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CONCENTRATION AND SOME CHARACTERISTICS OF PROTEINS IN CALF URINE AFTER COLOSTRUM INTAKE

FRATRIĆ NATALIJA and STOJIĆ V.

Faculty of Veterinary Medicine, Belgrade, Yugoslavia

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Quantitative and qualitative characteristics of urinary proteins excreted after colostrum intake under physiological conditions by calves were investigated in this study. Tests were carried out on 7 black and white spotted calves from birth to 38 hours old.

The mean concentration of calf urinary proteins, after colostrum intake up to 16 hours after birth was 2,7 ± 2,4 g/l, while after 30 hours it was 2,8 ± 3,5 g/l. The high values of SD indicated great individual variation in concentrations of urinary proteins from the examined calves. The concentration of urinary proteins of newborn calves cannot be analysed using variation measures derived from normal distribution.

Two categories of proteins of different origin were shown to appear in the urine of newborn calves. One was derived from blood plasma proteins, but the other category of proteins was not identified in blood plasma. IgG and albumin were the predominant components among proteins of serum origin.

Albumins excreted in the urine after colostrum intake were observed. They were shown to be heterogeneous with regard to molecular mass and had a higher electrophoretic mobility than blood serum albumins.

Three protein fractions with antigenic properties similar to serum IgG were detected. These fractions differed from complete IgG molecules with respect to electrophoretic mobility and molecular mass. It was concluded that they represented fragments and subunits.

The results interpreted in this study indicate that the urine samples of calves after colostrum intake have at least 2 proteins which could not be found in the blood sera.

Key words: proteinuria, newborn calves, protein fractions, serum proteins, colostral proteins.

INTRODUCTION

The young of some species such as calves, kids, lambs, foals and piglets are born without antibodies (hypogammaglobulinemia) and with only traces of gamma globulin in their blood. The newborn calf derives passive immunity by absorbing gamma globulins from the colostrum. This ability to absorb whole proteins is limited

to the first 24 - 30 hours of life. In fact, most macromolecules are pinocytosed by intestinal (jejunum, anterior ileum) epithelial cells (Brambel, 1970).

Molecules greater than approximately 70000 daltons (which include all Ig molecules) are taken up by the lymphatics. Smaller molecules are absorbed into the circulation. Very small protein molecules, including Ig fragments, are rapidly excreted in the kidneys, giving rise to transient proteinuria in newborn calves. Intestinal Ig absorption significantly decreases with age. A 50% decrease occurs by 9 hours (Selman, 1973) with marked decreases noted as early as 6 hours after birth (Kruse, 1970). The decrease in Ig absorption has several causes, including changes in the epithelial lining cells of the intestine, increases in proteolytic enzymes in the intestinal lumen, and decreases in forestomach pH. The increase in plasma protein concentration is due mainly to the absorption of immunoglobulins which is assumed to be the reason for transient proteinuria.

Numerous authors (Smith & Little, 1924; Pierce, 1959; Pierce & Johnson 1960; Pierce, 1961a and 1961b; McDougal, 1965) have established that the proteinuria of newborn calves developed after colostrum ingestion persisted during the period when the intestine was permeable to proteins (48 hours after birth). The results obtained by these authors showed that the characteristics of certain proteins contributing to proteinuria were not identical with those of serum proteins. Most of these proteins were of small molecularl weight and might be derived from colostral proteins, absorbed by the small intestine. The maximal protein concentration in the urine of newborn calves (2g/100 ml) and piglets (1,3 g/100 ml) was detected within 24 hours after birth, whereas after 72 hours almost no protein could be detected (Pierce & Johnson, 1960; Martinson, 1972). A transient proteinuria was also found in newborn lambs, kids and puppies.

Stojić (1980) reported that protein concentration in morning urine specimens from healthy cattle was 64 ± 27,5 mg/l. Investigations of the physicochemical and immunochemical properties of proteins in normal bovine urine samples (Stojić, 1980) showed that these proteins possess features that make them significantly different from corresponding blood plasma proteins, primarily IgG and albumins. IgG and albumins in the bovine urine are in the form of fragments and not complete molecules. The results obtained by Stojić (1980) also showed that protein components with antigenic properties similar to lambda light chains of human immunoglobulins exist in fractions of comparatively low molecular weight.

