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STUDIES ON THE PROTEINURIA OF THE NEW-BORN CALF

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The sequence of the protein changes in the serum or plasma of the suckling calf, contingent upon the passive absorption of immune lactoglobulin, has been studied in some detail by electrophoretic analysis (Jameson, Alvarez-Tostado & Sortor, 1942; San Clemente & Huddleson, 1943; Pedersen, 1945; Hansen & Phillips, 1947; Smith & Holm, 1948; Polson, 1952; Pierce, 1955*a*, *b*). However, a concomitant proteinuria first reported by Langstein & Neuberg (1907) and studied by Smith & Little (1924) and Howe (1924) had received little further attention until Deutsch & Smith (1957) showed that some of the protein in the urine was β lactoglobulin.

The new-born lamb also develops a transient albuminuria when it is suckled. Salting-out techniques and the detection of specific antibodies showed that globulins were also present (McCarthy & McDougall, 1949, 1953).

The α globulin of the new-born calf is composed largely of fetuin, a muco-protein (Pedersen, 1944, 1945, 1947; Deutsch, 1954) with a molecular weight of 48,700–50,600, which is significantly lower than that calculated for bovine serum albumin of 69,000 (Scatchard, Batchelder & Brown, 1946) or 66,600–68,800 (Rowe & Abrams, 1957). Rowe (1957) has shown that the selective protein filtration of the smaller plasma protein molecules characteristic of the nephrotic kidney glomerulus can be reproduced artificially by the filtration of normal serum through suitable nitrocellulose membranes. Therefore, assuming that the excess proteins in the urine of the calf were derived from the plasma, the small-molecular-weight fetuin would make an appreciable contribution (see Pierce, 1954).

The present investigation examines the proteinuria which develops in the colostrum-fed calf in terms of the renal clearance rate of the proteins and their electrophoretic analysis. As the work proceeded characteristics of certain urinary proteins showed that they were not identical with the normal serum proteins, and the study was extended to the electrophoretic examination of the colostral whey proteins as a possible alternative source. Serological studies and solubility measurements were also used to characterize further some of the urinary proteins.

METHODS

Management of calves. The calving was observed and the calves (shorthorn or shorthorn cross) were immediately removed from their dams. A blood sample was collected and thereafter the calves were fed by hand. They were housed throughout the experiment in pens with floors artificially heated during the winter months. Certain calves from which complete urine samples were collected for 52–56 hr were maintained for this period in isolation, without bedding, in centrally heated, well ventilated pens.

Collection and preparation of sera. Blood samples, about 25 ml., were collected from the jugular vein with aseptic precautions and allowed to stand during the night at room temperature. The serum was then separated from the clot by centrifugation and stored at $-10°$ C.

Collection and storage of urine. Urine samples were collected from the calves from birth onwards. Micturition was initiated by digital manipulation of the inferior aspect of the prepuce or vulva. The volume was measured and the sample filtered and cooled to 4° C, and within 12 hr stored at $-10°$ C.

Protein concentration. Urine samples were dialysed against running tap water for at least 120 hr and finally against 0·15 M-NaCl for a further 24 hr to salt-in any precipitated protein. Non-dialysable nitrogen values were determined by a semi-micro-Kjeldahl technique and corrected for any volume changes during dialysis. Serum protein concentrations were determined similarly although no allowance was made for non-protein nitrogen.

Serum and urine protein concentrations were adjusted refractometrically for electrophoretic runs. The samples were dialysed against phosphate buffer solution (ionic strength, $I = 0{\cdot}2$, pH 8·0) and 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). When there was sufficient material the concentration for electrophoresis was adjusted to approximately 2·0% ($n_1 - n_0 = 0{\cdot}00400$) for the Hilger & Watts and 1·0% ($n_1 - n_0 = 0{\cdot}00200$) for the Perkin-Elmer apparatus.

Electrophoretic technique. Electrophoretic experiments were made in either Hilger & Watts (H.W.) or Perkin-Elmer (P-E) classical apparatus (Tiselius, 1937) with the modified Philpot optical system. After temperature equilibration and compensation of the boundaries, a current from a stabilized valve rectifier was applied.

The following procedure was adopted for routine runs:

H.W.: 10 mA for 30 min followed by 20 mA for 150 min or 20 mA for 165 min, long middle section U tube, cross-sectional area 0·75 cm²; P-E: 15 mA for 45 or 60 min, long middle section U tube, cross-sectional area 0·3 cm². The starting and final boundaries of each run were photographed.

Analysis of electrophoretic patterns. The electrophoretic pattern was projected at P-E × 5 and H.W. × 8 diameters on to sheets of millimetre squared paper, and the outline traced. The lower contour was used for tracing the P-E pattern. The area of each electrophoretic component was calculated from mean planimetric values derived from the ascending and descending limb analyses (see Pierce, 1955*a*). Final concentrations of each component were derived from the mean of these analyses.

Mobility measurements. The mobilities of the various electrophoretic components were calculated from measurements of their displacement from the starting boundary on the descending side. The method of determining the conductivity of the buffer has already been described (Pierce, 1955*a*).

Serological tests. Brucella agglutination tests. Serum, whey and urine were serially diluted with carbol saline in tubes of 1 cm diameter. An equal volume of standardized *Brucella abortus* antigen was added, and after mixing, the tubes were incubated at 37° C for 20–24 hr. The arbitrary end-point chosen, + +, indicated about 50% clearing of the supernatant with marked sedimentation. When the antigen and methods described are used to test the International Standard *Brucella abortus* antiserum, 50% agglutination occurs at a dilution of 1/480 (final dilution in the serum-antigen mixture).

Fractionation procedures. The albumin fraction was prepared from adult bovine serum and the α globulin (fetuin) from neonatal calf serum. The fractions were used as markers in electrophoretic experiments to identify the mobility of the various proteins. The fetuin fraction showed an electrophoretic homogeneity > 98%.

Albumin. Bovine serum was treated with Na_2SO_4 30–31 g/100 ml. at 37° C, and the supernatant which contained the albumin fraction dialysed against distilled water and freeze-dried.

Fetuin. The method described by Deutsch (1954) was used. The final precipitate '*D*' was obtained after equilibration during the night at −10° C in 50% ethanol (v/v) at pH 4·5. A solution of the precipitate was dialysed against distilled water and freeze-dried.

