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Ultracentrifuge and electrophoretic studies on the proteinuria of the new-born calf

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INTRODUCTION

Much work has been directed in the past to a study of the different effects of the ingestion of colostrum. Amongst these the pronounced rise in the concentration of serum γ-globulin is most striking, and the passage in largely unaltered form of immune lactoglobulin through the intestinal mucosa to the lymph and serum is generally accepted. The role of the lower molecular weight proteins of colostrum has not been so much studied and the present work is largely concerned with this problem.

Previous studies (Pierce, 1959) have shown that the proteinuria of the new-born calf, first reported by Langstein & Neuberg (1907) and further studied by Smith & Little (1924) and Howe (1924), was contingent upon the ingestion of colostrum and persisted only during the period when the intestine was permeable to protein. Further, Pierce (1959) showed that the characteristics of certain proteins contributing to the proteinuria were not identical with those of the serum proteins. Evidence was presented which showed that most of this protein was of small molecular weight and might be derived from colostral protein absorbed with the immune lactoglobulin from the small intestine. The possibility was strengthened because Bangham, Ingram, Roy, Shillam & Terry (1958) had shown that the gut of the new-born calf was non-selective and absorbed colostral proteins in addition to immune lactoglobulin.

Pedersen (1936) and Deutsch (1947) showed that a considerable proportion of whey colostral protein was slowly sedimenting and heterogenous. This was confirmed by Johnson & Pierce (1959) who showed that the components of colostral protein other than the immune lactoglobulin sedimented more slowly than serum albumin ($< 3{\cdot}0S$) and were probably of lower molecular weight. These components, if absorbed across the intestinal mucosa into the circulation, might not be retained by the kidney glomerulus.

The present investigation is a continuation of earlier studies (Pierce, 1959; Johnson & Pierce, 1959). Electrophoretic, ultracentrifuge and immunological methods have been carefully used in parallel to identify the colostrum components and to compare them with those contributing to the proteinuria of the new-born calf.

MATERIALS AND METHODS

Management of calves and the collection and preparation of materials

The management of the calves and the method of collection, preparation and storage of the serum and urine samples have been described already (Pierce, 1959).

Urine was collected from calves at three different stages:

(1) After birth and before ingestion of colostrum. Protein concentration was approximately 0·1 g./100 ml. and the urine was concentrated by pervaporation at 18° C. to 1/10 of initial volume before examination.

(2) After receiving colostrum and during the period of proteinuria (up to 36 hr. after birth). Protein concentration was usually between 0·2 and 2 g./100 ml.

(3) Between the third and eighth week after receiving colostrum. This urine (termed normal urine) contained approximately 0·01 g. protein/100 ml. and after reducing to 1/40 of initial volume by pervaporation at 18° C. was examined.

Lymph was collected from the thoracic duct under deep anaesthesia and was allowed to clot at room temperature; the defibrinated lymph was stored at $-10°$ C. Colostral and milk proteins were clarified for electrophoretic and ultracentrifugal analysis by centrifugation for 30 min. at approximately 18,800 *g*. Whey was obtained as the supernatant after precipitation of the casein from milk or colostrum with rennin. γ-Globulin was obtained as the precipitate from adult bovine serum at 12 g. Na_2SO_4/100 ml. at 37° C. After dissolving in dilute aqueous Na_2SO_4 it was reprecipitated twice as above.

Protein determinations

Protein concentrations in serum, lymph, colostrum and whey were calculated from micro-Kjeldahl nitrogen determinations using a conversion factor of 6·25, no allowance being made for non-protein nitrogen. Urine samples were extensively dialysed before analysis, the nitrogen values corrected for any volume changes and protein concentrations evaluated as above.

Protein concentrations for ultracentrifuge and electrophoretic analyses were determined and adjusted refractometrically to approximately 1·0 % ($n_1 - n_0 = 0{\cdot}00200$), after dialysis against phosphate buffer at pH 8·0, ionic strength (I) 0·2 (KH_2PO_4, 0·5962 g./l.; $Na_2HPO_4.2H_2O$, 11·6108 g./l.).

Electrophoretic and ultracentrifuge techniques

Electrophoresis was carried out in the Perkin–Elmer version of the classical Tiselius apparatus (Tiselius, 1937). The procedure adopted for routine runs has already been described together with the method of analysis (Pierce, 1959).

Ultracentrifuge analyses were made in the Spinco analytical ultracentrifuge with diagonal schlieren optics; the technique and the method of analysis have already been described (Johnson & Pierce, 1959), sedimentation constants (s^0_{20}) being given in terms of Svedberg units ($S = 1 \times 10^{-13}$ c.g.s. units).

Agar-gel precipitin tests

The double-diffusion precipitin method of Ouchterlony (1949, 1953) was used to establish immunological relationships between certain proteins in the maternal colostrum and those present in the serum, lymph and urine of the new-born calf.

1·0 % (w/v) 'Hyflo' agar containing 0·15 M-NaCl and 0·1 % (w/v) sodium azide was poured to a depth of 0·5–0·7 mm. into an area bounded by brass formers 1·3 cm. deep on a plate-glass base. Circular reservoirs, 1·0 cm. in diameter, were cut accurately by cork borer to occupy the corners of a hexagon of side 2·2 cm. around a central reservoir of similar size. After introducing the reagents, the agar plates were covered by a second sheet of plate glass resting on the brass formers, and maintained at room temperature for 14 days during which time the development of the lines of precipitate were recorded photographically using indirect illumination, and by drawings to determine the course of the less distinct lines and zones.

The antisera were prepared from rabbits immunized by the intravenous or intramuscular injection of several series of graded doses of antigen.

