CHANGES IN THE CONCENTRATION OF LIPIDS AND SOME OTHER CONSTITUENTS IN THE BLOOD PLASMA OF CALVES FROM BIRTH TO 6 MONTHS OF AGE

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Summary

This paper describes the changes in concentration of the major lipid fractions, glucose, and protein in plasma from the jugular vein of six calves from birth to 6 months of age. Changes in haematocrit over this period are also presented.

Two- to threefold increases were observed in the plasma concentration of total fat, phospholipids, free cholesterol, and cholesterol esters within the first week after birth, with a further increase until 1 month of age. The concentration of these lipids remained high between 1 and 3 months of age but was lower in samples collected subsequently. Plasma triglycerides, glucose, and total protein increased from their lowest to their highest values over the first 2 days after birth. The haematocrit decreased markedly during the first week after birth and then increased somewhat over the next 3 months. The concentration of free fatty acids was relatively high in samples collected at birth but had halved in value by 2 days of age with a further gradual decrease over the next few months. The ratio of free : total cholesterol decreased from a value of approximately 0·27 at birth to 0·20 at 15 days of age, after which it remained constant.

The results suggested that, in the new-born calf, glucose reserves were quickly exhausted and free fatty acids were an important source of energy at this time.

I. INTRODUCTION

Although it has been reported that the concentration of lipids in the plasma of new-born calves is low relative to that in the adult (Zaletel, Allen, and Jacobson 1952; Riis 1964), there is little known about the relative concentration of triglycerides and the other lipid fractions at birth or the changes which occur over the first few months of life. During the first 10–14 days after birth, milk or a milk substitute is the sole source of nourishment of the calf and fat comprises 50% or more of the energy consumed. During the subsequent weeks an increasing amount of fodder is ingested and by 8–12 weeks a fully functional rumen has developed.

This paper describes the changes in the major lipid fractions and some non-lipid constituents in the plasma of calves over the first 6 months of life. The work is preliminary to more detailed studies on the absorption of fat and the metabolism of chylomicrons in the calf.

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II. Methods

(a) *Animals*

Observations were made on a total of 13 dairy calves of mixed breeds (Jersey, Guernsey, Friesian, and crossbreeds). The dams of these calves were in excellent condition throughout pregnancy, and parturition was not unduly prolonged. All calves were born during the winter of 1965 which was dry and mild.

Four heifer and two bull calves were used in the main experiment in which blood samples (100 ml) were collected at intervals from birth to 6 months of age to determine the variation with age in the concentration of the major lipid fractions and some non-lipid constituents. Three other heifer calves (2–4 weeks of age) were used to study the diurnal variation of a variety of constituents. The change in concentration of some constituents of plasma from birth to 2 months of age was also determined in another four calves (two heifer and two bull calves). From these calves only small samples of blood (10 ml) were collected in order to avoid the possible disturbance associated with the removal of larger volumes.

All calves were allowed to suckle their dams for 2 days after birth. Subsequently, they were fed from the bucket at the rate of 1 lb whole milk/10 lb body weight/day in two feedings which were given at 8 a.m. and 5 p.m. From 2–3 weeks of age calves were allowed access to a medium quality mixed pasture of subterranean and white clovers, ryegrass, and oats. After the calves were weaned at 3 months of age the diet was supplemented with a concentrate mixture in nut form and lucerne chaff. These calves remained in good health throughout the experimental period and gained weight at a satisfactory rate.

(b) *Collection of Samples*

Blood (100 ml) was obtained from the jugular vein by venipuncture. The collection of blood was carried out as quickly as possible and in most cases without noticeable disturbance of the calves. Samples were collected soon after birth before the calf had suckled its dam. Subsequent samples were taken at 10–10.30 a.m. at intervals up to 6 months of age. The blood was centrifuged at 7°C to remove the cells, the plasma transferred to polythene bottles and stored under nitrogen at −6°C until analysed. Heparin (Pularin Evans) was the anticoagulant used throughout.