Definition of terms. The term 'lactoglobulin' used in previous papers (Pierce, 1955*a*, *b*) when referring to the slowest electrophoretic component in bovine colostral whey, is now designated 'immune lactoglobulin', following the recommendations of the committee on milk protein nomenclature (Jenness, Larson, McMeekin, Swanson, Whitnah & Whitney, 1956) and is similar to that described by Smith (1946*a*, *b*). The mobility is similar to γ_1 globulin (Pierce, 1955*a*, *b*) and to the 'T' component (Smith, 1946*a*).

The main serum globulin components were identified as described previously (Pierce, 1955*a*, *b*). The method is similar to that of Bradish, Henderson & Brooksby (1954*a*, *b*) and Bradish & Brooksby (1954) who used a similar buffer system.

RESULTS

The determination of protein levels in the urine from calves fed on colostrum or boiled milk

Urine samples were examined daily for periods up to 49 days from thirteen calves fed on colostrum (fed group) and from three calves fed on boiled milk (deprived group). The results obtained on three animals in each group are shown in Fig. 1. Proteinuria was associated only with urine samples collected from the fed group during the first 48 hr of life. After this period the protein levels in the two groups were similar and appeared to have reached the normal level for the young calf.

One calf in the fed group examined for 10 days showed an exceptional pattern. After a normal rise in the protein concentration to 0·38% and a subsequent fall, a further proteinuria developed when the calf was 5 days old, reaching a maximum value of 0·31% and falling again to normal levels by the 8th day.

Protein concentrations in urine samples from the deprived group did not exceed 0·19 g/100 ml. compared with maximum values ranging from 0·44 to 2·14 g/100 ml. in the fed group. Protein levels determined on urine samples from the fed group after the termination of the proteinuria and from the deprived group up to 1 month of age gave a mean value of 0·0599 g/100 ml. (0·0017–0·2 g/100 ml.).

The ingestion of colostrum in the aetiology of proteinuria was indicated both by the absence of protein in the urine from the deprived group and by low protein levels, 0·097, 0·052 and 0·173 g/100 ml. in the urine of three calves in the fed group immediately before feeding colostrum. The electrophoretic patterns of pre-colostral calf sera showed very low values for γ globulin, so

that some indication of the amount of immune lactoglobulin passively absorbed from the gut could be determined by electrophoretic analysis (Table 1).

Although the maximum concentration of protein in the urine has little quantitative significance, the relationships between the total amount of protein fed to the calves, the amount of immune lactoglobulin passively

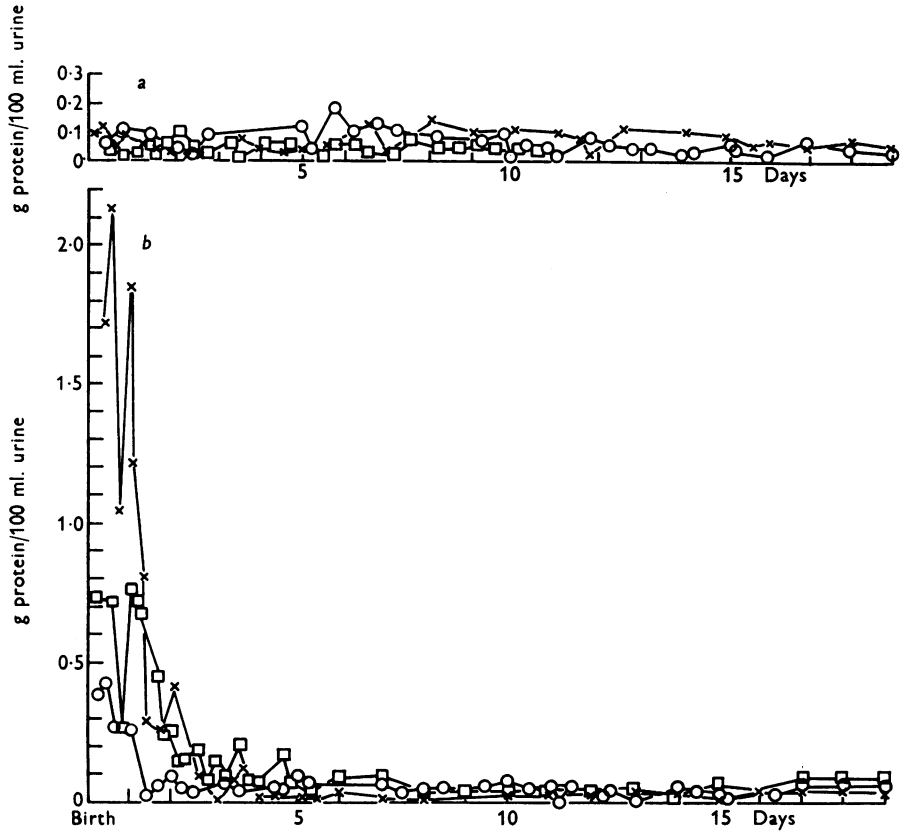

Fig. 1. The non-dialysable protein concentration in urine from new-born calves (*a*) O 5, ×–×; O 6, ○–○; O 9, □–□, fed on boiled milk, and (*b*) P 1, ×–×; O 51, ○–○; O 3, □–□, fed on colostrum.

absorbed, the rise in total serum protein and the total output of protein in the urine during the first 48 hr can be quantitatively evaluated. These results further emphasize the importance of the ingestion of colostral protein in the aetiology of the proteinuria (Tables 1 and 2).

Calf R 50 was fed on equal portions from a pooled colostrum sample (first and second milkings after calving) at a constant rate throughout the first 56 hr of life. Electrophoretic analysis of the serum proteins indicated that little further absorption of immune lactoglobulin occurred after the 31st hr,

and that the proteinuria terminated at the same time. Therefore the cessation of absorption of immune lactoglobulin from the gut and the termination of the proteinuria were not dependent upon the transition of colostrum to milk.