EXPERIMENTAL RESULTS

Physico-chemical examination

Text-fig. 1*a* contains sedimentation and electrophoretic diagrams for normal urine. The sedimentation diagram indicates the presence of slow sedimenting material ($s^0_{20} \approx 1S$), whose concentration in the original urine was calculated to have been about 0·01 g./100 ml. Electrophoretic diagrams were ill-defined, but showed the presence of a range of components whose mobilities were broadly similar to those of serum proteins, but with appreciable additional material moving considerably more rapidly than serum albumin. In spite of thorough dialysis against the supernatant buffer solution, the occurrence of pronounced boundary anomalies is shown by the presence of large δ and ϵ boundaries as well as by the difference in sharpness between descending and ascending boundaries. At the moderate total protein concentration and pH used, this is indicative of low mean molecular weight as compared with serum proteins. Such components are to be regarded as normal to urine and from now on are referred to as normal components. Without knowledge of the diffusion coefficient, a reliable mean molecular weight value is not available, but if the molecules are not widely divergent from spherical shape and of normal partial specific volume, the value may be taken as 5000–6000. Thus in spite of electrophoretic similarities, the components are not comparable with any main serum components.

A urine sample taken 6·75 hr. after birth from calf S 19 which did not receive colostrum was concentrated to 1/10 of its original volume and showed a similar slow sedimenting component ($s^0_{20} \approx 0{\cdot}7S$) but in addition an appreciable proportion of a component sedimenting ($s^0_{20} \approx 3S$) comparably with fetuin (Johnson & Pierce, 1959). Electrophoretic diagrams were again ill-defined but they contained considerably less γ-globulin-like material, and less of the components faster than serum albumin (5·85 cm.^{2} V^{-1} sec.$^{-1}$ $\times$ 10^{-5}) than normal adult urine (Text-fig. 1*b*).

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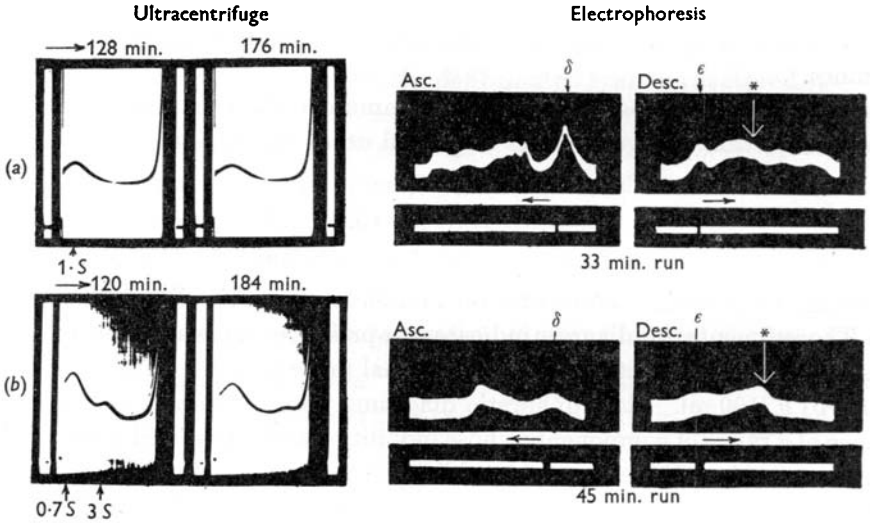
Urine samples taken after ingestion of colostrum were somewhat variable, but distinctive in that they usually possessed much more protein (up to 2 g./100 ml.) than occurred in normal and precolostral urines and of a wider range of sedimentation behaviour. In Text-fig. 2 sedimentation and electrophoretic patterns (at suitably adjusted protein concentrations), illustrating the range of possible behaviour, are included as well as the corresponding original protein concentrations in the urine. Whilst P 1 and S 3 (the more usual) contained sufficient protein for immediate examination, urine from E 25, which had been fed stored colostrum (see later) of somewhat low protein content, required concentrating to 1/10 of its initial volume. The latter procedure tends to accentuate the contribution of the normal components in this case and in comparing with unconcentrated urine (P 1 and S 3) this should be realized.



Text-fig. 1. Ultracentrifuge and electrophoretic patterns in phosphate buffer at pH8, $I = 0{\cdot}2$ for: (*a*) pooled calf (3–8 weeks) urine; original protein concentration 0·01 g./100 ml.; and (*b*) urine from calf S 19 (6·75 hr.) before feeding; original protein concentration 0·13 g./100 ml.

* The arrows on the descending electrophoretic patterns indicate protein with the mobility of albumin ($5{\cdot}85$ cm.^{2} V.$^{-1}$ sec.$^{-1} \times 10^{-5}$).

The ultracentrifuge pattern for E 25 is very similar to that for precolostral urine in Text-fig. 1*b* and the similar original protein concentration† is to be noted. On the other hand P 1 urine shows a high proportion of protein with the electrophoretic mobility range of the serum globulins and an increased proportion of the more rapidly sedimenting components including some material sedimenting with $s^0_{20} > 4S$ (comparably with albumin). As a further extreme, calf S 3 contains little of the slow-moving ($s^0_{20} \approx 1S$) component, but shows a peak with $s^0_{20} \approx 2{\cdot}3S$ which was better defined than for most of the urines examined.

In view of the considerably higher original protein concentrations of P 1 and S 3,

† The total solute excreted in a given time in the larger volume of E 25 urine was, however, much greater than for S 19 which was deprived of colostrum.

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the results are somewhat indicative of the addition of the more rapidly sedimenting components to different extents to a more or less constant background concentration of the slower-sedimenting normal components. Thus the most nearly normal of the post-colostral urines has by far the lowest protein content. Corresponding electrophoretic patterns show comparable variability and little direct correlation with sedimentation results. As with normal and pre-colostral urines, considerable boundary anomalies with large δ-boundaries (in the ascending limb) and blurred descending boundaries occurred. However, after allowing for the anomalous boundaries it is clear that a small proportion of γ-globulin-like material occurs in S 3 and E 25, but much more of albumin-like character.

