ULTRACENTRIFUGAL AND ELECTROPHORETIC STUDIES ON NEONATAL CALF SERA AND MATERNAL COLOSTRUM

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(With 5 Figures in the Text)

INTRODUCTION

The electrophoretic examination of sera from calves from birth onwards showed evidence of the early autogenous production of protein with γ-globulin properties, although the electrophoretic pattern was confused to some extent by the immune lactoglobulin passively acquired by the suckling calf (Pierce, 1955*b*). However, the development of γ globulin in calves deprived of colostrum could be more readily followed electrophoretically and the synthesis of autogenous γ globulin within the first 3 weeks of life was confirmed (Pierce, 1955*a*). Nevertheless, when an attempt was made to immunize young calves during the first 3 weeks of life using a freeze-dried preparation of the protozoan *Trichomonas foetus*, although large quantities of antigen were injected intramuscularly, no specific immune agglutinins were detected (Kerr & Robertson, 1954; Pierce, 1955*b*). On the other hand, the new-born lamb and human infant have been successfully immunized with alum precipitated diphtheria toxoid (A.P.T.) provided that the level of passively acquired specific antitoxin was minimal (Vahlquist, 1949; Barr, Glenny & Randall, 1950; Barr, Glenny, Hignett, Randall & Thomson, 1952). Halliday (1957) has also shown that rats aged 11 days produced antibodies within 3 days after a single injection of *Salmonella pullorum*. Thus the active production of antibody by the very young calf may be influenced by the presence of specific passively acquired maternal antibody, and also by the nature of the antigen injected rather than a physiological inability to synthesize specific γ globulins. The present investigation continues earlier studies by one of the authors (A.E.P.) and examines and relates the electrophoretic and ultracentrifugal behaviour of the proteins in the maternal colostrum, and serum from calves fed or deprived of colostrum. Particular reference is made to the physico-chemical characteristics of those proteins—the colostral immune lactoglobulin and calf autogenous γ globulin—which are associated with antibody activity.

MATERIALS AND METHODS

Ultracentrifugation

Preliminary experiments were performed in a Phywe air-driven ultracentrifuge at $26 \pm 2°$ C. and 150,000 g but in most of the work a Spinco Model E electrically driven analytical ultracentrifuge, providing fields of up to 300,000 g, was employed

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with cell temperatures of 15–18° C. The diagonal schlieren optical system, in one or other of its forms, was used in both instruments. Rotor temperatures, as indicated by a suitably placed thermistor (calibrated against an N.P.L. standard thermometer), were recorded continuously during experiments and in the Spinco instrument, at 60,000 rev./min., the temperature rise was not greater than 0·75° C. per hour. Sedimentation constants are reported here after correction by the normal procedure to the viscosity and density of water at 20° C.

Electrophoresis

Electrophoresis was carried out in phosphate buffer, pH 8·0, $I = 0{\cdot}2$ at $2 \pm 1°$ C. in the Perkin-Elmer (P.-E.) version of the Tiselius apparatus (Tiselius, 1937). The procedures adopted for routine runs and the methods of analysis have already been described (Pierce, 1959). The electrophoretically isolated serum and colostral fractions examined in the ultracentrifuge were withdrawn from the appropriate limb of the U-tube by means of a Pasteur pipette after the necessary separation of the individual components. In such experiments, the initial total protein concentration was about 4 g./100 ml.

Protein estimations

Protein concentrations were usually determined refractometrically after dialysis against phosphate buffer, pH 8·0, $I = 0{\cdot}2$. For electrophoretic and ultracentrifugal examination, such concentrations were adjusted approximately to 1·0 g./100 ml. ($d_n/d_x \approx 0{\cdot}00200$) for routine runs.

Serum and colostrum protein concentrations were otherwise calculated from semi-micro-kjeldahl determinations on duplicate samples assuming a protein/nitrogen ratio of 6·25 and making no allowance for non-protein nitrogen.

Management of calves

The calving was observed and the calves, shorthorn or shorthorn-cross were bled before feeding. Calves receiving colostrum were either bucket-fed or suckled their dams. Deprived calves were fed by hand from birth on boiled milk, calf O 6 for 4 days, calf O 5 for 6 days and calf O 9 for 7 days. Subsequently these calves were fed with fresh milk by hand. Intercurrent infections, frequent in the calves deprived of colostrum, were controlled by streptomycin given *per os*.

