Proteinuria of Newborn Sucking Ruminants

By E. I. McDOUGALL

Rowett Research Institute, Bucksburn, Aberdeen

(Received 11 May 1964)

1. Proteinuria was found in most sucking lambs and calves examined but was less prevalent in bottle-fed kids or bucket-fed calves. 2. This incidence of proteinuria corresponded to the presence of immune lactoglobulin in the serum. 3. The extent of proteinuria in lambs and calves rose to 20 g./l. or more, whereas in kids values of only 4 g./l. were obtained. 4. The proteins present in the urine of lambs and kids have been compared with those in the urine of calves by using boundary electrophoresis and paper electrophoresis. 5. Sedimentation analyses have been made on the proteins of lamb, kid and calf urine. Components with sedimentation coefficients 3s and less were present. The separated slow electrophoretic components were found to have sedimentation coefficients 3·3, 3·6 and 3·3s respectively. 6. The sedimentation analyses, and for calf urine the electrophoretic data, support the view that the slow-moving electrophoretic components of calf and lamb urine are degraded immune lactoglobulins. 7. There was electrophoretic evidence for other degraded proteins in some lamb urine which showed multiple zones that could not be related to serum or colostral proteins.

The main features of the transient proteinuria of the newborn ruminant were established by Smith & Little (1924). They showed that the urine of calves that had ingested colostrum contained coagulable protein up to 3 days after birth and that the urine of calves given no colostrum contained no protein. Deutsch & Smith (1957) identified one of the proteins in calf urine by crystallizing β -lactoglobulin from the urine. They showed that a protein of higher molecular weight and with a faster sedimentation coefficient (6·4s) was also present. This is near the value of 7s found by Smith (1946) for the immune lactoglobulin of bovine colostrum. Pierce (1959) and Pierce & Johnson (1960) studied the proteins of calf urine in detail. The proteinuria developed during the first 30–40 hr. of life. The protein clearance rate increased up to 17–26 hr. after birth and fell to low levels by 40 hr. Sedimentation analysis showed that the urine proteins resembled the slow-sedimenting colostral proteins α -lactalbumin and β -lactoglobulin. In contrast with the results of Deutsch & Smith (1957), no material with the sedimentation behaviour of the immune lactoglobulin of colostrum was found. They observed that there was antibody activity in the urine and that this was associated with a protein with the electrophoretic mobility, but not the sedimentation behaviour, of γ -globulin. It was suggested that this could be due to degradation of the immune lactoglobulin.

The proteinuria of newborn sucking lambs was studied by McDougall & McCarthy (1953). Salting-out techniques and detection of specific agglutinins showed that globulins with antibody activity were present in the urine. There is no information on the proteinuria of newborn kids.

The present work reports comparative studies on the proteinuria of calves, kids and lambs. The incidence and degree of proteinuria in these animals under practical conditions of management have been studied. The successful ingestion of colostrum by the young animals was detected electrophoretically by the presence of immune lactoglobulin in the serum obtained 3 days after birth. The proteins present in the urine of lambs and kids have been compared with those in the urine of calves by using boundary and paper electrophoresis. The proteins were also examined by sedimentation analysis. Both methods of examination have been correlated in experiments in which the proteins of the urine were separated by preparative electrophoresis and then examined in the ultracentrifuge.

MATERIAL

Samples of urine and serum were obtained from calves, kids and lambs at the Rowett Research Institute during three breeding seasons. The dams were Ayrshire cows, British Saanen goats and Blackface or Cheviot ewes. The number of young animals from which samples were obtained and their management at birth were as follows. Of 41 calves,

20 were suckled by their dams and 21 were fed the first milking of their dams from a bucket; 21 kids were all fed by bottle the first milking from their dams; 21 lambs were all suckled by their dams.

METHODS

The detection of proteinuria and estimation of urinary proteins were carried out by boiling and by Esbach's method, both according to the details given by Cole (1942).

For the preparation of solutions for electrophoretic and sedimentation analyses, urines with a low protein content and fractions from the preparative electrophoresis apparatus were concentrated by ultrafiltration through a collodion thimble and dialysed against the appropriate buffer.

Paper electrophoresis was carried out on horizontally suspended Whatman 3MM paper in sodium veronal buffer, pH 8.6 and I 0.05, for 16 hr. at 0.4 mA/cm. width of paper.