The colostrum fed to calf P 1 contained agglutinins to *Brucella abortus* which were associated with the immune lactoglobulin component. The pooled urine sample analysed from this calf also showed an agglutinin titre (1:36) which, assuming the same ratio of activity to total immune lactoglobulin as in the serum of the calf, would indicate a component accounting for approximately 16·0 % of the total urinary protein. Immune lactoglobulin at this concentration with a sedimentation constant of $s^0_{20} \approx 6$–$6{\cdot}5S$ (Johnson & Pierce, 1959) would be easily detected ultracentrifugally, but nothing sedimenting faster than $s^0_{20} \approx 4S$ was observed. However, an electrophoretic component in the urine showing a mobility ($2{\cdot}0 \pm 0{\cdot}8$ cm.^{2} V^{-1} sec.$^{-1}$ $\times 10^{-5}$) similar to immune lactoglobulin ($2{\cdot}2$ cm.^{2} V^{-1} sec.$^{-1}$ $\times 10^{-5}$) and comprising approximately 30 % of the total protein did occur. Owing to the boundary anomalies and poor resolution in the descending limb this component was not recovered with precision, but the slow-moving components as a whole were found to contain at least 75 % of the agglutinin activity (see Pierce, 1959).

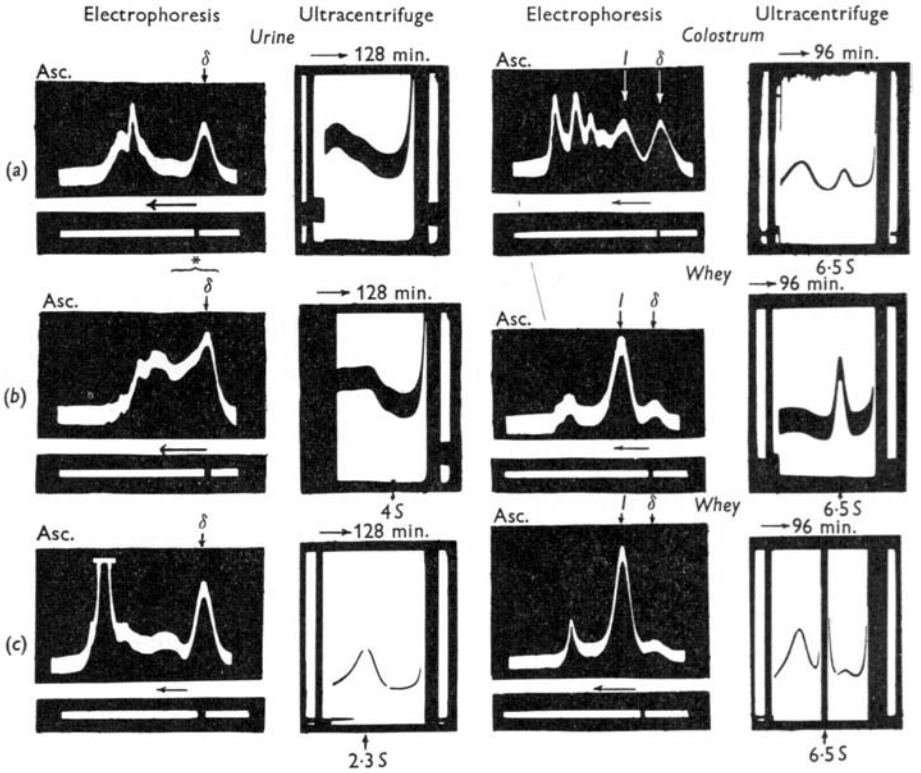
In attempting to explain the variability of the urine sedimentation and electrophoretic patterns, corresponding patterns for the different colostra which the calves received are shown also in Text-fig. 2 with other relevant information. It is to be noted that E 25 received considerable quantities of colostrum derived from fourth and fifth milkings after calving, and in which the content of immune lactoglobulin undoubtedly was low (probably *ca.* 1 g./100 ml.) and of electrophoretically fast components relatively high (*ca.* 3 g./100 ml.). On the other hand, the colostra fed to P 1 and S 3 were very rich in immune lactoglobulin (probably > 7 g./100 ml.) and relatively poor in slow sedimenting but electrophoretically fast proteins (< 3 g./100 ml.). However, it must be stressed that the faster sedimenting components occurring in urine from calves P 1 and S 3 are of much smaller sedimentation constant than immune lactoglobulin ($\approx 6{\cdot}5S$) and if derived from the latter must represent highly modified and probably degraded forms.

To aid in the interpretation of electrophoretic patterns, pure serum albumin and fetuin solutions were added separately to urine from calf S 3 before electrophoretic examination (Text-fig. 3). Whilst the fetuin was slower and readily distinguishable from a main urine component, albumin was quite indistinguishable electrophoretically from this same component.*

* The mobilities of bovine serum albumin and β_1-lactoglobulin of whey (Aschaffenburg & Drewry, 1955, 1957) was also observed to be very closely similar.

Ultracentrifuge examination shows the absence of any prominent albumin-like material, so that the resemblance applies only to electrophoresis. Further confirmation of this view was obtained by separating electrophoretically the fast and slow components of urine from calf S 3 followed by ultracentrifuge examination (Text-fig. 3). The electrophoretically fast component gave a sedimentation pattern similar to the whole urine with a main peak of $s^0_{20} \approx 2{\cdot}5S$, whilst the electrophoretically slower fraction (comparable electrophoretically with serum globulins) gave a peak of higher sedimentation rate ($s^0_{20} \approx 3{\cdot}7S$) and ill-defined slower components.

Similar fractionation experiments on different urines confirmed that the



Text-fig. 2. Electrophoretic and ultracentrifuge patterns in phosphate buffer at pH 8, $I = 0{\cdot}2$ for calf urines collected during period of proteinuria and corresponding ingested colostrum or whey:

	Calf no.	Age at urine collection (hr.)	Original protein concentration (g./100 ml.)	Remarks
(*a*)	E 25	9·5	0·10	Fed stored colostrum (fourth and fifth milking)
(*b*)	P 1	Pooled, 12·75–26	1·60	Fed mother's colostrum (first milking onwards)
(*c*)	S 3	26·3	1·34	Fed stored colostrum (first and second milkings)

* Slow-moving components fraction (see p. 251). *I* = Immune lactoglobulin.

