ELECTROPHORETIC AND IMMUNOLOGICAL STUDIES ON SERA FROM CALVES FROM BIRTH TO WEANING

I. ELECTROPHORETIC STUDIES

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(With 6 Figures in the Text)

CONTENTS

INTRODUCTION

The examination of the blood proteins of cattle by the 'classical' Tiselius electrophoresis apparatus has been concerned with the determination of the albumin and globulin values for normal adult serum and plasma (Deutsch & Goodloe, 1945; Hogness, Giffee & Koenig, 1946; Svensson, 1946; Bradish, Henderson & Brooksby, 1954*a*), with the changes in the different electrophoretic components resulting from natural infection or artificial immunization with bacterial and virus antigens (San Clemente & Huddleson, 1943; Wehmeyer, 1949; Janssen, 1951; Bradish, Henderson & Brooksby, 1954*b*; Bradish & Brooksby, 1954), and with the changes in the serum and plasma components of the young calf before and after suckling (Jameson, Alvarez-Tostado & Sortor, 1942; San Clemente & Huddleston, 1943; Pedersen, 1945; Hansen & Phillips, 1947; Polson, 1952).

The passive transference of globulin to the young calf following the ingestion of colostrum has been shown by electrophoretic examination. This demonstrates in broader terms a normal physiological process which had been observed earlier for individual antibodies by serological methods (Little & Orcutt, 1922; Smith & Little, 1922; Mason, Dalling & Gordon, 1930) and for the proteins associated with them by salting-out techniques (Howe, 1921).

In the present studies on the passive transference of the colostral globulin from

the mother to the calf, and on their subsequent elimination and replacement by autogenous globulin, the electrophoretic examinations have been carried out on serial samples from individual calves, and the changes in the different electrophoretic components have been traced during the early life of the calf.

The electrophoretic data have been divided into three sections: first, those derived from the sera of calves at birth and before suckling; secondly, those from the sera of colostrum-fed calves during the first 2–3 months after suckling; and thirdly, those from the sera of calves partially deprived of colostral protein during the neonatal period when passive absorption can occur. The latter group offers excellent opportunities for the study of the autogenous development of the globulin components.

MATERIALS AND METHODS

Management of calves

Shorthorn or shorthorn-cross calves were removed from their dams at birth. Apart from the special neonatal feeding arrangement for the calves in the deprived group the remainder were bucket-fed the colostrum from the dam and subsequently milk for 10–12 weeks. After the 4th week meal and hay were included in the diet. Each calving was observed and the calves remained under observation until the pre-colostral blood sample was taken. The calves remained housed during the period of the experiment.

Collection and preparation of sera

Blood samples (25–50 ml.) were collected from the jugular vein with aseptic precautions. Three to five samples were collected during the first week, and thereafter the calves were bled at weekly intervals. The samples were allowed to stand overnight at room temperature. The serum was then separated from the clot by centrifugation and stored at $-10°$ C.

Protein concentration

Protein concentrations for the different electrophoretic components were calculated from semi-micro-kjeldahl determinations on triplicate samples of serum using a protein/nitrogen ratio of 6·25; no allowance was made for non-protein nitrogen. Serum protein concentrations were determined and adjusted for the electrophoretic runs refractometrically. The sera were dialysed against six changes of phosphate buffer solution (I 0·2, pH 8·0), each change being 20 times the volume of the serum. The refraction of the buffer (n_0) and of the non-dialysable material (n_1) were measured at 25° C. with monochromatic light ($\lambda = 546$ mμ) derived from a high pressure mercury arc used in conjunction with a suitable light filter. The protein concentration was adjusted $n_1 - n_0 = 0{\cdot}00400$.

Electrophoretic technique

Routine electrophoretic experiments were made in the long cell of the Tiselius electrophoretic apparatus (Tiselius, 1937) modified to Philpot's (1938) optical system and using monochromatic light ($\lambda = 546$ mμ).

After temperature equilibration (1·5° C $\pm$ 0·75) and compensation of the boundaries, a current of 10 mA. derived from a stabilized valve rectifier was applied for

30 min., increased to 20 mA. and maintained at this level for a further 150 min.; the run was then terminated. The starting boundary was photographed, and as the different protein components separated, further photographs were taken at 60, 120 and 180 min.

Analysis of electrophoretic patterns

The electrophoretic pattern of the migrated protein components of the 180 min. photograph was projected at $\times 8$ diameters on to sheets of millimetre squared paper, and the outline traced. There are two well-established methods for the analysis of the electrophoretic pattern; both methods have been used.

Method 1. The protein boundaries of the electrophoretic pattern are separated by dropping perpendiculars to the base line from the minima of the curves (Tiselius & Kabat, 1939). This method was adopted where a continuous analysis of serial samples was required, since it can be readily applied to all electrophoretic patterns. A slight modification was incorporated which enabled the α_1 globulin to be more accurately determined. As the leading edge of the albumin component was uncomplicated by other protein boundaries, this peak was constructed geometrically (see below, Method 2) thus improving the separation of the albumin and α_1 globulin.