Calf K 7 was exceptional in that it received 250 ml. of a 0·15 M-NaCl solution containing 3·8 % of immune lactoglobulin (precipitated from colostral whey at 12 g. Na_2SO_4/100 ml.), after taking the initial blood sample. Glucose-saline provided additional feeds during the first 48 hr. of life, after which the calf was fed on fresh milk. The lactoglobulin provided was very small in comparison with the 48 hr. intake of a normal suckling calf and since subsequent electrophoretic analysis of the serum showed that the calf had absorbed less than 3·0 % of the total protein, it has been included in the deprived group.

Collection and preparation of sera

Blood (25 ml.) was collected from the jugular vein at varying intervals during the first week of life and thereafter weekly. Aseptic precautions were observed and

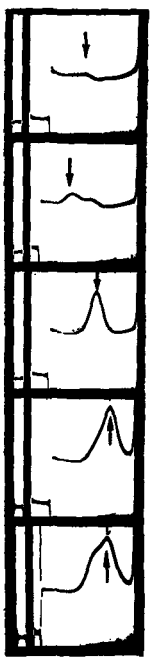
the blood was allowed to stand overnight at room temperature. The serum was then separated from the clot by centrifugation and stored at $-10°$ C.

EXPERIMENTAL RESULTS

Precolostral calf sera

The three major components occurring in the electrophoretic pattern of precolostral calf sera at pH 8 are, in descending order of electrophoretic mobility: albumin (57–69 %), α globulin (21–34 %) and β globulin (6–10 %). The respective mobilities of these components, 5·85 (6·28–5·57, S.D. (14)* 0·197), 4·45 (4·8–4·18, S.D. (14) 0·067) and 3·11 (3·37–2·91, S.D. (8) 0·082) cm.^{2} V.$^{-1}$ sec.$^{-1}$ $\times 10^{-5}$ correspond closely with those of the similarly named proteins from adult sera, but it is to be noted that the usual γ globulin of adult sera occurs to a very minor degree, contributing about 2·0 % of the total protein.

Table 1. *The ultracentrifugal examination of fractions isolated electrophoretically from precolostral calf sera*

Electrophoretic fraction	Ultracentrifuge pattern	Sedimentation† constant ($\times 10^{13}$)	Concentration* (g./100 ml.)
$\gamma+\beta$ globulin		5·2	0·04
$\gamma+\beta+\alpha$ globulin		3·0	0·17
Albumin		4·05	0·73
Albumin $+\alpha$ globulin		4·0	1·25
Albumin $+\alpha+\beta$ globulin		—	—

† Where otherwise ambiguous, a vertical arrow above the ultracentrifuge pattern denotes the component whose sedimentation constant is quoted for the concentration shown in the last column.

In order to correlate electrophoretic and ultracentrifuge findings satisfactorily, a series of electrophoretic fractions were prepared from precolostral serum and examined in the ultracentrifuge (Table 1).

* Numbers in parentheses refer to the number of experimental observations.

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The almost complete lack of globulin with a sedimentation constant of 6·9–7·0S is clearly shown in the ultracentrifuge pattern for the $\gamma+\beta$ fraction, in which the β component is thought to be responsible for the 5·2S boundary. The addition of the α fraction clearly gave rise to a slower component with sedimentation constant 3·0S and also to appreciable amounts of a much more rapidly sedimenting ('macroglobulin') material ($s^0_{20} \approx 16S$) not visible in the pattern shown. The albumin fraction sedimented as a well-defined component, but the detailed study of its sedimentation and the rather low sedimentation constant (4·05S at 0·73 g./100 ml.) (Harrington, Johnson & Ottewill, 1956) indicated contamination with α globulin. The albumin plus α-globulin fraction, in which the α globulin was apparent towards the end of sedimentation as a trailing edge on the main peak also showed a macroglobulin component, and confirmed this view. The addition of β globulin to the albumin plus α globulin clearly added to the slow trailing edge of the main boundary.