(d) *Analytical Technique*

Biochemical analyses on plasma samples were carried out as follows: total esterified fatty acids by the method of Stern and Shapiro (1953); free fatty acids by the method of Dole (1956); phospholipids by the method of Zilversmit and Davis (1950); total protein by the method of Gornall, Bardawill, and David (1949); and glucose by the glucose oxidase method of Huggett and Nixon (1957) in which Boehringer blood sugar kits were used.

Extraction of plasma lipids was similar to that described by Hartmann and Lascelles (1965*a*) except that the extraction mixture was transferred into a stoppered 250-ml measuring cylinder instead of a separating funnel. After layering with NaCl, the lower phase was removed by suction. It was possible to remove all but 0·2–0·5% of the lower phase without contaminating it with upper phase. Using

this method the standard deviation between duplicates for total fat in plasma was lower than that found with the previous method in which separating funnels were used. Analysis of lipids by thin layer chromatography followed the method of Hartmann and Lascelles (1965*a*). All estimations were carried out in duplicate.

III. Results

Preliminary observations were made on three 2–4-week-old calves to determine the change in the composition of plasma samples collected at intervals after the morning feeding of milk. The results for the plasma free fatty acids (F.F.A.), total esterified fatty acids, phospholipids, protein, and the haematocrit indicated that there was no consistent pattern of variation throughout the day for calves at this age at least. On the other hand, the concentration of plasma glucose was found to be consistently higher in samples collected 2 hr after the morning feeding than at any other time. This is in conformity with the results of other investigators working with milk fed calves (Preston and Ndumbe 1961; Mathieu and Barré 1964). This finding was not unexpected because milk contains a high concentration of lactose which is rapidly hydrolysed within the alimentary tract and the majority of the glucose and galactose absorbed within 2–3 hr of feeding. It was considered therefore that the proximity of the time of sampling to feeding would not have unduly biased the results (except for glucose) from the six calves in the main experiment which were sampled for convenience 2 hr after the morning feeding.

Analysis of variance of the results from the six calves sampled at intervals from birth to 6 months of age are summarized in Table1. The between-times mean squares were significant for all constituents indicating that there was a significant change in their concentration over the 6-month period of sampling. The mean squares attributable to differences between calves for the various constituents were of a much lower magnitude than the respective between-times mean squares. Significant differences between calves were found for total fat, phospholipids, cholesterol esters, and total protein.

The mean values and standard errors for the concentration of the various lipid fractions (except F.F.A.) in plasma collected between birth and 6 months of age are presented in Table 2. There were marked increases in the concentrations of total fat, phospholipids, free cholesterol, and cholesterol esters within the first week after birth, with further steady increases until 1 month of age. Their concentrations remained high between 1 and 3 months but were lower in the samples collected subsequently. Plasma triglycerides increased from the lowest to the highest values over the first 2 days after birth.

The relative changes in the concentration of free cholesterol and cholesterol esters during the first 15 days after birth are noteworthy (Table 2). It was found that the concentration of cholesterol esters increased at a significantly faster rate than that of free cholesterol. Thus the ratio of free : total cholesterol decreased steadily from birth to 15 days of age (see Table 3), after which it remained relatively constant until the age of 6 months. The value of the ratio after 15 days of age was approximately 0·20 which is similar to that found in the adult cow (Garton, Duncan, and Lough 1961; Hartmann and Lascelles 1965*a*).

TABLE 1

SUMMARY OF THE ANALYSES OF VARIANCE OF THE CONCENTRATIONS OF ALL PLASMA CONSTITUENTS OF THE SIX CALVES SAMPLED AT INTERVALS BETWEEN BIRTH AND 6 MONTHS OF AGE

Source of Variation	Degrees of Freedom	Mean Squares								
		Total Fat	Phospho-lipids	Triglyc-erides	Cholesterol	Cholesterol Esters	Glucose	Free Fatty Acids	Total Protein	Haemato-crit
Calves	5	9544·0*	1950·4*	207·8	26·7	2493·1*	254·5	5·4	2·4***	98·3***
Times	10	33449·0***	7756·6***	344·3*	129·6***	9009·5***	1914·5**	130·3***	4·4***	77·6***
Calves × times	50	3889·8	796·8	141·9	32·7	889·6	611·7	23·0	0·43	14·8