Method 2. The protein components of the electrophoretic pattern were geometrically resolved into a series of curves symmetrical about an axis through assumed Gaussian curves (Longsworth, 1942) and at right angles to the base-line. The analysis was commenced at the albumin peak and extended geometrically towards the salt boundary. These analyses were used for mobility measurements. The area of each electrophoretic component was determined three times planimetrically and the mean value calculated. Where the Longsworth method of analysis could be applied without difficulty the differences in results obtained by the two methods were small.

Mobility measurements

The mobilities of the various protein components were calculated from the boundary displacements on the descending side. Conductivity measurements on the buffer were made at intervals throughout each electrophoretic run. The conductivity cell was maintained in the electrophoretic tank. The ratio arms of the conductivity bridge were fixed at 2000 Ω and the cell resistance when balanced was read to the nearest ohm from the setting of a five-dial decade resistance box. Frequencies of 5, 7, and 9×10^2 cyc./sec. were matched to an amplifier with a variable gain control, and the signal detected by headphones.

Definition of terms

'Lacto-globulin' refers to the slowest electrophoretic component in colostral whey. This globulin is associated with antibody activity. The component is present in the absence of specific immunization, and for this reason the term *immune* lacto-globulin used by Smith (1946*a*, *b*) has been avoided, although the electrophoretic component referred to is identical.

'Autogenous globulin' refers to the globulin developed by the calf.

The description of the changes in the values of the different serum proteins is

based on data derived from four calves in each group. Mobility calculations, however, were frequently based on data from a larger group of calves; this is indicated by the number in parentheses before the mean values or standard deviation values.

EXPERIMENTAL RESULTS

The fractionation of colostrum

The lacto-globulin fractions fed to certain calves in the group partially deprived of colostral protein were prepared from colostrum collected on the day of calving. The casein was removed with rennin, and further protein fractions were separated by precipitation with Na_2SO_4 at concentrations of 12, 14 and 18 g./100 ml.

Several samples of whey and the Na_2SO_4 fractions prepared from them were examined electrophoretically (Fig. 1*a*, *b*). Only one of these fractions (Fig. 1*b* (*e*)) showed any evidence of a split in the lacto-globulin which could be related to the two γ globulin components usually evident in the electrophoretic pattern of adult bovine serum.

The mean mobility of the lacto-globulin after fractionation was 2·17 cm.^{2}/V./sec. $\times 10^{-5}$ at pH 8·0 (range of variation 2·28–2·11, standard deviation (6) 0·079).

The electrophoretic analysis of calf sera at birth and before suckling

The analysis of these sera has been given in detail, as the values obtained form the base-line against which subsequent changes are discussed.

The electrophoretic distribution of the various serum proteins of nine pre-colostral calf sera are summarized in Table 1.

The mean protein concentration of these sera was 4·24 g./100 ml. with a range of variation of 3·4–5·5 g./100 ml. (standard deviation (9) 0·596).

Table 1. *Summary of the electrophoretic analysis (method 1) of nine pre-colostral calf sera*

(Phosphate buffer pH 8·0, *I* 0·2.)

	Ascending (anode) limb				Descending (cathode) limb			
		Globulins				Globulins		
	Albumin	α	β	γ	Albumin	α	β	γ
Mean	64·4	25·7	8·4	1·5	62·2	29·1	7·4	1·3
Range of variation	60·6–70·8	18·8–30·1	7·0–11·0	0·7–2·0	58·4–66·4	22·9–32·6	5·8–9·3	0·4–2·2
Standard deviation	3·32	3·86	1·24	0·31	2·79	3·05	1·32	0·34
Standard deviation / Mean	0·052	0·15	0·148	0·207	0·045	0·105	0·178	0·262

		Globulins		
	Albumin	α	β	γ
Mean values ascending and descending	63·3	27·4	7·9	1·4
Range of variation	60·35–66·7	22·9–30·8	6·4–10·15	0·8–1·95
Standard deviation	2·43	2·56	1·16	0·45
Standard deviation / Mean	0·039	0·093	0·147	0·321

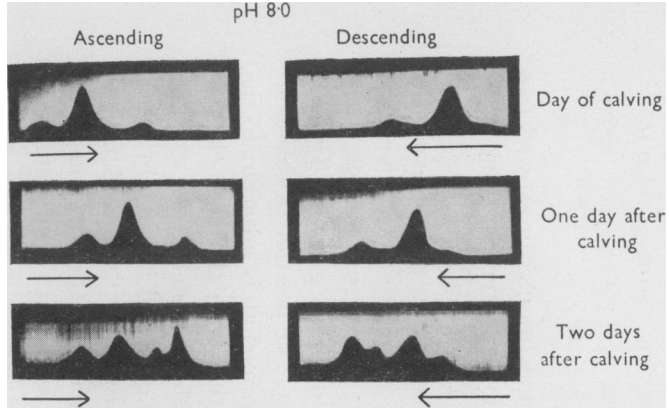

Fig. 1*a*. Electrophoretic pattern of colostral whey.