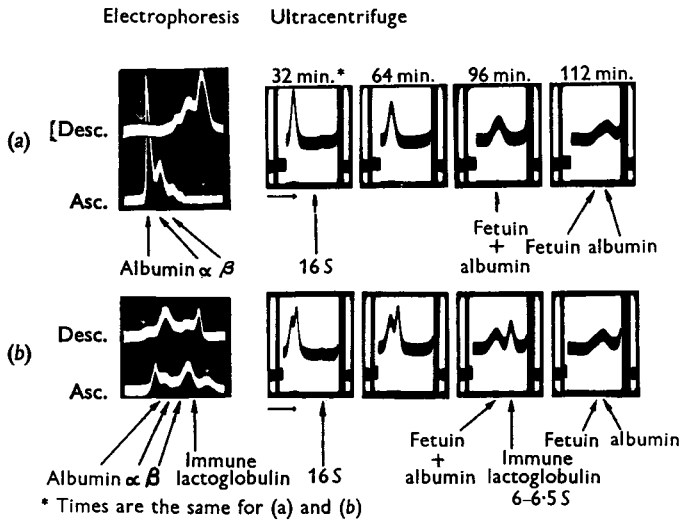

Fig. 1. Electrophoretic and ultracentrifuge patterns for: (*a*) precolostral, and (*b*) postcolostral calf serum (16 hr. after first suckling), in phosphate buffer, $I = 0{\cdot}2$, pH 8·0.

The whole precolostral serum pattern during sedimentation is now readily understood (Fig. 1*a*). The rapidly sedimenting 'macroglobulin' component is largely associated with the electrophoretic α component, which partly accounts for the trailing edge of the large main peak towards the end of sedimentation. The remainder of the large main peak contains not only albumin, but β globulin whose sedimentation rate depends (Pedersen, 1945) upon the solvent density. No component with sedimentation constant of *ca.* 7S is visible.

Postcolostral calf sera

The pronounced increase in a component with mobility similar to the γ_1 globulin and comparable with the immune lactoglobulin component in colostrum was detected in calf sera after ingestion of colostrum (Pierce 1955*a*) and is reflected clearly in the occurrence in the ultracentrifuge pattern of a new component with

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a sedimentation constant, as it occurs alongside other serum components, of *ca.* 6–6·5 (Fig. 1*b*). The presence of albumin, α and β globulins can be seen as in precolostral calf sera, and it is to be noted that the albumin peak now represents a much smaller proportion of the total protein (Table 2). Four calves showed a mean rise in total serum protein from 4·56 g./100 ml. at birth, before suckling, to 7·78 g./100 ml. 48 hr. after birth when absorption of immune lactoglobulin from the intestine had virtually ceased. This was accompanied by a fall in the albumin and α-globulin contents from 2·53 to 1·83 and 1·55 to 1·29 g./100 ml., respectively,

Table 2. *Electrophoretic and ultracentrifuge analyses of colostral protein, colostral whey protein, and of calf sera before and after suckling*

		% analysis							
		Electrophoretic					Ultracentrifuge (approximate sedimentation constants)		
		Component							
Calf no.		1 (casein)	2	3	4	Immune lactoglobulin	3*S* and lower	6*S*	18*S*
G 8	Total colostral protein pooled 1st, 2nd and 3rd milkings	7·8	21·8	11·5	12·0	46·9	54·0	45·0	1·0
M 3	Colostral whey protein pooled 1st, 2nd and 3rd milkings	2·5	14·4	6·5	8·7	67·8	21·8	76·8	1·4
	Pooled colostrum:								
	(*a*) before removal of rennin casein	24·2	18·1	8·9	10·6	38·2	—	—	—
	(*b*) after removal of rennin casein	4·7	25·1	11·3	16·2	42·7	—	—	—
			Globulin				Approximate sedimentation constants		
		Albumin	α	β	γ		4*S*	6*S*	16*S*
P 1	Calf serum before suckling	57·4	33·9	7·6	1·1		96·0	0·0	4·0
P 1	Calf serum 48 hr. after suckling	22·3	17·0	8·3	52·4		49·6	47·3	3·1

a small rise in the β globulin from 0·37 to 0·55 and a dramatic rise in γ globulin from 0·11 to 2·12 g./100 ml. The feeding of aliquots of a pooled colostrum to a calf did not prolong the period of obvious intestinal permeability assessed in terms of increase of immune lactoglobulin in the serum.

Colostrum

The ultracentrifuge pattern for colostral whey prepared by the precipitation of casein with rennin is shown in Fig. 2*a*. In addition to the main peak, sedimenting in the whey with a constant of 6·4*S* a small 'macroglobulin' type component ($s^0_{20} \approx 18S$) is apparent, as well as a small amount of material, with a sedimentation

constant of 9·5S which has no counterpart in the calf sera examined. Also clearly apparent are substantial amounts of much slower sedimenting material, with constants ranging downwards from *ca.* 3S to very low values. This material undoubtedly contains the α and β lactoglobulins described by Pedersen (1936*a*, *b*).