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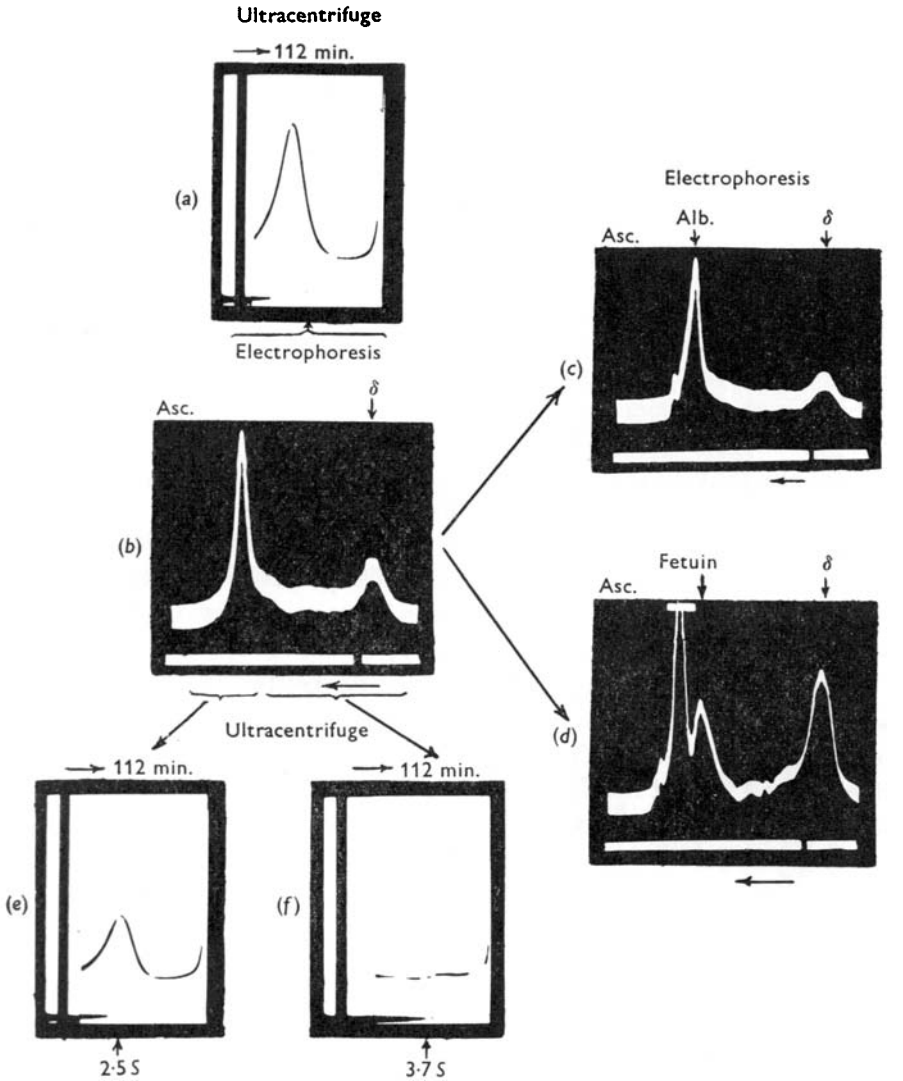
electrophoretically slow components sedimented with $s^0_{20} \approx 3{\cdot}7S$, occasionally with minor and somewhat faster peaks, but the electrophoretically faster components contained the very slow sedimenting urine components ($s^0_{20} < 1S$) as well as those of $s^0_{20} = 2{\cdot}5$–$3S$.

From the physico-chemical experiments described above, a large proportion (> 50 %) of the urine proteins are seen to resemble serum proteins electrophoretically but to differ unmistakably in sedimentation. Comparison with proteins of



Text-fig. 3. Electrophoretic and ultracentrifuge patterns for calf S 3 urine, pooled samples collected between 6·75 and 27 hr, mean concentration 1·7 ± 0·4 %, and derived solutions: (*a*) Ultracentrifuge pattern for S 3 urine. (*b*) Electrophoretic pattern for S 3 urine. (*c*) Electrophoretic pattern for S 3 urine plus bovine-serum albumin. (*d*) Electrophoretic pattern for S 3 urine plus bovine fetuin. (*e*) Ultracentrifuge pattern for electrophoretically fast components of S 3 urine. (*f*) Ultracentrifuge pattern for electrophoretically slow components of S 3 urine.

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colostral whey offers certain possibilities. In a previous paper (Johnson & Pierce, 1959), it has been shown that, in whey, the slow-sedimenting components (lactalbumin and β-lactoglobulin) correspond with the electrophoretically faster components (at pH 8·0). The urine components of $s^0_{20} = 1$–$3S$ show parallel physicochemical behaviour. On the other hand, no component with the sedimentation properties of immune lactoglobulin occurs in urine, though material with electrophoretic behaviour similar to that of this protein is sometimes present.

To investigate further these resemblances and differences, the agar gel-diffusion method has been applied, in which comparison is made between the components of serum (usually adult), colostrum and urine (taken during the period of proteinuria from a calf receiving colostrum unless otherwise stated).