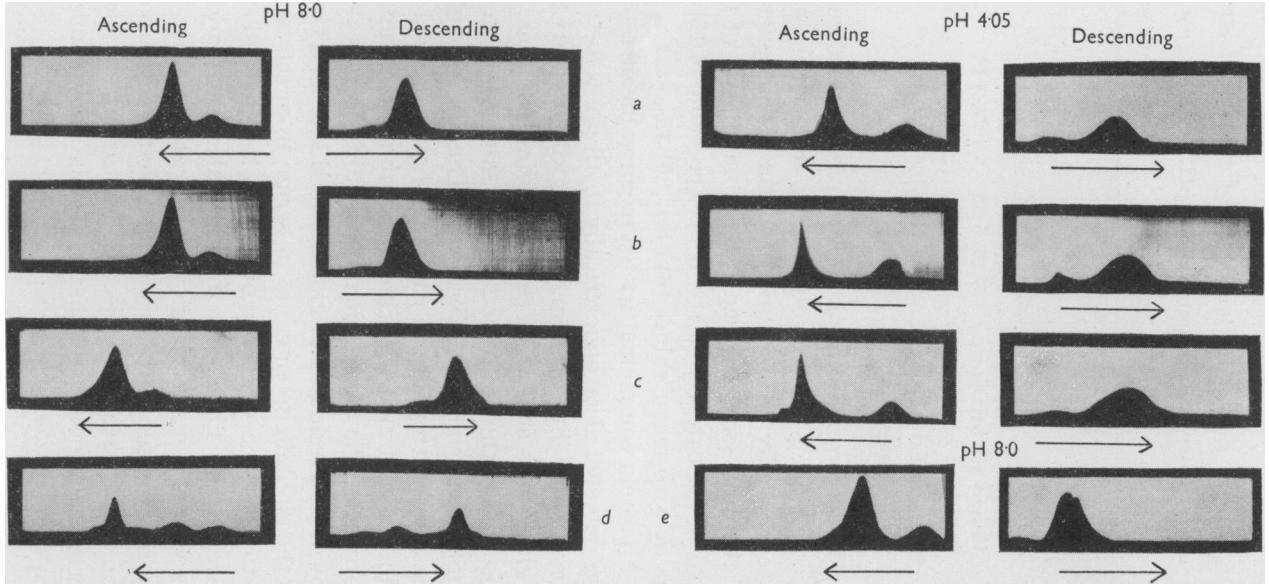

Fig. 1*b*. Electrophoretic patterns of lacto-globulin fractions of colostral whey precipitated at (**a**) 12, (**b**) 14, and (**c**) 18 g. Na_2SO_4/100 ml. whey. (**d**) Supernatant from precipitate at 18 g. Na_2SO_4/100 ml. whey. (**e**) Lacto-globulin precipitated at 18 g. Na_2SO_4/ml. whey and showing evidence of two components in the descending pattern.

The mobility data obtained from six of the above pre-colostral calf sera is shown in Table 2.

Table 2. *The mean mobility data calculated from the descending limb analysis (method 2) of six pre-colostral calf sera*

(Analysis made in phosphate buffer, pH 8·0, I 0·2, conductivity 0·005 Ω^{-1} cm.$^{-1}$ at 1·5° C.)

	Mobility (cm²/V./sec. × 10^{-5})				
		Globulins			
	Albumin	α_1	α_2	β	γ
Mean (6)	5·75	4·42	3·86	3·2	2·47
Range of variation	5·46–5·88	4·24–4·62	3·58–3·96	3·02–3·3	2·26–2·65
Standard deviation (6)	0·146	0·085	0·193	0·102	0·169
$\frac{\text{Standard deviation}}{\text{Mean}}$	0·0253	0·0192	0·0492	0·0319	0·0684

The electrophoretic pattern of pre-colostral calf serum frequently showed two α components on the descending side which were not apparent, or were indicated only by an asymmetry in the α component on the ascending side. In six of the nine sera analysed the α components of the descending pattern could be analysed separately (Method 2). The value of the α_1 was consistently higher than the α_2 with mean values (6) of α_1, 20·3 % and α_2 8·2 % total serum protein.

A protein component with a mean value of 1·4 % (shown as γ globulin, Table 1) was detected electrophoretically in all pre-colostral sera. The position of this component (mobility 2·47 cm.² /V./sec. × 10^{-5} at pH 8·0) immediately adjacent to the β globulin is similar to that of fibrinogen. This, together with the poor clotting observed in the pre-colostral blood samples, suggested that the so-called γ globulin might be unconverted fibrinogen. This component was obscured by the fibrinogen peak in electrophoretic patterns of pre-colostral citrated calf plasma. After the addition of 1 mg. of purified thrombin* to 20 ml. plasma and the removal of the fibrin clot, the elimination of the fibrinogen peak was apparent. There remained, however, a residuum of globulin in this area of the electrophoretic pattern, similar to the globulin detected in the serum from the same calf which was allowed to clot naturally. The serum removed from the clot and stored at 4° C. for 10 days did not develop any fibrin, nor did the subsequent addition of thrombin produce any detectable clot. Heating the serum at 56° C. for 15 min. did not alter the electrophoretic pattern. It is concluded that the component detected at low concentration in that area of the electrophoretic pattern ascribed to γ-globulin is not unconverted fibrinogen, and that it may be autogenous γ-globulin or globulin passively acquired *in utero*.

The electrophoretic analysis of sera from colostrum-fed calves

Four calves were examined in this group. Fig. 2 shows the sequence of changes in the different serum proteins of one of these calves and is typical of the other animals.