To ensure that the latter material was not formed by hydrolytic action arising from proteolytic contaminants in the rennin, a colostrum was clarified by centrifuging at 40,000 *g*. On examining in the ultracentrifuge (Fig. 2*b*) similar components to those for whey were visible, with constants of 6·2S and 18S as well as slower material with constants ranging downwards from 3S. The well-defined component of rennin-produced whey with s^0_{20} of 9·5S did not appear. Corresponding electrophoretic patterns are shown in Fig. 2 and the percentage distribution of peak areas in Table 2 where, apart from the main immune lactoglobulin

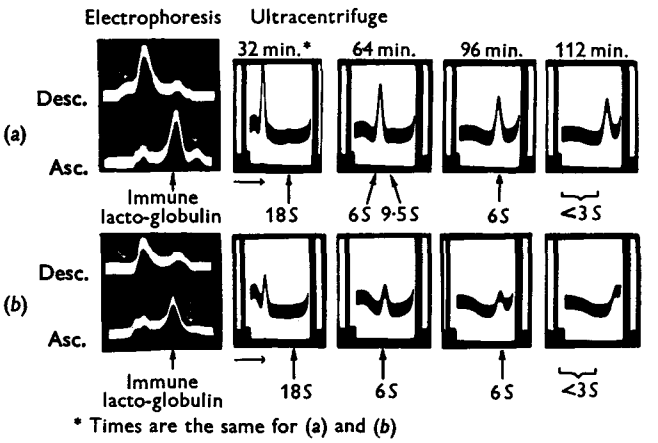

Fig. 2. Electrophoretic and ultracentrifuge patterns for: (*a*) rennin-produced colostral whey, (*b*) clarified colostrum, in phosphate buffer, $I = 0{\cdot}2$, pH 8·0.

peak, other components are denoted by numbers in order of decreasing electrophoretic mobility. The much increased contribution of component 1 in clarified colostrum is of note, and this component must therefore be largely associated with the casein.

Electrophoretically prepared immune lactoglobulin when examined in the ultracentrifuge gave a well-defined component (Fig. 3*a*) of sedimentation constant 6·6S at a concentration of 0·46 g./100 ml. and small amounts of the macroglobulin component ($s^0_{20} \approx 19S$). On the other hand, electrophoretic components 1–4 largely (> 95 %) contained (Fig. 3*b*) a prominent component of $s^0_{20} = 3{\cdot}2$ and slower sedimenting materials with constants ranging to very low values. Only a trace of the immune lactoglobulin (not visible in Fig. 3*b*) with $s^0_{20} = 5{\cdot}9S$ was visible.

It is clear that there is close agreement between sedimentation constants of the immune lactoglobulin and the globulin component which appears in calf serum after ingestion of colostrum. However, on the basis of the experiments reported here it cannot be said that the components are identical with one another.

Sera from calves deprived of colostrum

To aid in the further correlation of sedimentation and electrophoretic patterns, the following fractions were prepared by electrophoretic separation from a serum (Fig. 4*a*), collected 27 days after birth from a calf which was deprived of colostrum (O 9): albumin, γ globulin, and mixed albumin, α and β globulin (Fig. 4*b–d*). In the ultracentrifuge, the albumin component sedimented as a single well-defined