TABLE 1. Electrophoretic analyses of sera from four calves (*a*) at birth and before suckling, and (*b*) at their maximum passive immune lactoglobulin level approximately 48 hr after feeding colostrum*

(Phosphate buffer pH 8·0, *I* 0·2)

		Mean percentage distribution from ascending and descending limb analyses			
			Globulins		
Calf no.	Serum protein (g/100 ml.)	Albumin	α	β	γ and immune lactoglobulin
O 3 (*a*)	4·64	57·8	33·5	6·2	2·5
O 3 (*b*)	5·93	32·0	23·8	10·1	34·1
P 1 (*a*)	4·42	57·4	33·85	7·65	1·1
P 1 (*b*)	7·37	22·7	17·2	8·5	51·6
E 17 (*a*)	4·73	56·1	34·1	8·6	1·2
E 17 (*b*)	5·17	34·75	24·5	9·65	31·1
R 50 (*a*)	4·46	50·7	34·1	9·9	5·3
R 50 (*b*)	4·71	41·8	25·4	10·2	22·6

* Control deprived calves show little change in γ globulin level over this period (see Pierce, 1955*a*, *b*).

TABLE 2. The change in concentration of serum protein during the first 48 hr of calves (*a*) fed on boiled milk, and (*b*) fed on colostrum, together with the maximum concentration of urinary protein and the total protein excreted in the urine

Calf no.	Boiled milk fed (g)	Colostral protein fed in 48 hr* (g)	Maximum change in serum protein (g/100 ml.)	Maximum urinary protein (g/100 ml.)	Total protein excreted in urine in 48 hr (g)
(*a*) O 5	12870	–	+0·3	0·15	–
O 6	11530	–	+0·1	0·19	–
O 9	13210	–	−0·6	0·097	–
	Colostrum fed (g)				
(*b*) O 3	11840	597	+1·4	0·76	–
P 1	12168	831	+3·0	2·14	18·9
O 51	15400	–	+1·8	0·44	–
E 17	8290	198	+0·7	1·4	14·16
R 50	8000†	154	+0·21	0·63	4·64

* Excluding casein. † Pooled sample fed in equal portions over first 48 hr. – Not measured.

The determination of protein clearance rates

$U_p.V$ expresses the rate at which protein is excreted in the urine, when U_p is the concentration of protein in the urine in mg/ml. and V is the rate at which the urine is formed in ml./min. This analysis was applied to three calves (P 1, E 17, R 50) in the fed group from which complete urine samples were collected for the first 54–56 hr after birth. The results showed an increasing rate of protein excretion up to the 17th–28th hr and thereafter a fall. Since the rate of excretion appeared to be related, among other factors, to the concentration of serum proteins presented to the kidneys, the rate of excretion of protein

per unit of its concentration in the serum was calculated, i.e. the clearance rate. This is represented by $U_p . V/S_p$ where S_p is the concentration of serum protein in mg/ml.

In the adult kidney the clearance rate is normally a constant, the value being related to the substance. However, the clearance rate of protein in the new-born calf showed increasing values up to 17–26 hr. This period has been termed phase *a* and was followed by a sudden fall in clearance rate, phase *b* (Fig. 2).

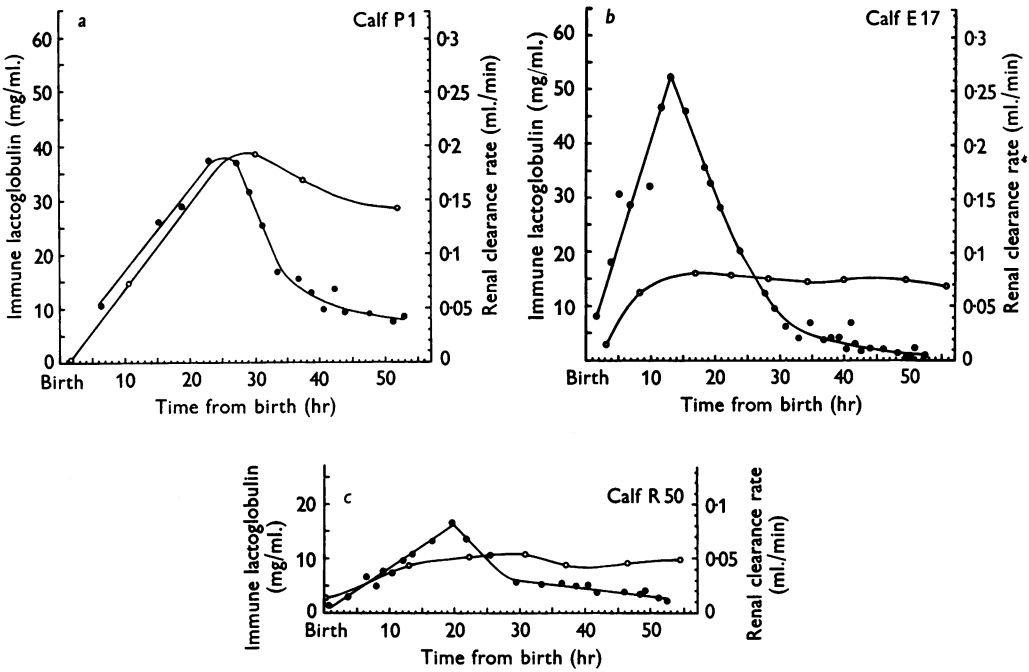

Fig. 2*a*, *b*, *c*. ●–●; renal clearance rates of total protein of three new-born calves fed on colostrum and ○–○; the concentration of immune lactoglobulin in the serum.

The rising serum protein concentration from birth was related to the passive acquisition of colostral protein, and the time at which the maximum concentration of immune lactoglobulin was attained in the calf serum coincided with the transition from *a* to *b*. No quantitative changes could be detected in the electrophoretic pattern of the serum proteins over the transitional period. Therefore, the period at which the intestine apparently became impermeable to globulin coincided with a sharp decline in the protein clearance rate (Fig. 2). Other data will be presented which show that S_p was not entirely representative of the proteins being excreted, so that the transition from phase *a* to *b* does not necessarily indicate a change in glomerular permeability.