* The thrombin for this experiment was kindly supplied by Dr W. H. Seegers. 1 mg. dry weight = 1330 units thrombin (see Murray, Johnson & Seegers, 1954).

Neonatal serum protein changes during the first 24 hr. The four calves showed the passive absorption of lacto-globulin into the circulatory system. One day after suckling the protein component in that part of the adult electrophoretic pattern associated with the γ globulins increased from a pre-colostral level of less than 2·2 % to 25–33 % (mean (4) 30·4 %). The passively acquired globulin showed considerable electrochemical spreading and only one peak could be distinguished

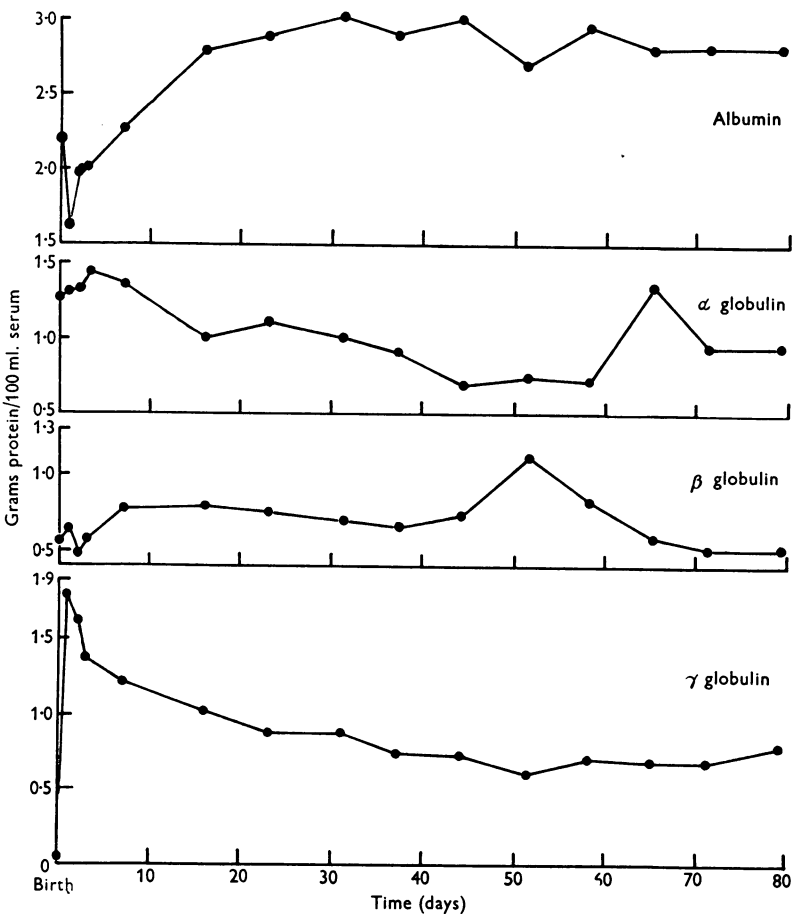

Fig. 2. The sequence of changes in the electrophoretic components of sera from a colostrum-fed calf from birth to 80 days.

(Fig. 3*b*). The mean value for the mobility of this component was 2·00 cm.^{2}/V./sec. $\times 10^{-5}$ at pH 8·0 (standard deviation (7) 0·152). The albumin values decreased by 580–900 mg. protein/100 ml. serum (mean (3) 715 mg.) while the lacto-globulin increased by 2090–1520 mg. protein/100 ml. serum (mean (3) 1738 mg.).

The ratio of the decrease in albumin to the increase in lacto-globulin in mg. protein/100 ml. gave a mean (3) of 1/2·43 one day *post partum*. The α and β globulins remained unaltered.

Serum protein changes between 2 and 40 days. The albumin was already recovering

by the second day, while the elimination of lacto-globulin exceeded absorption. The elimination of passively acquired antibody in adults is normally logarithmic, and this has been shown to be true in these calves (Pierce, 1955). However, the elimination of the passively acquired lacto-globulin as determined by quantitative electrophoretic analysis was complicated by the early autogenous production of γ globulin. By the 10th day *post partum* two components replaced the original single lacto-globulin component in all four calves (Fig. 3*c*, *d*), and the rate of elimination was no longer linear.

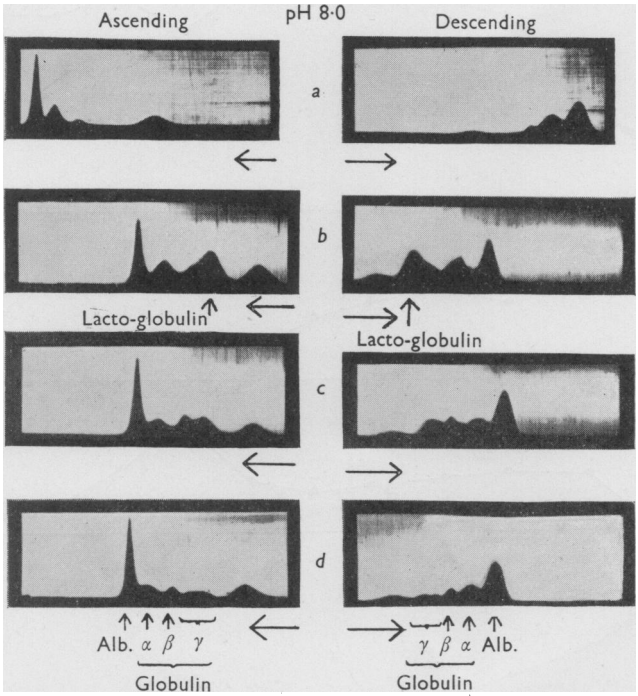

Fig. 3. The electrophoretic patterns at pH 8·0 of (*a*) pre-colostral calf serum, (*b*) calf serum 1 day after suckling, (*c*) calf serum 8 days *post partum*, and (*d*) 36 days *post partum*.

