 0·06	1·42± 0·17	1·40± 0·09	1·56± 0·06	1·43± 0·14
	Globulin	0·88± 0·04	0·87± 0·04	0·80± 0·08	0·86± 0·06	0·90± 0·06	0·93± 0·07	1·01± 0·09

(*e*) *Protein Concentration in Thoracic Duct Lymph of Newborn Calves*

The concentrations of albumin and globulin in the lymph during the first 6 hr after feeding calves colostrum, milk, and fatty whey are shown in Table 3. It is

evident that the concentration of globulin and albumin in lymph did not change significantly following milk feeding. On the other hand, in calves fed colostrum and fatty whey, globulin concentration rapidly increased from 1 hr after feeding, reaching peak values approximately three times greater than pre-feeding values. The increase in globulin concentration was clearly associated with the major increase in lymph flow (see Fig. 4). There was a distinct decrease in albumin concentration following the feeding of either colostrum or fatty whey which generally corresponded with the increase in globulin concentration in lymph. It was estimated that the output of globulin in lymph increased as much as fivefold. In two of the calves fed fatty whey there was a sharp decrease in albumin concentration as early as half an hour after feeding. In these calves there was a sharp transitory increase in lymph flow at this time.

IV. Discussion

The prompt, efficient absorption of lipid in calves fed fatty whey or fatty saline demonstrates clearly that newborn calves are capable of absorbing large quantities of dietary lipid. Indeed, comparison with previous work of Shannon and Lascelles (1967) shows that the ability to absorb lipid efficiently is not appreciably greater at 1 week than at 1 day of age. It is evident that mechanisms necessary for lipid absorption are fully functional at a very early age (cf. Lascelles and Wadsworth 1971).

The extremely prolonged absorption following the feeding of colostrum compared with milk is probably due to the fact that colostrum, which contains more than twice as much casein as milk [tabulation, Section II(*c*)], forms a very firm curd (White and Davies 1958) in the abomasum. It follows that the rate of release of the lipid entrapped in the casein curd will depend upon the rate at which the curd is broken down as a result of proteolysis. Hill (1956) attributed the slow breakdown of the casein curd in newborn lambs to a deficiency in proteolytic activity in the abomasum. Although this may be a contributing factor, the relatively rapid absorption of lipid in calves fed milk, observed in the present studies, indicates that the composition of the colostrum alone is of major importance. Moreover, the similarity in the pattern of absorption in newborn and 1–2-week-old calves (Shannon and Lascelles 1967) fed milk does not support the suggestion of a major role for a proteolytic deficiency in the newborn.

It is clear that the presence of casein in the diet prolongs lipid absorption and it was expected that this would be associated with a more efficient absorption. This was not found to be the case, at least at the levels of dietary lipid fed in the present experiments (Table 1). It is considered possible, however, that the presence of the casein curd would allow a greater efficiency of absorption under conditions of very high lipid intake.

There was usually a distinct increase in the flow of lymph immediately after feeding but the increase was not sustained and 1 hr later had returned to pre-feeding levels. This flow increase was particularly evident in calves fed fatty whey or fatty saline and it is significant that in the case of calves fed fatty whey, at least, it was associated with a sharp decrease in albumin concentration (Table 3), which suggests that the albumin in the capillary filtrate had been diluted by the water absorbed from the liquid diet. It would appear that this early flow response is comparable with the

first flow peak described by Hardy (1969) in his experiments on anaesthetized newborn calves infused intraduodenally with various liquid preparations. It seems reasonable to conclude, in agreement with Hardy, that this early flow increase is associated with the absorption of water.

A more substantial and prolonged increase in flow was observed 2–6 hr after feeding. Comparison of the patterns of flow following the feeding of milk and colostrum revealed distinct differences in both the timing and the extent of the increase in flow (Fig. 4). In the milk-fed calves the flow peak was smaller and occurred 4–6 hr after feeding, which corresponded with the maximum concentration and output of lipid in lymph. There was a similar correspondence between lymph flow and lipid output in calves fed fatty saline and fatty whey. It is suggested that the flow increase was stimulated by fat absorption, as has been described for older calves (Shannon and Lascelles 1967) and other animals (Simmonds 1955; Heath and Morris 1962). However, in colostrum-fed calves the major increase in flow was not associated with significant absorption of fat but closely followed the increase in concentration of globulin in lymph (cf. Shannon and Lascelles 1968). It should be pointed out that the major flow increase following feeding of fatty whey is associated with both globulin and fat absorption.

Immunoelectrophoretic analysis of lymph samples indicated that most of the globulin was immunoglobulin (IgG_1) which is the predominant whey protein in colostrum. Hardy (1969) observed a similar pattern of flow in calves given serum albumin or egg albumin in butyrate solution and considered that the osmotic activity of the absorbed protein was responsible for the profuse flow. On the other hand, in calves given serum immunoglobulin in boiled colostral whey, Hardy observed only a transient increase in lymph flow during the absorption of immunoglobulin in lymph. This as well as other evidence presented by this worker indicated that the quantity of immunoglobulin absorbed, which is less osmotically active than the smaller-molecular-weight proteins, was insufficient to increase lymph flow substantially. In the experiments reported in the present paper, however, 5–10 times more immunoglobulin was fed; preliminary evidence based on globulin recoveries in lymph (Table 3) indicates that there was a more efficient absorption of the dose administered in these unanaesthetized calves than in the anaesthetized calves used by Hardy. In these circumstances it is suggested that the osmotic effect of the absorbed immunoglobulin would have been substantial, and accordingly would have contributed significantly to the flow increase observed.

Nevertheless, Hardy's findings do suggest that factors other than the osmotic effects of the absorbed immunoglobulin contribute to increased lymph flow. In this connection, Yamazaki and Moriya (1969) have drawn attention to a substance referred to as colostrokininogen in the colostrum of cows. Incubation of colostrokininogen with salivary kallikrein converts it to an active kinin which is vasoactive and may also be capable of accelerating protein absorption. Thus it is conceivable that the feeding of colostrum may cause vasodilatation of the intestinal vasculature with a consequent increase in lymph flow. If vasodilatation of intestinal capillaries were solely responsible for the increases in lymph flow observed in the present experiments, it would be expected that the concentration of albumin (derived from the capillary filtrate) would have remained unaltered (cf. Shannon and Lascelles 1968). However,

there was an actual decrease in albumin concentration during the period of increased lymph flow, indicating that factors other than vasodilatation, such as the osmotic effect of absorbed immunoglobulin, were contributing to the increase in flow.

V. Acknowledgments

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