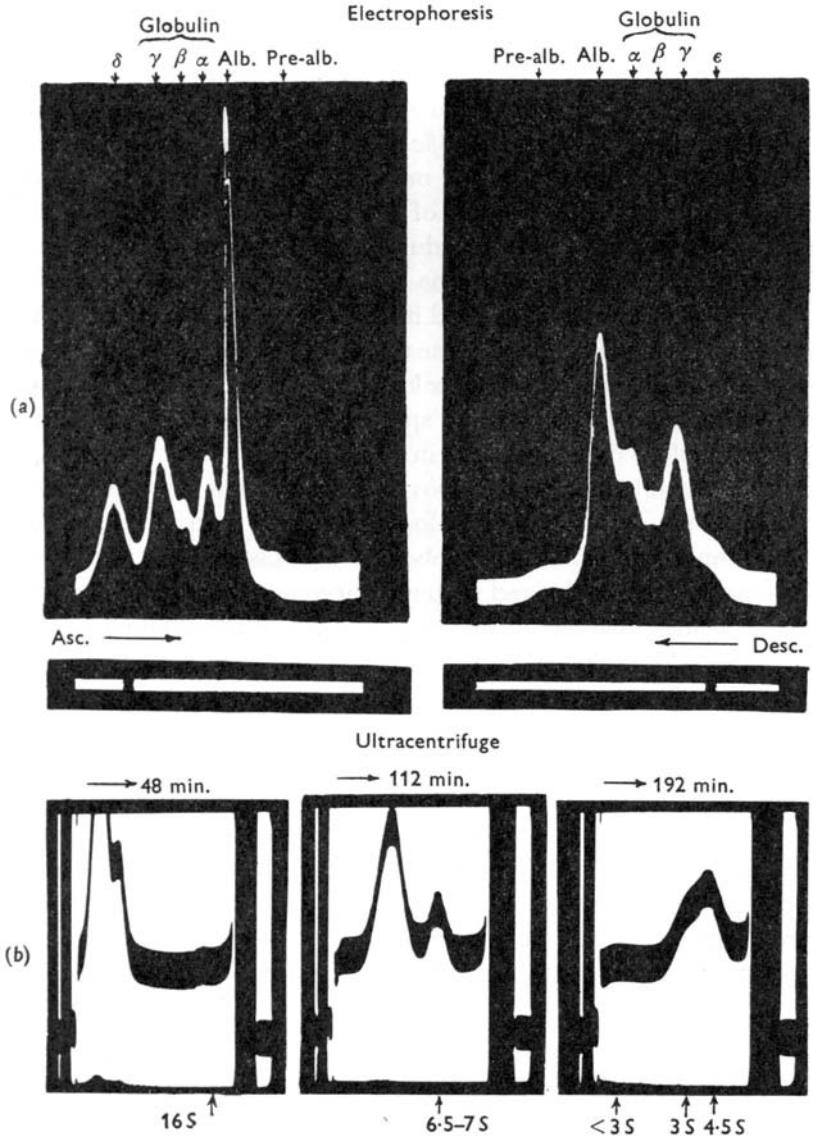
Agar-gel precipitin tests

Preliminary tests using serum from rabbits immunized against adult bovine serum (*as*) or bovine colostrum (*ac*) showed a multiplicity of precipitating lines against their respective antigens in the serum (*s*) and colostrum (*c*), several of which appeared to be shared between the two systems and with urine (*u*). Nevertheless, each system showed some lines which appeared to be specific and Plate 1 A shows one zone (1) comprising at least two diffuse bands of precipitate (*a*, *b*) and one line (2) both of which appeared to be specific to colostrum. Zone (1) was, however, also shared with the urinary proteins during proteinuria. From Plate 1 A it would seem also that the urine proteins cross-react much more with *ac* than with *as*.

By repeated cross-absorption of the undiluted rabbit antisera to colostrum or serum with freeze-dried adult bovine serum or colostrum, respectively, specific antisera which reacted only with colostrum (ac_1), or bovine serum (as_1) were produced. The absence of precipitating lines between, on the one hand, ac_1 and *s* (Plate 1 B), and on the other as_1 and *c* (Plate 1 C), demonstrates the reliability of these sera. Plate 1 C is of interest in that the components specific to adult and precolostral calf sera (i.e. absent in colostrum) were not detected in urine during proteinuria. On the other hand, of the specific components of colostrum certain are also common to urine (Plate 1 B). Of itself, this would appear to indicate, at some stage, direct access of colostrum to urine, which is denied to serum.

To investigate further, two electrophoretic fractions of colostrum (c_1, c_2) were prepared as indicated in Plate 2 I. Plate 1 B shows that the broad zone (1) arises from specific components in the electrophoretically faster colostrum proteins (c_1) and that these occur in abundance in urine, but are absent in serum. The well-defined line (2) must arise from specific, electrophoretically slower components of colostrum (c_2) (see later, Plate 1 E) which are apparently largely absent in urine as well as in adult or pre-colostral calf sera. The latter observation clearly implies that at least part of the electrophoretically slow colostrum fraction (possibly a small component comparable with serum β-globulin, i.e. mobility of 3·27 cm.^{2} V^{-1} sec.$^{-1}$ $\times 10^{-5}$; Pierce, 1959) is distinctly different immunologically from serum γ-globulin. It is to be recalled, however, that proteins in urine which were common to both serum and colostrum would not give rise to lines of precipitation in Plate 1 B in view of the cross-absorption stage (by serum) in preparing ac_1. That such components occur,

however, is clear from Plate 1 A. The origin of line (2) in the electrophoretically separated immune lactoglobulin fraction (c_2) is confirmed in Plate 1 E which also demonstrates the complexity of zone (1) with definite indications of subdivision



Text-fig. 4. Electrophoretic (*a*) and ultracentrifuge (*b*) patterns in phosphate buffer at pH 8, $I = 0{\cdot}2$ for post-colostral lymph from calf E 29 (12 hr.).

into at least 2 lines (*a* and *b*). Comparison of electrophoretically separated urine (u_3, u_4) and colostrum (c_1, c_2) proteins in their reactions against ac_1 serum were also made (Plate 1 F). To a great extent the fast urine (u_3) and colostrum (c_1) fractions show a reaction identity (zone 1) but it is clear that antibodies to the specific slow-colostrum components (c_2 line 2) give only the faintest (if any) reaction with the

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slow urine fraction (u_4) or with total urine. Thus if specific, electrophoretically slow colostrum components occur in urine at all, they do so only in very low concentrations.