The electrophoretic analysis of the proteins in the urine of colostrum-fed calves

The electrophoretic analysis of the proteins found in the urine usually showed five components, and frequently further components were easily identified from the ascending limb pattern where there was a characteristic

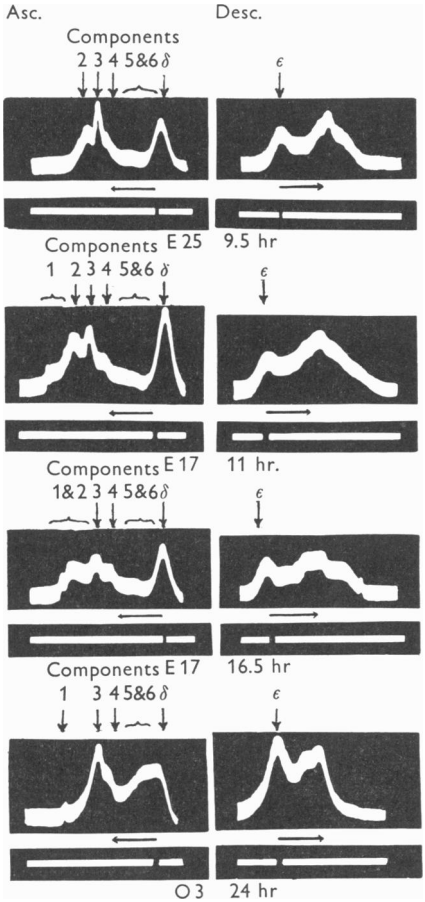

Fig. 3. Electrophoretic patterns of proteins in the urine of new-born calves E 25, E 17 and O 3 fed on colostrum. Time (hr) from birth. Component 3 has a mobility comparable with serum albumin.

sharpening of the boundaries. However, the descending limb pattern showed a concomitant boundary spreading and the components were difficult to analyse individually. When the difference in the degree of boundary spreading between the two patterns was marked, the δ and ε boundaries were excessively large (see Fig. 3) and this suggested a high proportion of non-dialysable low-molecular-weight material, which was accentuating the Donnan equilibrium

effect. A mean molecular weight determination confirmed this, giving a value of only 21,000 based on osmotic pressure and protein nitrogen measurements using the 6·25 conversion factor.

No nitrogen was detected in the salt boundary when recovered with a Pasteur pipette from the P-E electrophoretic cell after prolonged electrophoresis at pH 8·0. It is concluded that protein did not contribute significantly to the salt boundaries.

The mobilities shown in Table 3 were derived from the electrophoretic analyses of urine samples from four different calves and not all components could be analysed from every urine sample owing to excessive boundary

TABLE 3. Mobility data calculated from descending limb analyses of post-colostral calf serum, colostrum and calf urine. Analysis made in phosphate buffer, pH 8·0, I 0·2, conductivity 0·0049 Ω^{-1} cm^{-1} at 2·5° C

		Mobility (cm^2/V sec $\times 10^{-5}$)			
		Serum protein components			
			Globulins		
		Albumin	α	β	γ
		5·85	4·45	3·2	2·04
		Colostral protein components			
1	2	3	4	5	6
	(Casein)	(β lacto-globulin)			(Immune lacto-globulin)
7·26–9·38*	6·34 (10)†	5·63 (8)	4·21 (13)	3·27 (14)	2·24 (15)
		Urinary protein components			
1	2	3	4	5	6
7·32–9·4	6·62 (11)	5·85 (17)	4·34 (20)	3·24 (14)	1·95 (23)
Range of variation	6·06–7·1	5·4–6·27	4·00–5·03	2·9–3·8	1·1–2·77

* Measurements of mobility range of small components (see p. 479). † Number of observations in brackets.

spreading. There was some similarity in the mobility of components 3, 4, 5 and 6 with those of the calf serum components albumin, α, β and γ globulins, while components 1 and 2 when present had mobilities in excess of any demonstrable serum proteins (Table 3).

The most consistent trend in the electrophoretic analyses of urine samples collected during phases *a* and *b* from the four calves studied was a gradual increase in component 6 with increasing age, accompanied by a fall in components 1 and 2. The percentage composition of component 3 was usually greater than 4, and 4 was greater than 5 (Table 4).

Serial electrophoretic analyses on the colostrum fed to calf P 1 during the first 24 hr of life and on the calf serum over the same period when absorption from the gut was maximal, showed that 309 g of the immune lactoglobulin was ingested and 70 g was present in the plasma (plasma vol. equivalent to 4·9% of body weight). Assuming an identity between urine component 6 and

immune lactoglobulin (see p. 481, antibody activity), by the 24th hr approximately 4·5 g was excreted in the urine. Therefore, since these figures exclude extravascular globulin, at least 24% of the immune lactoglobulin fed was absorbed, of which a maximum of 6·0% was excreted in the urine.

The proteins involved in proteinuria are derived usually from the plasma and there is frequently a relationship between the relative amount of any one plasma protein in the urine and its molecular weight. Reference to Table 4

TABLE 4. Electrophoretic analyses of urine samples from calves which were fed on colostrum

(Phosphate buffer pH 8·0, *I* 0·2)

Calf no.	Time after birth (hr)	Urinary protein (g/100 ml.)	Mean percentage distribution from ascending and descending limb analyses				
			Component				
			1 and 2	3	4	5	6
E 17	5·75	1·21	54·1	20·7	15·1	6·9	3·2
	6·75	0·92	43·8	25·3	16·2	8·6	6·1
	8·0	0·55	47·7	27·4	13·2	7·1	4·6
	26·25	0·15	29·3	31·1	12·4	5·8	21·4
	28·5	0·22	17·1	30·0	17·7	8·3	26·9
P 1	17·66	1·05	22·2	26·6	16·7	12·2	22·3
	24·75	1·86	17·4	35·7	13·7	11·1	22·1
	26·0	1·22	15·4	24·2	21·9	13·4	24·1
	30·0	0·61	14·6	15·0	15·1	12·6	42·7
	32·25	0·81	7·5	23·8	19·4	12·2	37·2
	34·75	0·30	11·0	14·2	13·1	13·4	48·3
	39·25–41·6†	0·39	13·6	17·4	10·6	15·3	43·1
O 3	15·5	0·72	0·0*	35·3	28·1	13·9	22·7
	25·5	0·76	0·0*	51·0	14·9	13·4	20·7
	31·0	0·67	0·0*	40·0	12·9	14·5	32·6
	40·5	0·44	5·3	23·3	10·7	12·7	48·0
R 50	5·6	0·20	37·5	23·9	17·7	12·6	8·3
	7·3	0·27	25·0	31·8	22·5	8·9	11·8
	16·25	0·21	14·7	15·0	25·6	14·4	30·3
	18·1	0·19	13·3	16·1	24·5	11·8	34·3
	20·7	0·23	16·1	20·8	20·0	13·8	29·3
	22·75	0·38	19·3	19·0	16·3	13·0	32·4
	24·8	0·25	13·4	14·1	21·6	8·3	42·5
	27·7	0·62	9·7	18·1	18·3	17·6	36·3

* A small component could not be analysed separately from component 3. † Pooled sample.

shows that component 4 in the urine, with a mobility similar to fetuin with a molecular weight considerably lower than serum albumin, did not contribute a major proportion of the total protein.