The α globulins decreased to approximately half the neonatal value 20–40 days *post partum* (mean (4) 1417 mg. to 626 mg. protein/100 ml.).

In all four animals the β globulin increased during the first 10 days and a sudden rise, usually transient, was associated with low α globulin values. The α_1 and α_2 components were difficult to distinguish in post-colostral sera. In three calves, however, at the period of highest β and lowest α, the most depleted component was the α_1 (Fig. 4), which, in the pre-colostral sera was the larger of the two α components.

Forty days onwards. The four calves were examined *post partum* for 61, 65, 79 and 80 days respectively. After the 40th day the α globulin increased, and the β globulin decreased to percentage values approaching those of the adult (mean (4) β 11·5 %, α 18·2 %). The γ globulin remained fairly constant or showed a slight and sustained increase. At the termination of the observations the γ globulin values were approximately half (mean (4) 14·1 %) the normal adult level. The albumin remained relatively constant over this period.

The electrophoretic analysis of sera from calves partially deprived of colostrum

It was difficult to rear calves deprived of colostrum. However a small amount of colostrum or of lacto-globulin enabled the calves to survive.

Fractions precipitated from colostrum at 12 and 14 g. Na_2SO_4/100 ml. were given to three calves (maximum 15·75 g. protein in 0·85 % saline *per os*). The fourth calf was allowed to suckle colostrum after a delay of 17 hr. from birth, by which time the absorption was found to be very poor (γ globulin at birth 80 mg./100 ml., maximum passive increase 370 mg./100 ml.). Glucose saline or boiled milk replaced the normal colostrum and fresh milk requirements during the first 48 hr. of life, after this period the calves were bucket-fed milk in a similar manner to the calves in the colostrum-fed group.

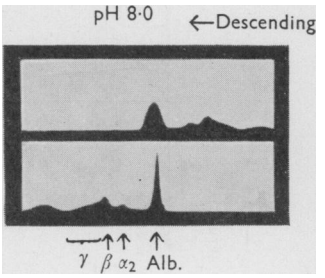

Fig. 4. The electrophoretic pattern at pH 8·0 of calf serum 19 days *post partum*, showing a prominent β component, a well-defined α_2 component, and the absence of the α_1 component.

Fig. 5 shows a typical sequence of changes in the serum proteins of one of the four calves.

Neonatal changes during the first three days. The passive increase due to the absorption of the lacto-globulin was only just detectable. The α globulins increased during the 2 days after birth in the three calves which received the colostral whey fraction. In the fourth calf, which was fed whole colostrum after 17 hr. delay, the α globulin remained constant during the first 2 days and then increased. The albumin values of the four calves remained constant 1 day after birth, and the subsequent alterations appeared as in the colostrum-fed group to be related to the changes in the other serum globulins particularly the γ globulins.

The β globulins increased in all four animals.

Serum protein changes between 3 *and* 30 *days.* Two calves, which had eliminated much of the passively acquired lacto-globulin 2–5 days *post partum*, rapidly developed autogenous γ globulin reaching values between 1170 and 1230 mg./100 ml. when 28–31 days old. In these two calves the albumin values decreased during the 30-day period. Both these animals showed fluctuations in their α and β globulin values. High β-globulin values were observed, on the 7th day *post partum* in one calf and the 28th in the other, and as in the calves in the colostrum-fed groups they were accompanied by low α globulin values.

The other two calves showed a rise in the albumin value which was associated with a gradual fall in α globulin. The γ globulin showed only a moderate autogenous production to 360–500 mg. respectively at the 29th and 30th day of life. In both of these animals the β- globulins remained constant.

The γ globulins were of particular interest in this group since their autogenous development could be followed without the masking effect of large quantities of passively acquired lacto-globulin. All four calves showed evidence of autogenous development of γ globulin by the 10th day. In three of the calves the production of γ globulin began within the first 5 days of life with an increase in the γ_1 globulin. Between the 7th and the 16th day, however, the γ_2 component could be distin-

guished, and this component gradually increased so that by the 24–31st day the γ_2 exceeded the γ_1 component. From the 28th day or later, a small component that can be detected in some adult sera could frequently be distinguished between the γ_2 and the salt boundary and has been termed γ_3 globulin (Fig. 6).