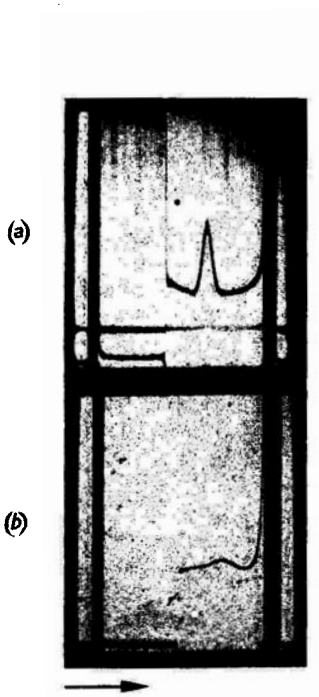

Fig. 3

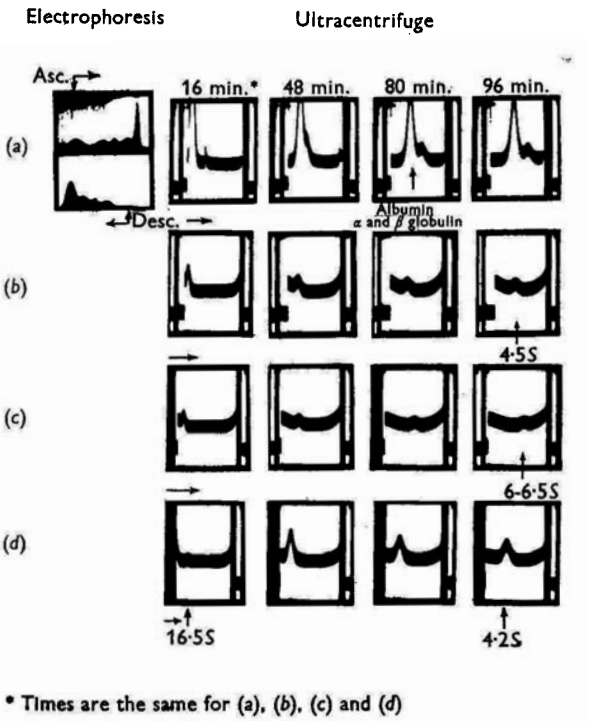

Fig. 4

Fig. 3. Ultracentrifuge patterns of electrophoretic fractions from clarified colostrum: (*a*) immune lactoglobulin, (*b*) colostral protein minus immune lactoglobulin, in phosphate buffer, $I = 0{\cdot}2$, pH 8·0.

Fig. 4. Electrophoretic and ultracentrifuge patterns for: (*a*) serum from 27-day-old deprived calf (O 9); and (*b*)–(*d*) its electrophoretically isolated fractions, in phosphate buffer, pH 8·0. (*b*) Albumin, (*c*) γ globulin and (*d*) albumin + $\alpha + \beta$ globulin. [(*a*) and (*d*), $I = 0{\cdot}2$, (*b*) and (*c*), $I = 0{\cdot}45$.]

boundary with a sedimentation constant at 0·11 g./100 ml. of $4{\cdot}5 \pm 0{\cdot}2S$, identical within experimental error to that of normal bovine serum albumin (Harrington *et al.* 1956). The γ globulin also sedimented as a single boundary with a constant at 0·10 g./100 ml. normal to bovine γ globulin. On the other hand, the mixed fraction contained a small quantity of rapidly sedimenting material ($16{\cdot}5S$) and the main peak was now slower ($4{\cdot}2S$ at 0·40 g./100 ml.) and somewhat broader. No component of sedimentation constant $7S$ was observed in the mixed fraction. Clearly the bulk of the α and β globulins sediment closely alongside albumin in phosphate

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buffer at pH 8 and I 0·2. The rapidly sedimenting macroglobulin material, in conformity with previous indications, is most probably associated with α globulin.

Sera from several deprived calves, collected at various times between birth and 77 days, were examined in detail by electrophoresis and ultracentrifugation; combined results are contained in Table 3. The most striking feature is the rise with time in the percentage of γ globulin, which largely runs parallel with the 6S component observed in the ultracentrifuge. In the main, the agreement between γ globulin and 6S component is within experimental error, but at very short and very long times, the γ-globulin content was consistently in excess, and it seems likely that the electrophoretic γ globulin must contain a small proportion of some component which sediments differently from the 6S component. It seems

Table 3. *Percentage compositions by electrophoretic and ultracentrifuge analysis of calf sera taken at different times after birth. K 7—partially deprived, O 5, O 6, O 9—deprived*