Kickofen et al. (1971) indicated that the urine of newborn calves contains Fc and Fab - like fragments originating from IgG_S, the main component of colostrum. The same samples likewise revealed the presence of at least 2 categories of Fc fragments. One of them resembles in size and antigenic characteristics the papain Fc, while the other is evidently smaller and antigenically deficient when compared to the papain Fc fragment.

The subject of this paper is protein heterogeneity in calfs urine sampls. The results obtained indicate that the protein level in calf urine specimens is different from that in adult cattle. Namely, the protein concentration in urine samples is significantly higher than that in adult bovine urine samples.

MATERIALS AND METHODS

Samples. Urine and blood samples were obtained from newborn calves from birth to 38 hours old. Serum samples were obtained after allowing venous blood to clot at room temperature, followed by centrifugation. Two urine samples from each calf were obtained with the specially constructed model. The samples were taken after colostrum ingestion, the first 16 hours after birth, and the second 30 hours after birth. The urine samples were immediately preserved with sodium azide. The total protein concentrations in urine samples were determined by a turbidimetric method using tannic acid (Mejbaum - Katzenelenbogen, 1955). The proteins in these samples were concentrated using the tannin caffeine method. All concentrated specimens were stored at -20^{0}C until investigation.

Analytical techniques

Electrophoresis. Paper electrophoresis was performed on Whatman No. 1 strips (4.0 x 24,0 cm) in 0,1 M veronal buffer (pH 8.6) at 3 - 4 V/cm for 18 hours.

Immunoelectrophoresis. The microtechnique of Scheidigeer (1955) was used. The precipitation lines were developed with the antisera listed in Table 1.

Table 1. List of antisera used in the immunodiffusion technique

	Immunogenical material	Specificity
Rabbit anti bovine antisera	Bovine serum proteins	Polyspecific
Anti urinary antisera (rabbit)	Bovine urinary proteins	Polyspecific
Rabbit anti-bovine IgG	INEP - Zemun	Monospecific
Sheep anti-bovine IgG1	INEP - Zemun	Monospecific
Rabbit anti-bovine IgG2	INEP - Zemun	Monospecific
Anti-albumin antisera (rabbit)	Bovine serum albumin	Monospecific

Gelfiltration. Serum and urinary proteins were fractionated on a Sephadex G-200 (Pharmacia, Uppsala, Sweden) column (2.0 x 100 cm) using a 0.1 M TRIS (1.0M NaCl/pH 8.0) buffer as eluent at a constant flow rate of 16 ml/hour. Protein distribution in the eluates was determined at 280 nm using a Beckman DU-2 spectrophotometer. Gel filtration was carried out according to the technique describd by Porter et al. (1967).

RESULTS AND DISCUSSION

Protein concentration in calf urine

An analysis of the vaues obtained for protein concentration in the urine of calves after colostrum intake up to 38 hours old is given in Table 2.

The mean protein concentration in the sample 16 hours after birth was 2,7 ± 2,4 g/l, while after 30 hours it was 2,8 ± 3,5 g/l. Individual values ranged from 0,107 - 5,850 g/l for the first sample and 0,139 -8,950 g/l for the second sample. These data showed that the concentration of urinary proteins of newborn calves cannot be analysed using variation measures derived from normal distribution. This is in agreement with the findings of Stojić (1977, 1980) who demonstrated that protein concentrations in bovine urine are not distributed normally. A more precise

evaluation of the proteinuria range in cattle can be given using a Poisson type of distribution.

Table 2. Values for total protein concentration in the urine of newborn calves

	The time of sampling	
	First sample-to 16 hours	Second sample-to 30 hours
Number of samples	7	7
Mean SD	2.7± 2.4 g/l	2.8±3.5 g/l
SE	0.92 g/l	1.35 g/l
CV	80%	125%
Range	0.107 - 5.850 g/l	0.139 - 8.950 g/l

Types of heterogeneity of urinary proteins

Electrophoretic heterogeneity. The electrophoretic heterogeneity of urinary proteins of calves was determined by paper electrophoresis (Figure 1).