Plate 1 D shows the occurrence to a small extent in post-colostral calf serum, but more so in lymph of material related to the electrophoretically fast components of colostrum and urine which are absent in pre-colostral and adult sera. The almost complete absence of line (2) between ac_1 and post-colostral serum (s_2) and lymph (l), where the immune lactoglobulin is present at high concentration, is somewhat surprising and indicates that the *specific* components responsible in the colostrum immune lactoglobulin fraction occur only as a small proportion of the whole. This is readily confirmed by the use of the non-absorbed *ac* (Plate 2G) against colostrum fractions (c_1, c_2), s and an adult γ-globulin fraction (γ). The slow colostrum fraction now gives a broad zone (3) resembling zone (1) but clearly very different in origin, and the well-defined line (2) which must occur within it is quite obscured. Further the same broad zone which occurs strongly in adult serum and adult γ-globulin, is absent in the fast colostrum fraction. The latter does, however, give rise to a less strong zone which in spite of appearances is to be identified with zone (1) and therefore does not occur in adult serum.

The picture emerging from the above results is that the predominant urine protein components are related to colostrum rather than to serum, and, of the colostrum components, the electrophoretically faster (but ultracentrifugally slower) or closely similar, derived materials account for a large proportion of the urine protein whilst little or no unchanged immune lactoglobulin is observable. Degraded forms of the latter may, however, occur. On the other hand, a pre-colostral urine (from calf S 19) after concentration by pervaporation to 1/10 of original volume (final protein concentration 1 g./100 ml.), gave a definite reaction with *as* (Plate 2H). Since the calf had not received colostrum, it is probable that some of the normal components of this urine are related to the serum proteins. Pooled urine from 3- to 8-week-old calves reacted similarly, and both urines gave little or no reaction with ac_1. Thus it seems that at least some of the *normal* protein components are probably related to and may be derived from serum, but that during proteinuria, a large amount of colostrally derived protein appears, which to a large extent submerges the reactions of the normal components.

Lymph proteins

Since it seems clear that colostrum is the origin of the proteins in urine at proteinuria, the route by which this passage occurs is of some interest. Comline, Roberts & Titchen (1951*a*, *b*) have demonstrated the initial absorption from the gut into the lymphatic system, and lymph has therefore been examined by electrophoresis and ultracentrifuge (Text-fig. 4) alongside corresponding pre- and post-colostral sera from the same animals (Table 1). Superficially electrophoretic and sedimentation patterns resemble those of the sera, but the occurrence of appreciable amounts (up to 2·7 % of total protein) of electrophoretically faster but slower sedimenting material in the lymphs is to be noted. Further, in most of the cases examined and particularly for calf S 4, the immune lactoglobulin content was

much higher in the lymph. Nevertheless, as has already been noted from gel-diffusion (Plate 1 D), the specific slow colostrum components (not present in adult serum) do not occur appreciably in lymph.

Table 1. *Mean ascending and descending electrophoretic analyses of:* (*a*) *pre-colostral calf serum*, (*b*) *post-colostral calf serum, and* (*c*) *lymph.* (*b and c were sampled during the period of intestinal permeability*)

			Component			
				Globulin		
Calf no.	Age (hr.)	Protein concn. (g./100 ml.)	Albumin	α	β	γ and immune lactoglobulin
E 25	2·25	4·20 (*a*)	—	—	—	—
	24·5	5·04 (*b*)	34·6	35·0	9·7	20·7
	26·5	— (*c*)	37·9	34·1	10·2	17·8
E 29	3·5	5·52 (*a*)	—	—	—	—
	10·25	5·59 (*b*)	34·1	29·9	9·2	26·8
	10·25	3·68 (*c*)	40·3	21·2	8·1	30·4
S 4	1·0	4·55 (*a*)	58·1	25·9	12·0	4·0
	5·25	5·93 (*b*)	36·4	28·3	8·7	26·6
	5·25	5·94 (*c*)	17·1	7·2	5·8	69·9
S 5	3·0	3·81 (*a*)	55·8	28·8	12·4	3·0
	9·0	4·21 (*b*)	56·5	27·2	11·8	4·5
	9·0	2·27 (*c*) 5·6*	57·7	19·6	10·5	6·6

— = no values. * = pre-albumin component.

DISCUSSION

From the experimental results it will be clear that the electrophoretic, ultra-centrifugal and gel-diffusion techniques have been used to a large extent in parallel in studying the various biological fluids and derived products. Ultra-centrifugation in the main provides information on molecular size and state of aggregation, electrophoresis to a large extent ignores such properties, but discriminates on the basis of electrical and to some extent chemical properties, whilst behaviour in gel-diffusion, a technique with great powers of discrimination and sensitivity, involves probably all such properties in a complex fashion.

To some extent the problem of deciding on serum or colostrum as the source of a particular urinary protein may seem of academic interest. However, where it can be shown that one or other source is predominant, such knowledge is clearly important in establishing the mechanism by which the proteins reach their destination.

Pre-colostral urine and pooled urines from normal 3- to 8-week-old calves contain low concentrations of protein which, in spite of electrophoretic indications and immunological similarity, is much more slowly sedimenting and therefore probably of much lower molecular weight than any of the major serum components. Undoubtedly, however, this protein is not derived from colostrum and is immunologically related to certain serum components. Such protein could possibly be derived by degradation of serum proteins, or alternatively could represent precursors or steps in the synthesis of serum proteins.