		Electrophoresis				Ultracentrifuge (approximate sedimentation constants)		
			Globulins					
Calf no.	Age in days	Albumin	α	β	γ	4S	6S	16S
K 7	Birth	68·8	20·6	9·1	1·5	94·1	0·0	5·9
	7	45·3	21·3	26·6	6·7	93·8	0·0	6·2
	22	46·9	33·0	11·3	8·8	87·9	7·8	4·3
	31	34·2	27·2	13·8	24·8	70·2	26·9	2·9
	77	36·0	18·6	9·9	35·5	62·4	31·7	5·9
O 5	26	58·5	19·4	13·0	9·1	85·0	9·8	5·1
	33	56·1	20·1	11·3	12·5	87·5	8·9	3·7
	40	58·1	16·9	14·2	10·8	84·3	9·2	6·5
	55	58·7	14·9	11·2	15·2	88·2	8·3	3·5
	60	56·9	14·5	11·5	17·0	83·5	12·9	3·7
	68	52·4	21·0	8·8	17·8	85·4	12·1	2·5
O 6	31	61·2	17·5	13·1	8·2	90·5	4·3	5·2
	38	63·2	15·5	11·8	9·6	88·3	8·1	3·6
	45	59·3	15·6	13·4	11·7	85·3	7·7	7·1
O 9	13	43·8	21·2	17·8	17·2	82·9	13·5	3·6
	20	52·5	15·6	14·5	17·4	82·7	14·8	2·5
	27	47·6	19·9	14·0	18·4	77·4	15·8	6·8

unlikely that this can be the macroglobulin (16S) component, which is associated with the α globulin, and it probably occurs in the main (4S) peak.

In view of the increasing total protein concentration of the sera with time, the less prominent changes in the sera are better considered in terms of concentrations rather than percentage contributions and an example of these changes in a deprived calf (O 5) is shown in Fig. 5. The gradual increase in the autogenous γ globulin with time is accompanied by a comparable rise in albumin. There is a gradual fall in the α-globulin component which is probably associated with decreasing concentration of the foetal α-globulin (fetuin) and the concentration of β globulin remains effectively constant.

DISCUSSION

The ultracentrifuge examination of electrophoretically isolated fractions makes possible a detailed comparison of ultracentrifuge and electrophoretic patterns, particularly in the simpler case of precolostral sera. The macroglobulin component, which has been observed in all bovine sera examined, has consistently been identified with α globulin; it will be recalled that Müller-Eberhard & Kunkel (1956) observed that a macroglobulin component of human serum occurred in the γ-globulin fraction. There is no evidence to show that it is a 'denatured globulin' as has been suggested by Cann (1953) in a study of human γ globulins.

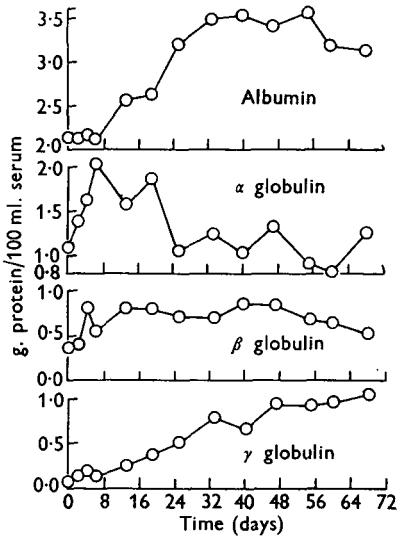

Fig. 5. Changes with time in the serum proteins of calf (O 5) deprived of colostrum.

The single prominent sedimenting boundary of precolostral sera which, at the later stages of sedimentation, shows unmistakable signs of the separation of differently sedimenting components, contains the slower sedimenting part of the α globulin ($s^0_{20} \approx 3S$) as well as β globulin whose sedimentation depends somewhat critically on the solvent density (Pedersen, 1945). In postcolostral sera, though γ globulin sediments mainly with $s^0_{20} \approx 7S$, it seems possible that a small fraction may sediment differently. Further electrophoretic fractionation experiments are planned to test this point. Apart from the γ and macroglobulin, the other electrophoretically separable components sediment closely together in the prominent main peak as in the precolostral sera.

The ultracentrifuge patterns of clarified colostrum or colostral whey, which are in good general agreement with the findings of Pedersen (1936*a*), appear much simpler than the corresponding electrophoretic diagrams, but, as with the main peak in serum, the slow and heterogeneous sedimenting material contains several (at least four) electrophoretically different components. On the other hand, the lactoglobulin, isolated electrophoretically contains one main sedimenting species

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only. On ingestion of colostrum before the age of 48 hr., the new component appearing in the calf serum is very comparable in physical properties with the lactoglobulin. To investigate possible differences it will be necessary to isolate both components and to subject to detailed physico-chemical characterization as well as to gel-diffusion techniques. No evidence for the presence in postcolostral sera of the slower sedimenting components of colostrum was obtained, and further work, still in progress, has indicated that some of these proteins are absorbed but do not reach concentrations in the plasma detected by the present methods. However, the findings would appear at first sight to be in disagreement with those of Bangham, Ingram, Roy, Shillam & Terry (1958) that colostral proteins are non-selectively absorbed by the neonatal calf. The dose level used in their experiments was at least 1000 times lower than the normal protein intake of a calf and of that used in the present experiments, and one dose only was given. Under these conditions it is unlikely that the 'albumin' or globulin fraction would be detected ultracentrifugally or electrophoretically.