Paper electrophoresis (Figure 1) showed that serum of newborn calves does not contain any protein with an electrophoretic mobility of γ - globulin. After

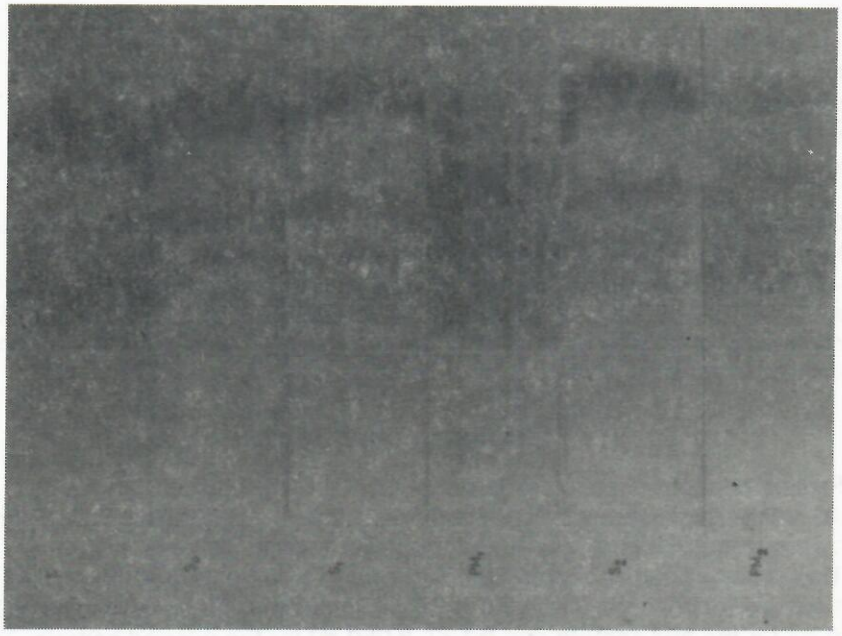

Figure 1. Proteinograms: S - blood serum; S_0 - newborn calf serum before the intake of colostrum; S_1 - calf serum after colostrum intake; PM_1 -, urinary proteins of the first sample; S_2 - calf serum 30 hours after birth; PM_2 - urinary proteins of the second sample.

ingestion of colostrum, however, appreciable amounts of colostral IgG appeared in calf serum. At this time electrophoresis separated the proteins of calf urine into four fractions corresponding to blood serum albumin, alpha, beta and gamma globulins. Their relative amounts are given in Table 3.

Table 3. The relative amounts of protein fractions

sample	albumins %	globulins %		
		alpha	beta	gama
1	17.79±5,77	35.96±7,27	18.6±4,36	27.6±6.42
2	19.8± 6.08	35.87±4.38	20.01±4.81	27.46±6.30

The relative amount of the albumin fractions was significantly lower than the globulin fractions (A/G = 0.22 for the first sample, A/G = 0.24 for the second sample). Đurđević (1963) also showed that the excretion of serum albumin is much smaller than for serum globulins (whose molecular weight is twice bigger). Thus, in addition to the molecular weight and configuration, the electrical charge of macromolecules and of the capillaries, plays a vital role in regulating the transglomerular passage of various macromolecules.

The relative amount of the alpha globulin fraction was higher than the other globulin fractions. This results from the elimination of fetal serum proteins (mainly α - glycoproteins, termed fetuin) by the urine of newborn calves (Pedersen, 1944). This is in agreement with the findings of Jamesson et al. (1942) and Spiro (1960), who showed that the sera of newborn calves contained large amounts of α - globulins, and that the level of these decreased to essentially adult values within a few days.

The relative amount of the gamma globulin fraction was high as a result of the absorption of colostral IgG.