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

The mean values and standard errors for the concentration of glucose, F.F.A., and total protein in the plasma collected between birth and 6 months or age, together with the haematocrit values over this period, are presented in Table 4. The

TABLE 2

MEAN VALUES AND STANDARD ERRORS FOR THE CONCENTRATIONS OF TOTAL FAT, PHOSPHOLIPIDS, TRIGLYCERIDES, FREE CHOLESTEROL, AND CHOLESTEROL ESTERS IN PLASMA COLLECTED FROM THE SIX CALVES AT INTERVALS BETWEEN BIRTH AND 6 MONTHS OF AGE

The standard errors were calculated from the results of the six calves for each time of sampling. The between-duplicate standard deviations, also presented, were calculated from duplicate determinations of each constituent in 66 plasma samples

Age (days)	Total Fat (mg/100 ml)	Phospholipids (mg/100 ml)	Triglycerides (mg/100 ml)	Cholesterol (mg/100 ml)	Cholesterol Esters (mg/100 ml)
Birth	140·0±13·8	50·9± 3·6	6·75±1·71	7·95±1·01	37·2± 4·6
2	250·7±18·7	110·7±17·8	33·45±9·80	13·30±1·91	67·5± 8·0
6	295·0±22·6	129·2± 7·6	24·95±6·56	18·17±2·65	103·3±11·8
10	348·3±29·2	149·4±12·2	29·30±7·87	19·92±2·31	131·8±12·7
15	337·7±30·6	146·8±16·1	14·70±2·08	19·87±1·40	133·8±14·6
30	392·0±31·1	168·8±18·3	13·38±1·80	23·97±3·49	161·3±17·0
45	378·0±35·0	170·2±16·7	15·87±2·19	21·17±3·18	153·7±18·6
60	359·3±40·8	156·4±15·8	21·10±5·37	20·53±2·75	139·2±19·7
3 months	401·3±25·5	179·2± 9·4	22·55±4·14	23·30±2·22	159·0±12·6
4 months	312·0±57·1	159·7±12·0	23·45±2·63	18·20±1·05	128·7±10·1
6 months	314·2±15·9	142·4± 6·2	21·53±2·59	15·48±1·88	118·3± 2·7
S.D.*	4·3	2·8	0·60	0·50	3·0

* Between-duplicate standard deviation.

concentration of F.F.A. was relatively high in samples collected immediately after birth but decreased sharply within 2 days with a further gradual decrease over the next few months. On the other hand, the concentration of plasma glucose which

TABLE 3

MEAN VALUES AND STANDARD ERRORS FOR THE RATIO OF FREE TO TOTAL CHOLESTEROL

Data calculated from samples collected from the six calves at intervals between birth and 6 months of age

Age (days)	Ratio of Free to Total Cholesterol (±S.E.)	Age (days)	Ratio of Free to Total Cholesterol (±S.E.)
Birth	0·269±0·010	15	0·204±0·013
2	0·254±0·016	30	0·204±0·011
6	0·233±0·013	45–6 months	0·195±0·010
10	0·207±0·010		

was relatively low at birth increased sharply to reach a peak at 2 days of age. This was followed by a decrease to 2 months of age, after which there was no significant trend. At 6 months the concentration was still considerably higher than that in

the adult cow. In this connection, Kennedy *et al.* (1939) have reported a high concentration of blood glucose in calves during the first week after birth with a gradual decrease to the adult level by 1 year of age. Total protein increased from the lowest to the highest values over the first 2 days after birth, whereas the haematocrit decreased markedly during the first week and then increased gradually over the next 3 months (see Table 4).