Evidence is not available to show whether the large amounts of protein normally ingested over the whole period of intestinal permeability would be absorbed also non-selectively. The reduction in the serum by the 20th hr. of the 'albumin' relative to the lactoglobulin fraction observed by Bangham *et al.* (1958) would also reduce the chances of these proteins being detected by the ultracentrifuge analyses which were carried out in these experiments towards the end of the period of intestinal permeability.

The changes with time in the sera of colostrum-deprived calves (Table 3 and Fig. 5) are in good general agreement with those already published (Pierce, 1955*a*), the main feature being the slow rise in γ-globulin concentration accompanied by a similar concentration increase in albumin. Even so, the proportion of albumin decreases simultaneously owing to the absence of γ globulin initially. This should be remembered alongside the rapid fall in albumin concentration which occurs simultaneously with the rise in immune lactoglobulin concentration after ingestion of colostrum. Whilst osmotic regulation largely accounts for the latter observations, it is clear that in the deprived calves, the total protein osmotic pressure must rise significantly with age. The fall in α-globulin concentration, probably a reflexion of the disappearance of the foetal component fetuin (Pedersen, 1944, 1947), opposes such a rise but, with β globulin rising significantly also, cannot affect the net rise in pressure. Whilst area measurements are subject to considerable error, the macroglobulin component does not show a pronounced trend with time.

In terms of ultracentrifuge components, the main feature in the serum from deprived calves is the appearance and rising concentration with time of a component with $s^0_{20} \approx 6\text{–}6{\cdot}5S$ and which, as already noted, does not quite account for all the autogenous γ globulin.

It cannot be said without further examination that the early autogenous γ globulin is identical with that occurring in adult serum; in fact the various adult γ-globulin components, of which at least three have been identified (Pierce, 1955*b*), develop at different periods during the first few weeks of life. Bradish, Henderson

& Brooksby (1954) showed that following infection with the virus of foot-and-mouth disease there was an increment in those γ-globulin components with the lowest mobilities.

In the present experiments no attempt has been made to subfractionate the γ globulin or to investigate antibody activity. Thus in early life, it is possible that the autogenous γ globulin may be largely devoid of antibody activity but it is clear that the very young calf (under 3 weeks old) is capable of synthesizing γ globulin with molecular weight, shape and general properties very similar to those of adult serum.

SUMMARY

1. Neonatal serum proteins and those of maternal colostrum have been examined in the Spinco analytical ultracentrifuge and the Perkin-Elmer electrophoresis apparatus. To facilitate correlations between the two types of result, certain protein fractions have been prepared electrophoretically and examined in the ultracentrifuge.

2. The electrophoretically distinguishable components of precolostral serum, albumin, α and β globulin and traces of γ globulin sedimented mainly as a single boundary which showed evidence of a slower component (probably fetuin) with a sedimentation constant of about $3S$. A small proportion of a macroglobulin component ($s^0_{20} \approx 16S$), associated with the α globulin, was also present. In conformity with the very low γ globulin, there was a complete lack of globulin with $s^0_{20} \approx 6{\cdot}5$–$7{\cdot}0S$. The β globulin and the bulk of the α globulin sedimented closely with albumin, whose sedimentation constant in an electrophoretically isolated fraction was somewhat lower than normal, probably as a result of α-globulin contamination.

3. The electrophoretic and ultracentrifuge analysis of postcolostral calf serum showed evidence of passive absorption from the maternal colostrum of immune lactoglobulin. There was a rise in the concentration of total serum protein of which up to 51·6% was immune lactoglobulin, of sedimentation constant 6–6·5S. No such component occurs in precolostral sera. The electrophoretic and ultracentrifugal characteristics of this globulin were not significantly altered as the result of absorption. The acquisition of immune lactoglobulin was accompanied by a simultaneous fall in serum albumin concentration.