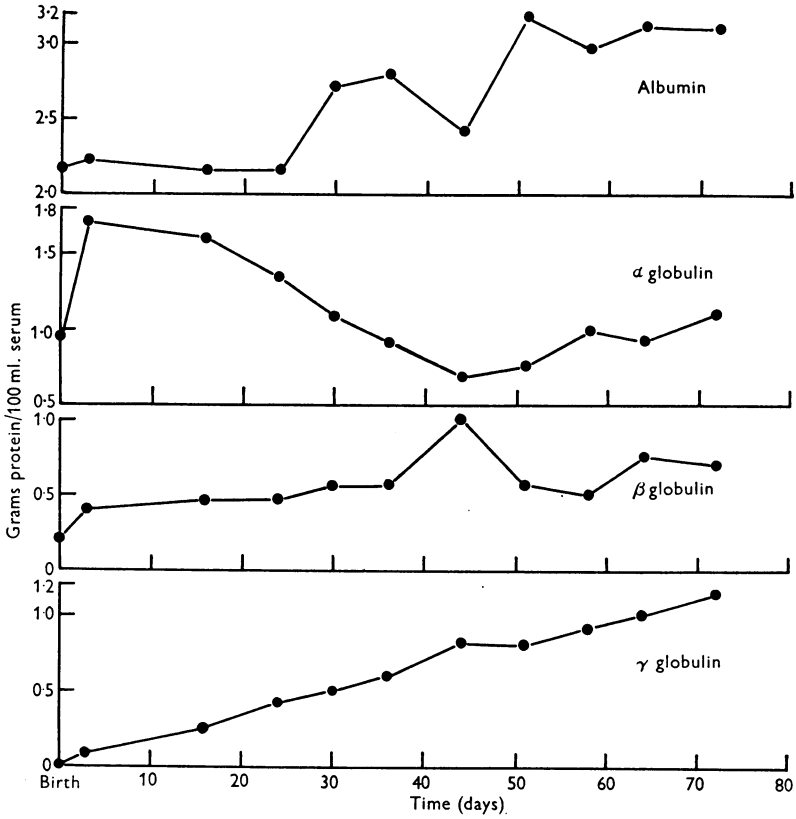

Fig. 5. The sequence of changes in the electrophoretic components of sera from a calf from birth to 72 days, and from which the colostral globulins were partially withheld.

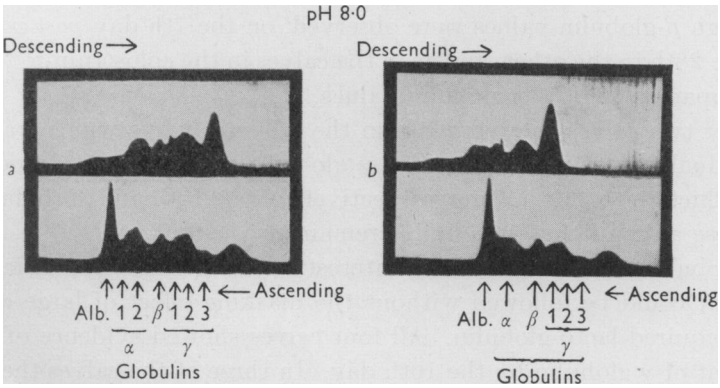

Fig. 6. The electrophoretic patterns at pH 8·0 of calf sera at (*a*) 50 days and (*b*) 63 days *post partum*, and showing the three γ globulin components.

The individual γ globulin components were poorly defined and showed considerable electrochemical spreading. In the fourth calf, in which the autogenous production of γ globulin proceeded slowly, although γ_1 and γ_2 components could be distinguished by the 8th day, they could not be analysed separately and no γ_3 could be identified.

Serum protein changes from 30 *days onwards.* The calves were examined for 63–78 days *post partum.* The gradual increase in the γ globulins was maintained except in one calf in which the γ globulin decreased from 1900 to 960 mg./100 ml. from the 50th to the 78th day. The albumin values in this calf therefore decreased until the 50th day and then increased up to the 78th day. Fig. 5 shows a transient rise in β globulin which coincided with the lowest α globulin value.

Mobility measurements. Prolonged electrophoresis of mixtures of pre- and post-colostral calf sera failed to show any changes in the mobilities of the major electrophoretic components during the first 6 weeks of life, nor were the mobilities of the calf serum proteins significantly different from those of the adult (Table 3).

Table 3. *Mobility measurements on the electrophoretic components of sera from calves* 2–6 *weeks of age, and from adult cattle*

(Electrophoresis in phosphate buffer pH 8·0, I 0·2, conductivity 0·005 Ω^{-1} cm.$^{-1}$ at 1·5° C.)

	Mean mobility (cm.^{2}/V./sec. $\times 10^{-5}$)				
	Albumin	α	β	γ_1	γ_2
Adult cattle sera (6)	5·99	4·4	3·4	2·49	1·65
Range variation	5·76–6·35	4·19–4·5	3·05–3·55	2·28–2·68	1·18–1·88
Standard deviation (6)	0·228	0·117	0·210	0·155	0·283
Standard deviation / Mean	0·0381	0·0266	0·0618	0·0622	0·172
Calf sera (7)	5·94	4·42	3·29	2·43	1·79
Range of variation	5·73–6·21	4·29–4·62	3·10–3·52	2·19–2·64	1·60–1·93
Standard deviation (7)	0·187	0·193	0·138	0·136	0·119
Standard deviation / Mean	0·0315	0·0437	0·0419	0·0560	0·0665