TABLE 4

MEAN VALUES AND STANDARD ERRORS FOR THE CONCENTRATIONS OF GLUCOSE, FREE FATTY ACIDS, AND TOTAL PROTEIN IN PLASMA, TOGETHER WITH HAEMATOCRIT VALUES

The blood samples were collected from the six calves at intervals between birth and 6 months of age. The standard errors were calculated from the results of the six calves for each time of sampling. The between-duplicate standard deviations, also presented, were calculated from duplicate determinations of each constituent in 66 plasma samples

Age (days)	Glucose (mg/100 ml)	Free Fatty Acids (mg/100 ml)	Total Protein (g/100 ml)	Haematocrit (%)
Birth	71·7±12·3	20·71±4·76	4·81±0·14	47·2±2·17
2	133·3±18·9	9·61±1·64	7·82±0·62	36·7±2·07
6	125·3± 6·4	8·12±1·74	7·65±0·51	36·9±2·63
10	114·5±14·3	9·06±2·39	7·58±0·41	39·8±2·48
15	117·6±8·3	5·06±0·68	6·97±0·44	40·7±2·26
30	97·0± 7·8	7·86±2·01	6·36±0·17	40·7±1·36
45	98·8±10·1	5·58±0·72	6·22±0·16	37·3±0·71
60	87·8± 4·3	5·62±1·18	6·18±0·06	37·2±1·84
3 months	106·8± 5·1	5·09±0·57	6·33±0·07	44·5±1·93
4 months	94·1± 2·6	4·32±0·78	6·80±0·01	44·7±1·44
6 months	94·4± 5·3	4·83±0·51	6·78±0·13	41·9±1·18
S.D.*	1·2	0·20	0·07	0·10

* Between-duplicate standard deviation.

The between-calves, within-calves, and between-times correlations were calculated from the respective between-calves, calves×times, and between-times variances and covariances for the various plasma constituents. There were significant positive correlations between the plasma concentrations of phospholipids and free cholesterol ($r = 0{\cdot}83$, $P<0{\cdot}001$), phospholipids and cholesterol esters ($r = 0{\cdot}90$, $P<0{\cdot}001$), and free cholesterol and cholesterol esters ($r = 0{\cdot}85$, $P<0{\cdot}001$). The between-times correlations between these above constituents were 0·94, 0·97, and 0·96 respectively ($P<0{\cdot}001$).

It was considered that the collection of large volumes of blood in the main experiment might have disturbed the calves with a consequent alteration in the concentration of the plasma constituents, particularly glucose and F.F.A. (and haematocrit). Thus further observations were made on four calves from which blood samples (10 ml only) were collected at intervals from birth to 2 months of age. These calves were particularly quiet and samples were taken quickly without noticeable disturbance. The changes in glucose, F.F.A., and haematocrit showed similar trends to those given in Table 4.

IV. Discussion

The plasma collected from the calves immediately after birth was transparent whereas some of the samples collected after milk feeding were cloudy in appearance. This cloudiness was most striking in the samples collected 2 days after birth and was found to be associated with a high concentration of triglyceride. It seems almost certain that the cloudy appearance was due to the presence of chylomicrons. The very high concentrations of triglycerides in the samples collected at 2 days (Table 2) may have been due to the higher consumption of milk fat by the calves which had been suckling their dams up to this time.

The finding that the concentration of total esterified fatty acids and phospholipids in the plasma of calves 2–4 weeks old did not change significantly with time after feeding suggests that the digestion and absorption of fat in the calf is a slow process. In fact, the results of preliminary experiments (Shannon and Lascelles, unpublished data) have indicated that the output of chylomicron triglyceride in the thoracic duct lymph of the milk-fed calf does not vary markedly with time after feeding.