The percentages for urine shown in Table 5 were calculated from the values shown in Table 4; components 1 and 2 were omitted since there are no corresponding components in the serum. The percentage composition of the serum over the period during which the urine samples were collected was estimated from serial electrophoretic analyses over the comparable period. The results show that component 5 rather than component 4 was more consistently excreted in excess of the comparable β globulin in the serum.

Analyses of variance carried out on these percentages transformed to angles ($\sin^{-1}\sqrt{p}$, where p stands for 'proportion') tested whether the distribution of the various components was the same for serum as for urine and whether if the distribution were not the same, the differences were the same for all calves. The analyses showed: (*a*) The differences between the albumin and urine 3 component were not the same for all calves; the difference between calves being highly significant ($P < 0{\cdot}001$). Reference to Table 5 shows that the difference for calf R 50 (19·3%) is appreciably different from the differences for the other three calves. (*b*) The serum α globulin and urine 4 component differences again varied between calves although not to the same extent (significant level $P < 0{\cdot}05$). (*c*) The differences between serum β globulin and urine 5 component, on the other hand, did not differ significantly between the four calves.

TABLE 5. A comparison of the percentages of the protein components albumin, α, β and γ globulins in the serum with the percentages of the components 3, 4, 5 and 6 in the urine*

Calf no.	E 17	R 50	P 1	O 3	Mean
Serum albumin	41·8	44·0	25·2	37·7	36·8
Urine 3	44·4	24·7	26·1	37·5	31·1
Difference	−2·6	19·3	−0·9	0·2	5·7
Serum α	26·5	28·0	19·6	25·9	24·8
Urine 4	25·2	25·6	18·4	16·3	21·7
Difference	1·3	2·4	1·2	9·6	3·1
Serum β	9·0	9·3	8·3	9·3	8·9
Urine 5	12·2	15·3	15·1	13·8	14·3
Difference	−3·2	−6·0	−6·8	−4·5	−5·4
Serum γ	22·2	18·3	46·7	26·5	28·1
Urine 6	16·5	32·9	39·5	31·2	30·7
Difference	5·7	−14·6	7·2	−4·7	−2·6

* In calculating the percentages for urine, components 1 and 2 have been omitted since there are no components of corresponding mobility in the serum.

The mean percentage of the urine component was 5·4 higher than the mean percentage of β globulin, this difference being highly significant ($P < 0{\cdot}001$). (*d*) The differences between serum γ globulin and urine 6 component differed significantly between calves ($P < 0{\cdot}001$). Again the difference for calf R 50 (14·6%) is appreciably higher than the differences for the other three calves. The feeding regimen differed slightly for calf R 50 and calves E 17, P 1 and O 3 (see p. 472). This does not, however, at present appear to be an adequate explanation for the different results obtained.

The presence in the electrophoretic pattern of protein components with mobilities in excess of serum albumin, the low mean molecular weight of the urinary protein compared with serum protein and the absence of any selective excretion of serum protein components relative to their molecular weights, suggested that the electrophoretic mobility was insufficient evidence on which to identify urine protein components with those of serum.

Further studies on the identity of the proteins in the urine of calves in the fed group

Protein marker experiments. The complexity of the electrophoretic patterns of the urinary proteins and the boundary spreading in the descending limb made the geometrical analysis of patterns for mobility determinations difficult and the values approximate. Therefore the similarity of mobility between individual components in the urine and serum was confirmed by incorporating specific serum protein fractions with urine samples. Figure 4 shows the electrophoretic pattern of the fetuin fraction, and identifies the mobility with that of the α globulin in calf serum.

Figure 5 confirms the similarity between the mobilities of fetuin and the urine component 4, and serum albumin and urine component 3. The large proportion of protein in urine samples from E 17 with a mobility faster than serum albumin (see Table 4) is also confirmed.

Paper electrophoresis. The analysis of certain urine samples by free electrophoresis suggested a considerable heterogeneity in the mobility of the slowest component, which was frequently poorly defined from the salt boundary (see Fig. 3, calf O 3). Paper electrophoresis showed that some of this protein had a mobility slower than the immune lactoglobulin in the colostral whey, or after absorption by the calf, in the post-colostral serum (Fig. 6*a*).

Urine components 1 and 2, which migrated faster than the serum albumin and which were particularly evident in urine samples from E 17, were very faintly stained for protein by azo-carmine. However, they stained intensely with the periodic acid-Schiff reagent for carbohydrate (Fig. 6*b*).

The variation in the electrophoretic distribution of the protein in different urine samples is shown by the comparison of the electrophoretograms in Fig. 6. E 17 at 6·75 hr shows a large component 3 with the mobility of serum albumin, while a pooled sample collected from P 1 during the later stages of the proteinuria shows a large slow component, a well defined 4th component, but very little component 3.

The salting-out characteristics of the protein in the urine of calves O 3 and E 25 were examined. Sodium sulphate was added (26·8 g/100 ml. urine) at 37° C and the precipitate and supernatant examined electrophoretically (Fig. 7). In both urine samples most of the protein in components 4, 5 and 6 was precipitated, and those components with the mobility of serum albumin (component 3) or faster remained soluble. A small, very fast component (included in component 1) in both urine samples was precipitated.