DISCUSSION

Recent research into the synthesis of serum protein in the rat has shown that the liver is responsible for the production of the albumin and α and β globulins, while the non-hepatic tissues of the caudal half of the rat synthesized the γ globulins (Miller & Bale, 1954; Miller, Bly & Bale, 1954). By comparison it is interesting to note that in the present studies and in those of other workers (San Clemente & Huddleson, 1943; Jameson *et al.* 1942; Hansen & Phillips, 1947; Polson, 1952), the serum of the newborn calf possesses proteins the mobilities of which are similar to the albumin and α and β globulins of the adult, while the γ globulins are almost absent. The traces which are present may be passively acquired *in utero.* Lactoglobulin appears to be absorbed rapidly and within the first 24 hr of life, since the passively acquired globulin in the colostrum-fed group showed an appreciable drop on the 2nd day after birth. Also the calf from which colostrum was withheld during the first 17 hr. of life subsequently showed very poor absorption.

The α globulin is of particular interest and is composed largely of a protein different from the albumin or serum globulins of the adult. This protein, fetuin, was first studied by Pedersen (1944, 1945, 1947) who showed that the molecular weight was only 48,700–50,600, which is less than that of the serum albumin (69,000; Scatchard, Batchelder & Brown, 1946), and that the iso-electric point was very low (pH 3·5; Pedersen, 1947). More recent investigations by Deutsch (1954) have confirmed these observations and have shown that fetuin is mucoprotein in nature.

Both the albumin and presumably the fetuin make the major contribution to the plasma osmotic pressure. However, the compensation in response to the large and rapid absorption of the lacto-globulin appears to be confined to the albumin, since on the day after calving in the colostrum-fed calves the albumin values declined sharply while the fetuin or α component remained relatively constant. Only subsequently did the fetuin component decline, and this was accompanied by a recovery in the albumin values.

Smith & Little (1924) and Howe (1924) have shown that provided the calf suckles there is a transient albuminuria during the first few days of life, which may account in part for the decrease in serum albumin. The variation of the serum albumin concentration appears to be the mechanism controlling the protein osmotic pressure. A consideration of the relative molecular weights of the albumin (69,000) and of the lacto-globulin (180,000; Smith, 1946*b*) tends to confirm this, since the quantitative decrease in albumin required to balance the acquired lacto-globulin would be approximately in the ratio of their molecular weights, 1/2·6; the experimental value derived from the mean of the three colostrum-fed calves one day after suckling was 1/2·43.

In the present studies, and in those of Wehmeyer (1949), there was a transient rise in fetuin immediately after birth in calves deprived of colostrum, while the albumin remained constant. Wehmeyer considered that the colostrum suckled by the calf inhibited the production of fetuin; this, however, is not in accord with Deutsch (1954) who thought that the accumulation of the fetuin might be a reflexion of the rapid cell proliferation in embryonic growth. However, in view of the molecular size of the fetuin, this protein may also be partially eliminated in the urine when the calf suckles colostrum.

Bradish *et al.* (1954*b*) examined adult cattle sera electrophoretically and detected two major γ globulin components, and occasionally a smaller third component. The electrophoretic conditions were closely similar to those of the present experiments, where the autogenous production of two major γ globulin components and frequently of the third component could be detected within the first few weeks of life. These three components appear to form the basic electrophoretic pattern of the bovine γ globulins. In most electrophoretic examinations the lacto-globulin associated with antibody activity in whole colostrum, fractionated colostrum or in post-colostral serum of the neonatal calf showed only one component. A review of the literature, however, shows some difference of opinion. Smith (1946*a*) considered that the lacto-globulin resembled the *T* component (γ_1) in mobility rather than the γ_2 and also failed to detect any split in the lacto-globulin. Polson (1952)

found a considerable amount of a component with a mobility of γ_2 in the lacto-globulin, which also showed electrophoretic evidence of being composed of two components. Only on one occasion in the present experiments was there clear evidence of two components in the lacto-globulin. The mobility measurements, however, suggested that the lacto-globulin might be a mixture of the two main γ globulin components in adult serum, since the value obtained was approximately the mean of the two. Polson suggested that the mobility determinations of Smith (1946*a*) may have been influenced by denaturation of the lacto-globulins during the iso-electric precipitation of the casein. In the present experiments, and in those of Polson (1952), rennin was used. The two major γ globulin components in the adult serum show electro-chemical inhomogeneity and poor resolution. Therefore the possible denaturation during accumulation in the udder and subsequent absorption by the neonatal calf may destroy their individuality. This may also account for the asymmetries observed in certain electrophoretic patterns of lacto-globulin fractions. The early appearance of the γ_1 and γ_2 components in suckling calves, and the rapid decrease in the rate of fall of the globulin passively acquired by the colostrum-fed calves, suggest that the autogenous development of γ globulin is not inhibited by the acquired lacto-globulin. Synthesis of γ globulin within a few days of birth was shown in the partially deprived group of calves.

SUMMARY

1. The serum proteins of calves from birth to weaning, and the maternal colostral whey have been examined with the 'classical' Tiselius electrophoresis apparatus. Differences were shown between calves fed colostrum and those partially deprived of colostrum.