4. Examination of colostrum, rennin-produced colostral whey, and of electrophoretic fractions prepared from colostrum showed the presence of a small proportion of a macroglobulin ($s^0_{20} \approx 18S$), a well-defined immune lactoglobulin of $s^0_{20} \approx 6{\cdot}4S$ and electrophoretic properties in the γ-range, and a group of electrophoretically more mobile components with sedimentation constants ranging downwards from $3S$.

5. Electrophoretically prepared fractions from the serum of a deprived calf 27 days old showed sedimentation properties in conformity with the results on precolostral sera, with the macroglobulin component being again associated with α globulin. The remainder of α and β globulin sedimented alongside albumin and the additional γ-globulin component in a single boundary of $s^0_{20} \approx 6{\cdot}5$–$7S$.

6. The concentration of autogenous γ globulin developed by calves which had been deprived of colostrum increased with time and was generally in fair agreement with that of the 6·5–7S component.

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REFERENCES

BANGHAM, D. R., INGRAM, P. L., ROY, J. H. B., SHILLAM, K. W. G. & TERRY, R. J. (1958). The absorption of ^{131}I-labelled serum and colostral proteins from the gut of the young calf. *Proc. Roy. Soc.* B, **149**, 184.

BARR, M., GLENNY, A. T. & RANDALL, K. J. (1950). Diphtheria immunization in young babies. Study of some factors involved. *Lancet*, i, 6.

BARR, M., GLENNY, A. T., HIGNETT, S., RANDALL, K. J. & THOMSON, A. (1952). Antigenic efficiency of fluid and precipitated diphtheria prophylactics in very young babies and lambs. *Lancet*, ii, 803.

BRADISH, C. J., HENDERSON, W. M. & BROOKSBY, J. B. (1954). Electrophoretic studies of ox serum. 2. The sera of cattle infected with the virus of foot-and-mouth disease. *Biochem. J.* **56**, 335.

CANN, J. R. (1953). Ultracentrifugal properties of human gamma-globulins prepared by electrophoresis-convection. *J. Amer. Chem. Soc.* **75**, 4213.

HALLIDAY, R. (1957). The production of antibodies by young rats. *Proc. Roy. Soc.* B, **147**, 140.

HARRINGTON, W. F., JOHNSON, P. & OTTEWILL, R. H. (1956). Bovine serum albumin and its behaviour in acid solution. *Biochem. J.* **62**, 569.

KERR, W. R. & ROBERTSON, M. (1954). Passively and actively acquired antibodies for *Trichomonas foetus* in very young calves. *J. Hyg., Camb.*, **52**, 253.

MÜLLER-EBERHARD, H. J. & KUNKEL, H. G. (1956). The carbohydrate of γ-globulin and myeloma proteins. *J. Exp. Med.* **104**, 253.

PEDERSEN, K. O. (1936*a*). Ultracentrifugal and electrophoretic studies on the milk proteins. I. Introduction and preliminary results with fractions from skim milk. *Biochem. J.* **30**, 948.

PEDERSEN, K. O. (1936*b*). Ultracentrifugal and electrophoretic studies on the milk proteins. II. The lactoglobulin of Palmer. *Biochem. J.* **30**, 961.

PEDERSEN, K. O. (1944). Fetuin, a new globulin isolated from serum. *Nature, Lond.*, **154**, 575.

PEDERSEN, K. O. (1945). *Ultracentrifugal studies on serum and serum fractions.* Uppsala.

PEDERSEN, K. O. (1947). Ultracentrifugal and electrophoretic studies on fetuin. *J. phys. Chem.* **51**, 164.

PIERCE, A. E. (1955*a*). Electrophoretic and immunological studies on sera from calves from birth to weaning. I. Electrophoretic studies. *J. Hyg., Camb.*, **53**, 247.

PIERCE, A. E. (1955*b*). Electrophoretic and immunological studies on sera from calves from birth to weaning. II. Electrophoretic and serological studies with special reference to the normal and induced agglutinins to *Trichomonas foetus*. *J. Hyg., Camb.*, **53**, 261.

PIERCE, A. E. (1959). *J. Physiol.* (In the Press.)

TISELIUS, A. (1937). A new apparatus for electrophoretic analysis of colloidal mixtures. *Trans. Faraday Soc.* **33**, 524.

VAHLQUIST, B. (1949). Response of infants to diphtheria immunization. *Lancet*, i, 16.

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