Boundary electrophoresis was carried out in the Tiselius apparatus (Hilger) at 1° in sodium veronal buffer, pH 8.6 and I 0.1.

Preparative electrophoresis was carried out in the continuous apparatus of Hannig (1961) (Bender and Hobein) in tris-acetate buffer, pH 8.2 and I 0.1.

Sedimentation analysis was carried out in the Spinco model E analytical ultracentrifuge at 59780 rev./min. in phosphate buffer, pH 7.4 and I 0.2.

The protein concentration of solutions for boundary electrophoresis or sedimentation analysis was estimated from the refractive increment measured in a differential refractometer based on the design of Cecil & Ogston (1951).

RESULTS

The incidence of proteinuria in young calves, kids and lambs and the presence of absorbed immune lactoglobulin in their serum is shown in Table 1, and the extent of proteinuria in these animals is shown in Fig. 1.

The proteins present in the urine of kids and lambs are compared with those in calf urine in the examples shown in the paper electrophoretograms of Fig. 2. These include an appropriate serum sample for reference. Paper electrophoretograms of the colostrum proteins for comparison with the urine proteins are given by McDougall (1965). Early samples of urine usually contain the faster-migrating proteins. The faster-migrating proteins

of kid and lamb urine have a lower mobility than those of the faster-migrating proteins of calf urine. The mobilities of the fast-migrating urine proteins are close to those of the faster components of the corresponding colostrum. Later samples contain in addition a slow component with a mobility close to that of γ -globulin of serum. In kid urine this protein was present only in traces. In calf urine its mobility was slightly less than that of γ -globulin and the immune lactoglobulin. A typical comparison of these proteins is shown in the paper electrophoretogram of Fig. 3. Some samples of lamb urine contained, instead of the single fast component, a series of fast components that could not be directly related to proteins of colostrum or serum. An example of this is shown in Fig. 2.

Two examples of diagrams obtained by moving-boundary electrophoresis of lamb urine are shown in Fig. 4. In one, the main component is a fast electrophoretic component; in the other, the main component is slow-moving. Traces of a faster component are also present. The average mobilities of these proteins in three samples of lamb urine are included in the legend.

Sedimentation diagrams and sedimentation coefficients of the proteins in calf, kid and lamb urine are given in Fig. 5.

The electrophoretic separation of the components of a calf urine containing fast and slow electrophoretic components is shown in Fig. 6. Fractions I

Table 1. Incidence of proteinuria and absorption of immune lactoglobulin in young calves, kids and lambs

Animal and method of feeding	Presence of immune lactoglobulin in serum	Transient proteinuria (> 1 g./l.)
Kids, bottle-fed	24 out of 50	12 out of 24
Calves, bucket-fed	15 out of 25	7 out of 24
Calves, sucking	36 out of 41	18 out of 20
Lambs, sucking	18 out of 20	19 out of 20

Fig. 1. Scatter diagram showing concentration of protein in urine of newborn ruminants after the ingestion of colostrum. Samples were from lambs (a), kids (b) and calves (c) from the herds of the Rowett Research Institute.

Fig. 2. Paper electrophoresis of urinary proteins collected at different times from newborn ruminants. Veronal buffer, pH 8.6 and I 0.05, was used. The current was 0.4 mA/cm. width of paper for 16 hr. (a) Calf urine (OS, ox serum); (b) kid urine (GS, goat serum); (c) lamb urine (SS, sheep serum). Alb, Albumin; α , β and γ , globulins. The numbers beneath the diagrams indicate hours from birth.

and II were found, on sedimentation analysis, to contain components with sedimentation coefficients 2.7 and 3.3s respectively. Similar experiments on kid-urine proteins gave values of 2.9 and 3.6s, and on lamb-urine proteins of 2.5 and 3.3s, for the fast and slow electrophoretic components respectively. The fast component of the lamb urine appeared to be polydisperse.

DISCUSSION

The incidence of proteinuria and the successful absorption of colostrum was almost universal in the sucking lambs and calves examined. This was not the case, however, for bucket-fed calves or bottle-fed kids. In the kids the low incidence may sometimes be due to the poor quality of some colostrum samples such as was noted by McDougall (1965).

It could also be due to the methods of management or to the personal factor involved in bottle- or bucket-feeding young animals.