On the other hand, at the period of intestinal permeability, the feeding of colostrum results in much higher protein concentrations in the urine, and the additional protein (over the normally occurring components) is clearly much more related to colostrum than to serum. Thus, though some material related to common components in colostrum and serum occurs, other protein is related to specific colostrum components (absent in serum), whilst nothing related to specific serum components was observable. On the basis of electrophoretic and sedimentation behaviour, it is conceivable that the slower sedimenting colostral whey components (β-lactoglobulin and lactalbumin) are passively absorbed and reach the urine without modification, but further work (now in progress) is required to prove this point. On the other hand, little if any of the electrophoretically slow immune lactoglobulin reaches the urine, and any material found there and resembling it (e.g. P 1 Text-fig. 2) must be in a highly degraded form of much lower sedimentation constant. The rather definite finding in urine from one calf during proteinuria of antibody activity which the calf's mother had possessed, is of great interest in this connexion and can only be explained if the activity is associated with protein of much lower sedimentation constant than normal γ-globulin. However, too much ought not to be inferred from this single case.

Though it is known that immune lactoglobulin, little changed if at all, reaches the serum of the newly born calf from the colostrum, it is also true that little if any of the slower sedimenting whey proteins (β-lactoglobulin and lactalbumin) can be detected in the serum. Since Bangham *et al.* (1958) indicated that the gut of the young calf absorbed non-selectively, it was of interest to examine lymph in this connexion, since protein passing through the intestinal mucosa might be expected to appear there. With the larger amounts of immune lactoglobulin observable there, small but definite amounts of slower sedimenting material occurred, with sedimentation and electrophoretic properties in reasonable agreement with the lower molecular weight whey proteins. When the volume of lymph and subsequent haemodilution is considered, as well as the continual loss of the lower molecular weight materials from the plasma through the kidney, the rather low concentration in lymph and non-appearance in serum is perhaps not surprising. On the other hand, the high concentration of immune lactoglobulin in serum arises from the impermeability of the kidney to this protein.

An important general point regarding experimental procedure towards complex biological fluids emerges. Any single physico-chemical method used and interpreted in isolation would have given incomplete or misleading results. Electrophoresis would have suggested identities between species which sedimentation behaviour disproves. On the other hand, sedimentation alone would have demonstrated differences between species without giving any indication of relatedness. Gel-diffusion in its fundamentals, particularly regarding its quantitative aspects, is not in itself firmly established. However, a combination of the different methods provides results of great reliability and is generally to be recommended, particularly for the examination of the more complex systems.

SUMMARY

1. Many immunologically related components occur in bovine serum and colostrum, but certain components in each are absent in the other (specific components). Two components (or groups) occur in colostrum which do not appear in serum, and these can be associated with electrophoretically slow and fast colostrum components.

2. Pre-colostral and adult urines contain low concentrations ($< 0{\cdot}2$ g./100 ml.) of protein which, though immunologically related to serum proteins, does not closely resemble any major serum component in physical properties.

3. Urines collected during the period of proteinuria contain increased concentrations of protein (up to 2 g./100 ml.) which is immunologically related to both serum and colostrum. From the presence of material related to specific colostrum components (and the absence of specific serum components), the added protein evidently originates from colostrum rather than serum. The total added protein possesses components with electrophoretic mobilities covering a wide range, with appreciable protein faster than albumin at pH 8. From sedimentation studies, the added proteins resemble the slower sedimenting colostrum proteins (β-lactoglobulin and lactalbumin) and contain no observable material with the sedimentation behaviour of immune lactoglobulin.

4. Immunologically, the added protein is related only to the electrophoretically faster of the specific colostrum components, and this applies also to post-colostral lymph in spite of its very high content of immune lactoglobulin. It follows that the specific electrophoretically slower colostrum components represent only a small proportion of the total immune lactoglobulin fraction.

5. The presence in urine, collected during the period of proteinuria, of antibody activity (deriving originally from the calf's mother), and of protein with the approximate electrophoretic mobility of, but without the sedimentation behaviour of γ-globulin, is to be explained probably on the basis of molecular degradation at some stage between absorption from the intestine and excretion in the urine.

6. The low molecular weight colostrum components, though present in low concentration in the lymph, are absent or below the detectable threshold in post-colostral sera (containing much material derived from immune lactoglobulin), and this is thought to be partly due to selective action by the kidney, allowing the passage of, and causing concentration of low molecular weight protein in the urine, whilst retaining higher molecular weight protein in the serum.

7. The results show that the proteinuria of the new-born calf arose mainly 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, the former protein, was subsequently cleared from the circulation probably by glomerular filtration, accounting for the difficulty of detecting them electrophoretically and ultracentrifugally. On the other hand, the immune lactoglobulin with its much higher molecular weight was not so cleared from the circulation and is readily detected there.