2. A study of the pre-colostral calf serum showed the presence of albumin and of two major components with mobilities similar to the α and β globulins of adult serum. A component forming approximately 1·4 % of the total serum proteins and with a mobility similar to that of γ_1 or fibrinogen represented the γ globulin. This globulin component was not unconverted fibrinogen and may be autogenous γ globulin or γ globulin passively acquired *in utero*.

3. Autogenous γ globulin was evident in colostrum-fed and colostrum-deprived calves shortly after birth. The γ_1 and γ_2 components could be distinguished by the 10th day after birth, at which time the γ_1 globulin was the greater. By the 30th day the γ_2 globulin exceeded the γ_1 globulin and a smaller component termed the γ_3 globulin could usually be detected between the γ_2 and the salt boundary.

4. Albumin concentrations generally fluctuated inversely to changes in the total serum globulins.

5. The α globulins associated with fetuin declined shortly after birth in the colostrum-fed group. In the deprived group α globulin first rose and then fell. In both groups minimum α globulin values were reached at about the 30th day, when the α_1 globulin, although initially the major component in pre-colostral calf serum, was more depleted than the α_2.

6. The β globulin frequently showed a transient though marked increase when the α globulins were at their lowest values.

7. No changes in the electrophoretic mobilities of the major serum proteins were detected as the calves matured, and no significant difference was found between the mobilities of the electrophoretic components of calf and adult sera.

8. The electrophoretic examination of colostral whey, colostral lacto-globulin fractions and calf serum immediately after suckling usually showed one lacto-globulin component. The relationship between the serum γ globulins and the lacto-globulin is discussed.

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REFERENCES

BRADISH, C. J. & BROOKSBY, J. B. (1954). *Biochem. J.* **56**, 342.
BRADISH, C. J., HENDERSON, W. M. & BROOKSBY, J. B. (1954*a*). *Biochem. J.* **56**, 329.
BRADISH, C. J., HENDERSON, W. M. & BROOKSBY, J. B. (1954*b*). *Biochem. J.* **56**, 335.
DEUTSCH, H. F. (1954). *J. biol. Chem.* **208**, 669.
DEUTSCH, H. F. & GOODLOE, M. B. (1945). *J. biol. Chem.* **161**, 1.
HANSEN, R. G. & PHILLIPS, P. H. (1947). *J. biol. Chem.* **171**, 223.
HOGNESS, K. R., GIFFEE, J. W. & KOENIG, V. L. (1946). *Arch. Biochem.* **10**, 281.
HOWE, P. E. (1921). *J. biol. Chem.* **49**, 115.
HOWE, P. E. (1924). *J. exp. Med.* **39**, 313.
JAMESON, E., ALVAREZ-TOSTADO, C. & SORTOR, H. H. (1942). *Proc. Soc. exp. Biol. N.Y.*, **51**, 163.
JANSSEN, L. W. (1951). *Verh. Akad. Wet. Amst.* **47**, 36.
LITTLE, R. B. & ORCUTT, M. L. (1922). *J. exp. Med.* **35**, 161.
LONGSWORTH, L. G. (1942). *Chem. Rev.* **30**, 323.
MASON, J. H., DALLING, T. & GORDON, W. S. (1930). *J. Path. Bact.* **33**, 783.
MILLER, L. L. & BALE, W. F. (1954). *J. exp. Med.* **99**, 125.
MILLER, L. L., BLY, C. G. & BALE, W. F. (1954). *J. exp. Med.* **99**, 133.
MURRAY, M., JOHNSON, S. A. & SEEGERS, W. H. (1954). *Science*, **119**, 293.
PEDERSEN, K. O. (1944). *Nature, Lond.*, **154**, 575.
PEDERSEN, K. O. (1945). *Ultracentrifugal Studies on Serum and Serum Fractions.* Upsala.
PEDERSEN, K. O. (1947). *J. phys. Chem.* **51**, 164.
PHILPOT, J. ST L. (1938). *Nature, Lond.*, **141**, 283.
PIERCE, A. E. (1955). *J. Hyg., Camb.*, **53**, 261.
POLSON, A. (1952). *Onderstepoort J. vet. Sci.* **25**, 7.
SAN CLEMENTE, C. L. & HUDDLESON, I. F. (1943). *Tech. Bull. Mich. agric. Exp. Sta.*, no. 182.
SCATCHARD, G., BATCHELDER, A. C. & BROWN, A. (1946). *J. Amer. chem. Soc.* **68**, 2320.
SMITH, E. L. (1946*a*). *J. biol. Chem.* **164**, 345.
SMITH, E. L. (1946*b*). *J. biol. Chem.* **165**, 665.
SMITH, T. & LITTLE, R. B. (1922). *J. exp. Med.* **36**, 181.
SMITH, T. & LITTLE, R. B. (1924). *J. exp. Med.* **39**, 303.
SVENSSON, H. (1946). *Ark. Kemi. Min. Geol.* **22A**, 156.
TISELIUS, A. (1937). *Trans. Faraday Soc.* **33**, 524.
TISELIUS, A. & KABAT, E. A. (1939). *J. exp. Med.* **69**, 119.
WEHMEYER, P. (1949). *Rev. Immunol.* **13**, 57.

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