The extent of proteinuria in lambs was similar to that in calves, 20 g./l. or more being found, whereas in kids values of only 4 g./l. were attained. This presumably means that the kid ingested relatively less colostrum. The difference observed in the electrophoretic mobility of the slow component of calf urine compared with the immune lactoglobulin of colostrum suggests that this protein has been altered between absorption into the serum and excretion in the urine.

The proteins present in lamb and kid urine differ from those in calf urine in that the fast electrophoretic components of the former are not so fast as in the latter. This is probably due to differences in the mobility of the fast components of the colostrum.

Fig. 3. Paper electrophoretograms of the proteins in a calf urine (CU) compared with those in calf serum (CS) and in colostral whey (W). Veronal buffer, pH 8.6 and I 0.05, was used. The current was 0.4 mA/cm. width of paper for 16 hr. Alb, Albumin; α and β , globulins; β -LG, β -lactoglobulin; ILG, immune lactoglobulin.

Fig. 4. Boundary electrophoresis diagrams of the proteins of lamb urine in veronal buffer, pH 8.6 and I 0.1, at 1° . (a) Main component fast, after 183 min. (E 5.9 v/cm.); (b) main component slow, after 122 min. (E 5.5 v/cm.). ϵ is the stationary boundary in the descending limb. Components 1, 2 and 3 had average mobilities, in three urine samples, of -1.35×10^{-5} , -4.09×10^{-5} and -6.72×10^{-5} cm.²sec.⁻¹v⁻¹.

Fig. 5. Sedimentation diagrams of the proteins of calf, kid and lamb urine in phosphate buffer, pH 7.4 and I 0.2. The direction of sedimentation is from right to left at 59780 rev./min. (a) Calf-urine proteins (1.3 g./100 ml.) after 96 min.; $S_{20,w}$ 2.9s. (b) Kid-urine proteins (1.8 g./100 ml.) after 64 min.; $S_{20,w}$ 3.0s. (c) Lamb-urine proteins (0.6 g./100 ml.) after 96 min.; $S_{20,w}$ 2.9s.

Fig. 6. Preparative electrophoresis of proteins of a calf urine in tris-acetate buffer, pH 8.2 and I 0.1. The current was 200 mA. The throughput time was 150 min. A, Slow component; B, fast component; C, coloured component. Fractions I and II were used for sedimentation analysis (see the text).

These components can be seen in the paper electrophoretograms shown by McDougall (1965).

The sedimentation analyses on calf, kid and lamb urine and on the separated electrophoretic components of these urines showed that only slow-sedimenting components were present. The slow electrophoretic component of the urine is therefore not immune lactoglobulin. This would be expected to have a sedimentation coefficient 7s, as reported by Smith (1946) for cow colostrum. The slow electrophoretic component could be a degraded immune lactoglobulin, as suggested for calf urine by Pierce & Johnson (1960). Degradation of a colostral protein may also explain the multiple electrophoretic components sometimes found in lamb urine.

I am grateful to Mr W. Thomson, Mr J. Gill and Dr Margaret I. Chalmers for access to calves, lambs and kids

under their care, Mr J. Stewart for technical assistance and Dr J. J. Connell, Torry Research Station, Aberdeen, for use of their analytical ultracentrifuge.

REFERENCES

- Cecil, R. & Ogston, A. G. (1951). *J. sci. Instrum.* **28**, 253.
Cole, S. W. (1942). *Practical Physiological Chemistry*, 9th ed., pp. 78, 311 and 363. Cambridge: W. Heffer and Son Ltd.
- Deutsch, H. F. & Smith, V. R. (1957). *Amer. J. Physiol.* **191**, 271.
Hannig, K. (1961). *Z. analyt. Chem.* **181**, 244.
McDougall, E. I. (1965). *Biochem. J.* **94**, 99.
McDougall, E. I. & McCarthy, E. F. (1953). *Biochem. J.* **55**, 177.
Pierce, A. E. (1959). *J. Physiol.* **148**, 469.
Pierce, A. E. & Johnson, P. (1960). *J. Hyg., Camb.*, **58**, 247.
Smith, E. L. (1946). *J. biol. Chem.* **165**, 665.
Smith, T. & Little, R. R. (1924). *J. exp. Med.* **39**, 303.