Proteolytic enzyme activity. Proteolysis in urine, significant in terms of these experiments, was investigated as a factor in the production of electrophoretic components with mobilities in excess of serum albumin. A urine sample collected during phase *a* was immediately cooled and maintained below 4° C

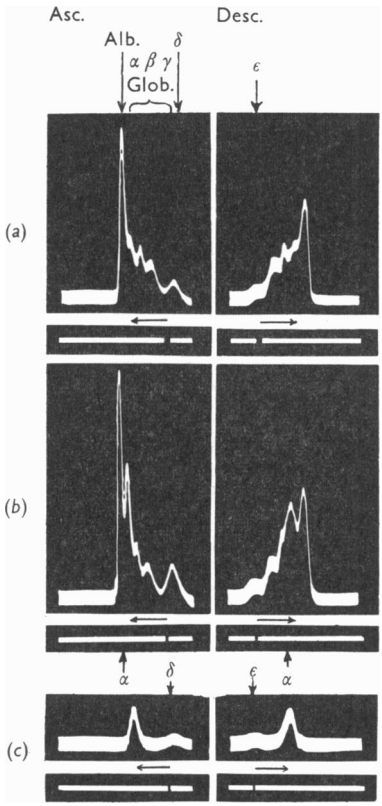

Fig. 4

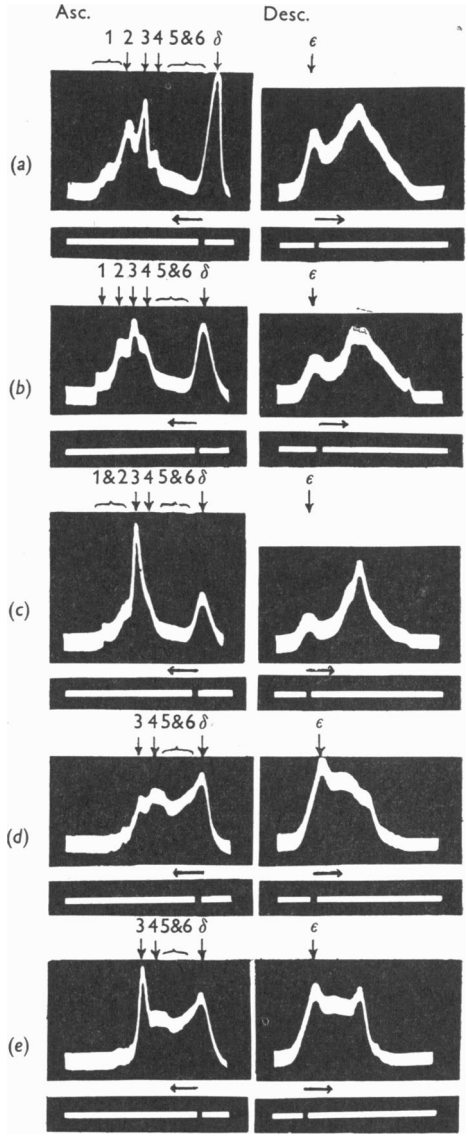

Fig. 5

Fig. 4. Electrophoretic patterns of post-colostral calf serum, O 9, (*a*) before, and (*b*) after the addition of the fetuin fraction (*c*) and showing the association of the fetuin with the α-globulin component.

Fig. 5. Electrophoretic patterns of proteins in the urine of calves (*a*) E 17 and (*d*) P 1 fed on colostrum: (*b*) shows the rise in component 4 after the addition of fetuin to (*a*); (*c*) and (*e*) show the rise in component 3 after the addition of serum albumin to (*b*) and (*d*).

until examined 18 hr later. Nevertheless a component comprising 37 % of the total protein was detected with a mobility in excess of serum albumin. In two further experiments the same urine and another from a calf 6 days old were used to dilute calf serum, obtained during the period of intestinal permeability, from 4·99 % to 1·5 % protein. The urinary protein contributed less than 5 % of the total protein and no component faster than serum albumin was detected in the electrophoretic pattern after 24 hr at 37° C.

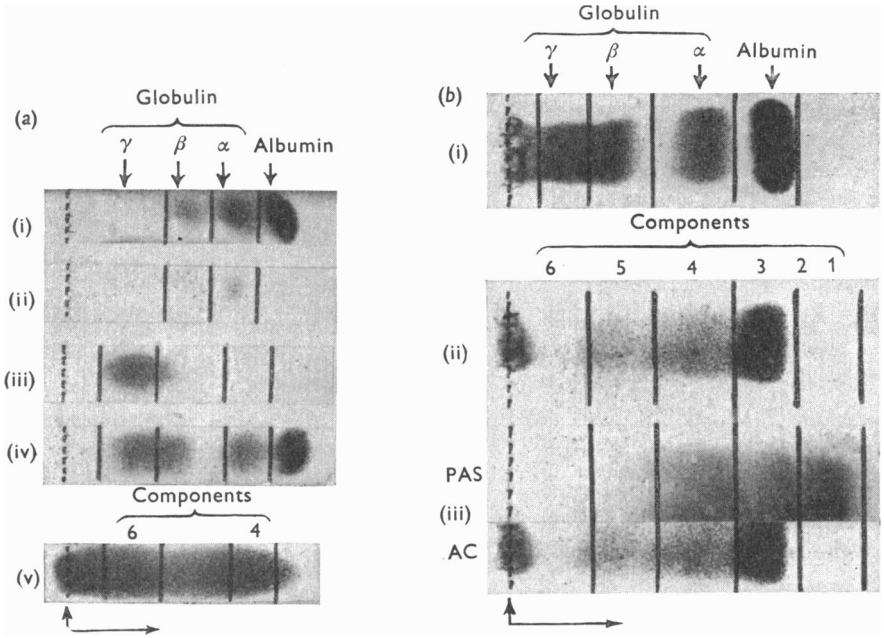

Fig. 6*a*, electrophoretograms showing the relative mobilities of proteins in (i) pre-colostral calf serum, (ii) fetuin, (iii) immune lactoglobulin, (iv) post-colostral calf serum and (v) P 1 urine (pooled).

b, electrophoretograms showing the relative mobilities of proteins in (i) E 17 post-colostral calf serum, (ii) E 17 urine 6·75 hr after birth, and (iii) E 17 urine stained with periodic acid-Schiff (PAS) and azo-carmine (AC).