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EXPLANATION OF PLATES

PLATES 1 AND 2

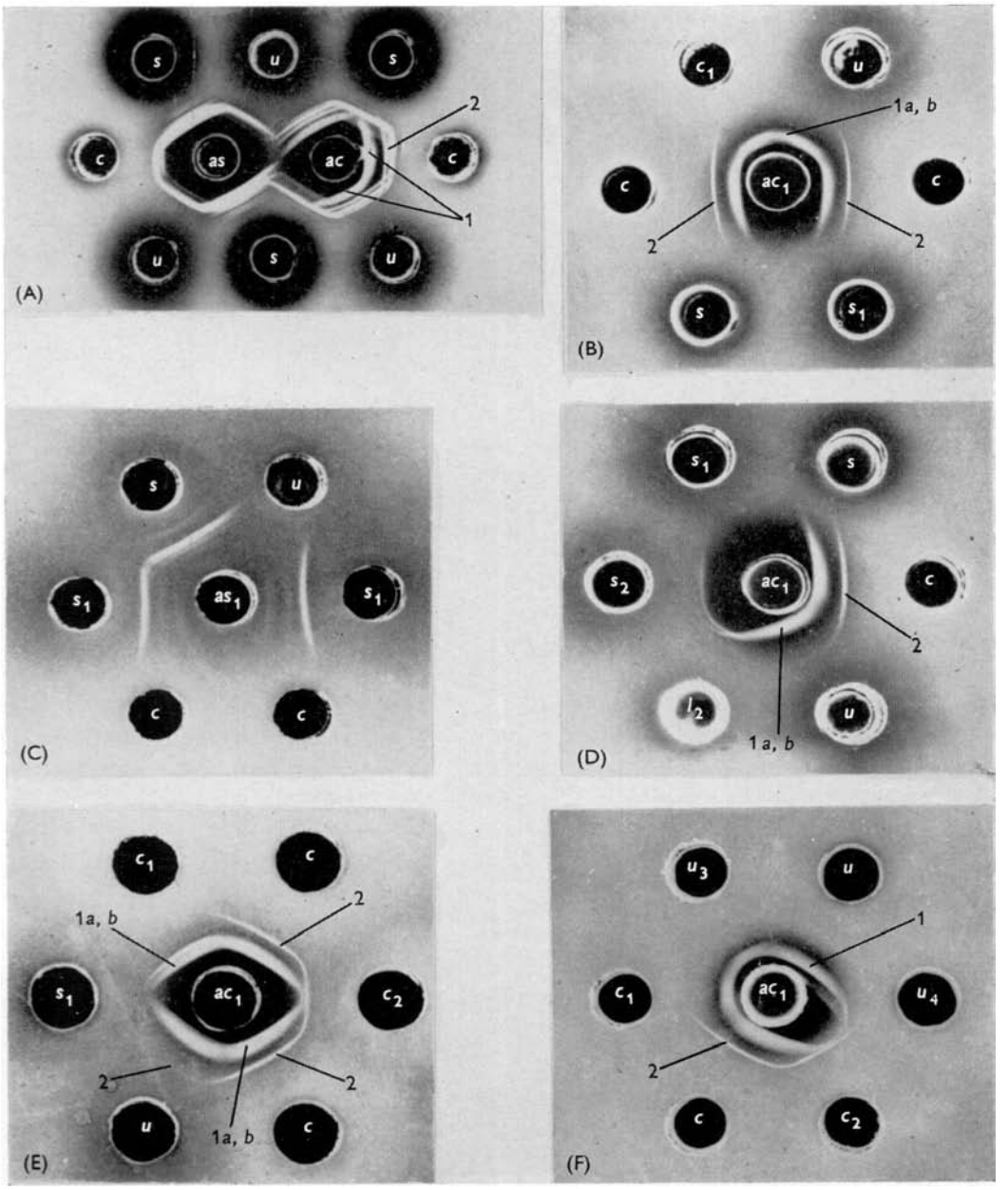
Precipitin patterns in agar-gel for adult serum (s), pre-colostral serum calf (s_1), post-colostral serum calf (s_2); colostrum (c), and derived fractions, fast components (c_1), slow components (c_2); whey (w); lymph post-colostral calf (l_2); urine collected during the period of proteinuria (u) and derived fractions, fast components (u_3), slow components (u_4); (u_1) $\equiv$ calf S 19 pre-colostral urine (protein concn. $\approx$ 1 g./100 ml.); (u_2) $\equiv$ pooled 3- to 8-week urine (protein concn. 0·24 g./100 ml.), against the following rabbit antisera.

PLATE 1

(A) Anti-bovine serum (as), anti-bovine colostrum (ac). (B), (D), (E), (F), Specific anti-bovine colostrum (ac_1). (C) Specific anti-bovine serum (as_1).

PLATE 2

(G) Anti-bovine colostrum. γ-Globulin derived from adult bovine serum by salt fractionation $\equiv \gamma$ (see p. 248). (H) Specific anti-bovine colostrum and anti-bovine serum. (I) Contains the electrophoretic pattern for bovine colostrum and illustrates the components representing the electrophoretic fractions (c_1) and (c_2).



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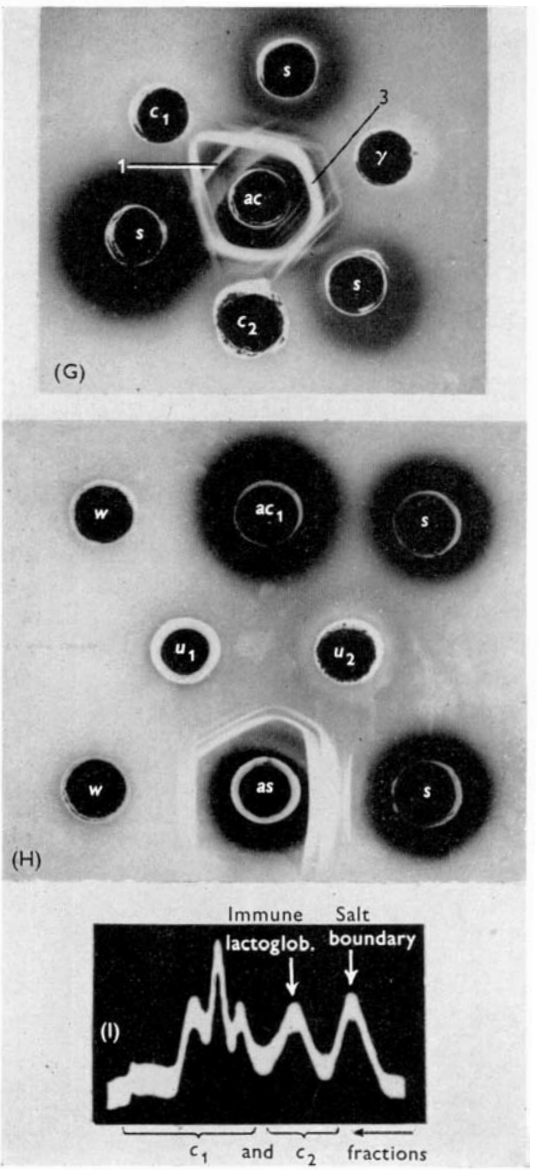

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