The concentration of all lipid fractions (except F.F.A.) was found to be very low in the new-born calf (Table 2). In contrast with our results is has been reported that the lipoid phosphorus content in the plasma of the calf at birth is at least as high as that of the mother (Green and Macaskill 1928) and the cholesterol esters are present in negligible concentration (Shope 1928). On the other hand, Zaletel, Allen, and Jacobson (1952) reported extremely low concentrations of phospholipids and cholesterol esters (and total lipids) in the plasma of the new-born calf. Their values were much lower than those described in the present paper. The alcohol–ether, light petroleum procedure which was adopted for the extraction of lipids by these workers was probably less efficient than the chloroform–methanol procedure used by us. Furthermore, they used the acetone–magnesium chloride precipitation method in their determination of phospholipids. However, Kaplan and Lee (1965) and Crowley, Ways, and Jones (1965), who used similar extraction procedures to those described in this paper, found the concentration of phospholipids in the plasma of the new-born infant was approximately 50% and total cholesterol 30–40% of that in plasma from adults.

A most striking feature of our data was the rapid increase in the concentration of all classes of lipid (except F.F.A.) during the first 15–30 days after birth (Table 2). Although earlier studies in man suggested that the increase in plasma lipid during the first few weeks of life occurred gradually (Deuell 1955), recent studies have indicated that the increase is quite rapid. Thus Kaplan and Lee (1965) found an almost threefold rise in triglyceride levels in the plasma of infants within the first 3 days following birth. During the same period they reported an increase of approximately 40% in the concentration of phospholipids and free cholesterol which is still considerably less than that found in the present study. In the calf, it would appear that the rapid and highly correlated increases in the concentration of phospholipids, free cholesterol, and cholesterol esters, which are components of the lipoprotein complexes, reflect a marked stimulus to the fat transport mechanisms as a consequence of the high fat diet. It is noteworthy that the concentration of these lipids

decreased between 15 and 40% after weaning at 3 months of age. The more rapid changes observed in the calves compared with infants may be associated with the higher intake of fat relative to carbohydrate in the former species.

The concentration of cholesterol esters was found to increase much more sharply than that of free cholesterol during the first 15 days after birth, resulting in a decrease in the ratio of free : total cholesterol over this period (Table 3). This is in agreement with the reports by Boyd (1936) of high concentrations of free cholesterol in the plasma of new-born infants (40% of the total cholesterol), and by Hodges, Sperry, and Anderson (1943) that the ratio of free : total cholesterol in infants has attained a constant value at the age of 2 months.

At birth the concentration of plasma glucose was lower and the F.F.A. higher than at any other time during the first 6 months of life (Table 4). The low concentration of glucose suggests a rapid exhaustion of the glucose reserves. Thus it would appear that the mechanisms for the control of glucose production in the new-born calf are much less efficient than those of the adult cow (cf. Hartmann and Lascelles 1965*b*). The higher concentration of F.F.A. in the plasma at birth almost certainly would be associated with the mobilization of depot fats (cf. Gordon and Cherkes 1956). Since the utilization of F.F.A. by a number of tissues is known to be a function of plasma concentration (Gordon and Cherkes 1956; Steinberg 1964) our results suggest that F.F.A., derived from the fat depots, are an important source of energy for the new-born calf. Similar conclusions for new-born infants have been presented by Smith (1945) and Van Duyne and Havel (1959). Further studies are in progress to determine the relationship of the levels of F.F.A. in the plasma of the dam and calf at parturition; in addition the effect of starvation and exposure to cold on the levels of F.F.A. and glucose in the plasma of the new-born calf is being studied.

The finding that the haematocrit values were high in the calves at birth (Table 4) is in conformity with the results obtained in calves by Wise *et al.* (1947) and in lambs by Evans and Blunt (1961). The gradual fall in haematocrit in calves and lambs over the first few weeks of life has been ascribed to the rapid growth of the body exceeding the rate of production of red cells, giving rise to a physiological anaemia (Wise *et al.* 1947; Evans and Blunt 1961). This explanation would not fully account for the sharp decline observed in our calves during the first 2–6 days after birth. This change may have been partly due to a rapid alteration in plasma volume associated with the intake of large amounts of liquid diet, particularly during the first 2 days when the calves were suckling their dams. Indeed, it was observed in the four calves from which only small volumes of blood were taken that the haematocrit had decreased from a mean value of 43·0% to a mean value of 39·7% within 7 hr of birth (approx. 4–6 hr after the first feeding).

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