Antibody activity. Component 6 in the urine showed an electrophoretic mobility similar to that of the immune lactoglobulin component (see Table 3) and increased with age, corresponding to a rising proportion of passively acquired immune lactoglobulin in the calf serum, thus suggesting a relationship between the two components. A new-born calf was fed on colostrum from a cow previously immunized with killed *Brucella abortus* strain 19 vaccine and the agglutination results shown in Table 6 were obtained on serial samples of urine. The second urine sample collected 17 hr 40 min after birth gave the highest end-point, 48; thereafter the agglutination end-point decreased to < 2

at 53 hr 15 min. A fraction withdrawn from the descending limb of the electrophoresis apparatus and comprising the salt boundary together with component 6 of the urine showed, on subsequent serological examination, at least 75% of the antibody activity. A significant correlation was also obtained

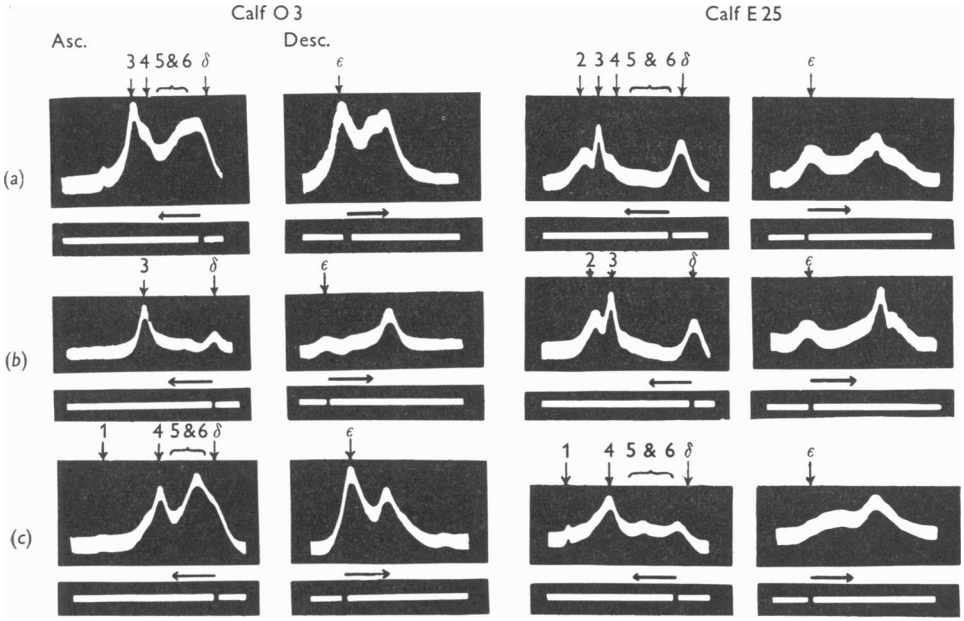

Fig. 7. Electrophoretic patterns of the proteins in (*a*) urine from calves O 3 and E 25, (*b*) the supernatant, and (*c*) the precipitate after fractionation with Na_2SO_4 26·8 g/100 ml.

TABLE 6. *Brucella abortus* agglutination by maternal serum, colostral whey, calf serum and serial samples of urine. Results shown as the reciprocal of the titre

Highest maternal serum titre during immunization	512
Colostral whey at calving	2048
Pre-colostral calf serum	nil
Highest post-colostral calf serum titre	640

Serial calf urine samples

Time after birth (min)	Titre	Time after birth (min)	Titre
770	32	2165	2
1060	48	2225	4
1195	16	2285	6
1485	24	2355	6
1560	12	2495	6
Sample lost	—	2555	4
1620	6	2700	6
1695	6	2895	8
1800	8	3000	8
1855	16	3045	2
1935	12	3135	2
1995	6	3195	<2
2085	6		

between component 6 (in mg protein/ml.) and the reciprocal of the agglutination end-point of the urine samples shown in Table 6.

The electrophoretic examination of proteins in the colostral whey

Proteins in the colostral whey after rennin digestion are presumably available for absorption in the small intestine. Whey samples prepared from colostrum collected from the first to the eighth milking after calving were analysed electrophoretically. Whey component 6, the immune lactoglobulin,

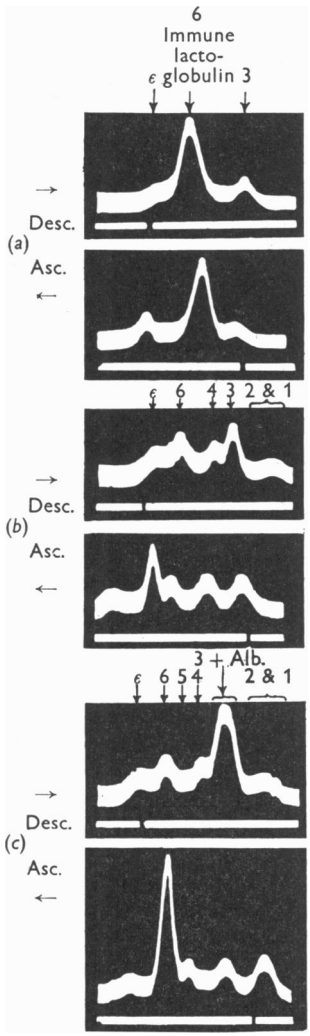

Fig. 8. Electrophoretic patterns of cow O 51 colostral whey proteins*: (*a*) at first milking, (*b*) at 7th milking and (*c*) after the addition of serum albumin to (*b*).

* The casein, component 2, is therefore much reduced.

predominated in the early samples (Fig. 8). The fourth to the eighth samples showed four components with mobilities similar to but not identical with the serum albumin, and α, β and γ globulins (Fig. 8, Table 3), together with components with mobilities faster than serum albumin with a range from 6·2 to 9·38 $cm^2/V\ sec \times 10^{-5}$. The casein component analysed from whole colostral protein also showed an electrophoretic mobility in excess of serum albumin (6·34 $cm^2/V\ sec \times 10^{-5}$) and a component of similar mobility was sometimes present in the urine (see E 17, component 2, Fig. 5). Although mixing experiments confirmed the similarity between whey component 3 and serum albumin, they were not identical and in some experiments could be resolved electrophoretically into two peaks, the colostrum component 3 being very slightly slower (see Table 3). The whey protein, with the exception of the immune lactoglobulin, has a low sedimentation velocity in the analytical ultracentrifuge compared with the serum proteins (Pedersen, 1936; Deutsch, 1947; Johnson & Pierce, 1959), so that again the electrophoretic similarity in the mobility measurements of certain serum and whey proteins is not evidence of a more general identity. The results show the difficulty in identifying, by electrophoretic mobility, any protein recovered in the urine with either serum or colostral proteins, particularly during the period when colostral proteins may be absorbed intact from the gut.