Heterogeneity with respect to molecular weight. Urinary protein heterogeneity with respect to molecular weight was evaluated by gel filtration on a Sephadex G-200 column (Figure 2). Urinary protein chromatograms are presented in Figure 3 which shows that urinary proteins of the first sample elute in two chromatographic peaks. The position of peak I on the urinary protein chromatogram of the first sample, indicates similarity to chromatographic peak I for serum proteins. Peak II elutes when serum protein elution is finished. Urinary proteins of the second sample elute into three chromatographic peaks. Peak I of the urinary protein chromatogram of the second sample corresponds to peak I of the serum protein chromatogram. Peak II of urinary proteins corresponds to peak III for serum proteins. Urinary proteins of peak III elute after all serum proteins had eluted.

The results showed that urinary proteins of calves are a heterogeneous set of molecules whose quantitative and qualitative relations vary depending on sampling time.

Immunoelectrophoretic heterogeneity. Immunoelectrophoretic assays of urinary proteins with specific antisera against serum and urinary proteins had a dual objective: firstly, to determine which blood serum proteins were excreted via

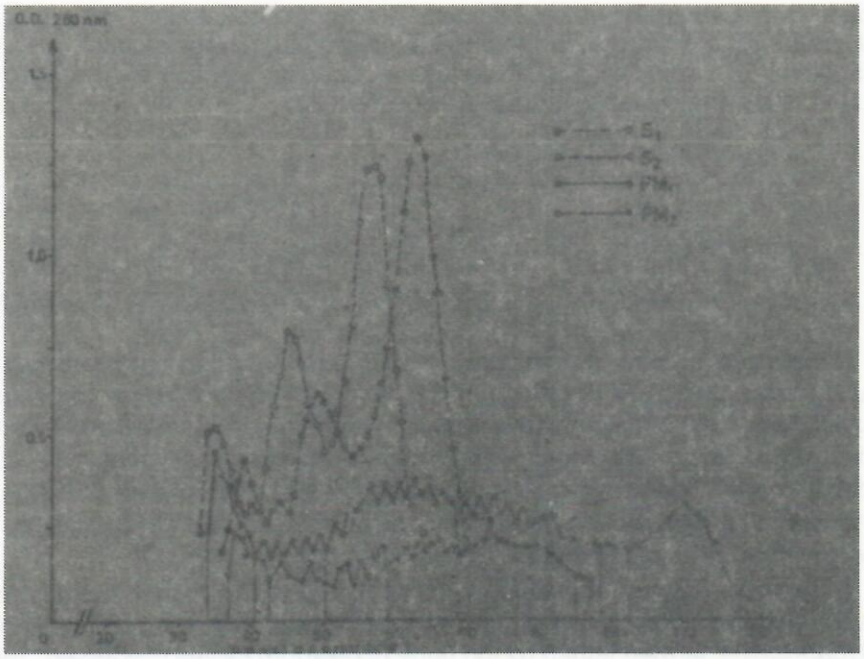

Figure 2. Chromatograms: S_1 - calf serum after colostrum intake; PM_1 - pooled urinary proteins of the first sample; S_2 - calf serum 30 hours after birth; PM_2 - pooled urinary proteins of the second sample. Depicted details mark eluate fractions separately analyzed later on.

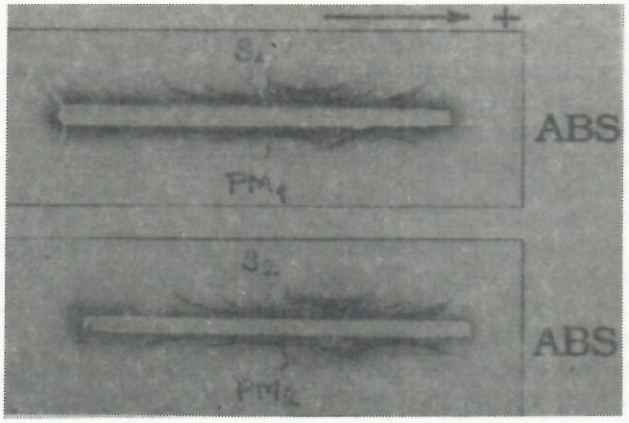

Figure 3. Immunoelectropherograms: S_1 - calf serum after colostrum intake; PM_1 - pooled urinary proteins of the first sample; S_2 - calf serum 30 hours after birth; PM_2 - pooled urinary proteins of the second sample developed with polyspecific antiserum (ABS).

the urine under physioloical conditions by calves and secondly, to estabish which proteins were present in urine, but not in serum.