DISCUSSION

The study covers a period of many inter-related physiological changes attendant upon birth. An explanation of the mechanism of the temporary proteinuria initiated by the ingestion of colostrum can be approached only tentatively on the results obtained.

Phase (*a*): *rising protein clearance rate.* The assumption that the proteinuria of the new-born calf was entirely dependent on a permeability of the neonatal glomerulus to serum macromolecules was not confirmed by the results. First, if the glomeruli were more permeable, some degree of proteinuria might be expected in urine samples collected before suckling and from calves in the deprived groups, whereas the protein levels in these samples fell within the normal limits for older calves. Secondly, there would not necessarily be a correlation between the cessation of absorption of immune lactoglobulin and the reduction in protein clearance rate. The clearance rate of serum protein would be expected to be greatest at the start, declining with time, whereas during phase (*a*) the clearance rate increased. Thirdly, certain proteins recovered from the urine showed molecular weight and electrophoretic mobility dissimilar from those of the serum proteins. And fourthly, there was no apparent preferential filtration of the serum protein with low molecular weight, fetuin.

A limited normal glomerular permeability to macromolecules, followed by tubular reabsorption (Bing, 1936), would offer some explanation for the rising

clearance rate, since an increase in plasma protein concentration, arising from the absorption of immune lactoglobulin, would be followed by increased protein concentrations in the glomerular filtrate. The threshold for the tubular reabsorption would be exceeded and this would result in an apparent increase in the filtration rate.

Assuming competition for tubular reabsorption (Hardwicke & Squire, 1955) not only would the immune lactoglobulin be recovered in the urine but also an equivalent proportion of all those proteins present in the glomerular transudate. However, the rise in total serum protein *per se* was not of primary importance in the production of the proteinuria. For example, calf O 5 in the deprived group showed a rise in serum protein of 0·3% at the 48th hr but developed no proteinuria, whereas calf R 50, which was fed on relatively small amounts of colostral protein, showed a rise of only 0·21% in plasma protein but developed a proteinuria.

The protein components in the urine with mobilities greater than serum albumin could be quantitatively assessed. For example, the protein concentration in a sample of urine of E 17 was 1·2 g/100 ml. when 54·1% or 0·65 g/100 ml. migrated faster than serum albumin. This concentration was considerably greater than any total urine protein concentration from deprived calves or from calves before suckling or after the termination of the proteinuria, so that it was unlikely that these particular proteins were derived entirely from sites within the urinary tract.

Phase (*b*): *falling protein clearance rate.* The inception of phase (*b*) could be correlated with the period when there was little further increase in the plasma of passively acquired immune lactoglobulin, and occurred during the period when the total protein concentration in the plasma presented to the kidney was maximal. Since the passive elimination of immune lactoglobulin from the circulation was relatively slow, the protein concentration in the plasma of calves fed on colostrum fell only slightly during phase (*b*) and exceeded that during much of phase (*a*). Therefore the concentration of serum protein was not of primary importance and the proteinuria appeared to be essentially dependent upon the continuing absorption of immune lactoglobulin from the gut. The absorption into the circulation of colostral proteins other than immune lactoglobulin and their subsequent clearance by the kidney seemed the probable source of some of the urinary proteins. This is supported first by the electrophoretic evidence of some whey protein with a mobility similar to urine protein and with a mobility in excess of any demonstrable serum protein and secondly by the presence of protein in colostrum and urine with a lower molecular weight than serum protein. Thirdly, the proteinuria was associated with not only the ingestion of colostrum, but more precisely with the period of intestinal permeability. Fourthly, there was no evidence of the preferential filtration of serum protein with a low molecular weight.

The glomerular membrane of the normal kidney is permeable to protein with a molecular weight below that of albumin. Kerridge & Bayliss (1932) and Bayliss, Kerridge & Russell (1933) found that gelatin, and Bence-Jones protein with a molecular weight of 35,000 and also egg albumin with a molecular weight of 34,000 were excreted by the cat and rabbit kidney and by the isolated dog kidney. Lowell, Colcher, Kendall, Patek & Seegal (1946) also found that gelatin given intravenously to man was excreted.

These results appear similar to those visualized when the calf absorbs small-molecular-weight colostral protein. Moreover, recent work by Bangham, Ingram, Roy, Shillam & Terry (1958) has shown that the gut of the calf is non-selective and will absorb colostral proteins other than immune lactoglobulin. The mean molecular weight of the protein in the urine sample was 21,000 so that it is probable that if this protein were present in plasma it would be cleared rapidly by the kidney and high plasma concentrations would not develop. This is in conformity with the experimental results, since no electrophoretic components faster than albumin were detected in the serum. The relative sedimentation velocities in the ultracentrifuge of calf serum, urine and colostral protein may be more revealing in studies on the neonatal proteinuria of the suckling calf and are the subject of a further study.

SUMMARY

1. A proteinuria developed during the first 30–40 hr of life in calves fed on colostrum and was absent in similar calves which were fed on boiled milk.

2. The transition from colostrum to milk was not the factor which terminated the absorption of immune lactoglobulin from the gut.

3. Calculations based on protein clearance rates showed two phases: (*a*) rising values up to the 17th–26th hr after birth, and (*b*) rapidly declining values reaching minimal levels by the 40th hr.

4. Termination of intestinal absorption of protein from the gut, as determined by steady or falling immune lactoglobulin levels in the serum, coincided with the transition from phase (*a*) to phase (*b*).

5. Electrophoretic analyses of the urine proteins showed at least six components, some of which were comparable on a mobility basis with the serum proteins. Urine components 1 and 2 had a mobility faster than serum albumin and composed up to 54·1 % of the total protein; proteins with similar characteristics were observed in colostral whey.

6. The results suggest that the proteinuria arose to some extent from low-molecular-weight protein in the colostrum which was absorbed from the gut together with the immune lactoglobulin. Owing to the small molecular weight, this protein was subsequently cleared from the circulation by glomerular filtration. High concentrations of these proteins were not developed in the circulation and so remain undetected electrophoretically.

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