Urinary proteins of blood origin

Immunoelectropherograms for urinary proteins obtained with polyspecific antisera against serum proteins always showed 5 precipitation arcs in the first sample and 7 precipitation arcs in the second sample. These were distributed on the electrophoretic field from the prealbumin zone to the gamma globulin zones. The characteristic appearance of the electropherogram is presented in Figure 3.

Due to their morphological characteristics it was always possible to detect precipitation arcs that correspond to serum albumins and two precipitation lines in the gamma globulin zones. The precipitation arc for urinary albumins always developed from a diffuse center of greater electrophoretic mobility than the precipitation arc for serum albumins. Similar characteristics were noted for adult bovine and human urinary albumins (Shultze and Heremans, 1968).

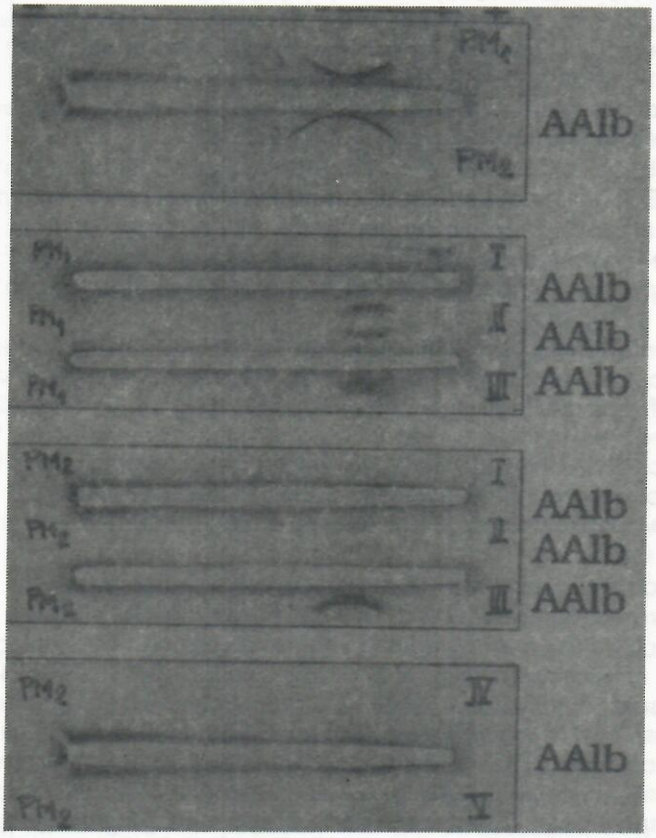

Figure 4. Immunoelectropherograms: PM_1 - pooled urinary proteins of the first sample; PM_2 - pooled urinary proteins of the second sample; I, II, III chromatogram protein fractions of the first sample: I, II, III, IV and V chromatogram protein fractions of the second sample (depicted in Fig.3); developed with monospecific antiserum against serum albumin (AAl).

The presence of albumin in peak II of the chromatogram (2,3,4 fractions) obtained by gelfiltration on Sephadex G-200 indicates that urinary albumins encompass three categories of molecules (Figure 4).

The first category, with a greater molecular weight than those in serum, could in part result from polymerization (Walevik, 1979) and in part from their tendency to form complexes with other proteins, primarily with immunoglobulins (Harboe and Folling, 1974) or their Fc fragments (Laurel, 1976). The second category had values close to the molcular weight of albumin and finally there was a category with a lower molecular weight than that of albumins. Since bovine blood serum albumins are comprised of one peptide chain and do not have a subunit structure (Brown, 1975), it can assumed that the albumins of low molecular weight found in calf urine are in fact serum albumin fragments. This supposition is based on data, that the sedimentation constant for urinary albumins is less than that for serum albumins (Schultze and Heremans, 1966). Walewik (1979) reported that the degradation of albumin occurs in the circulation or in the intercellular space. In investigations of immunochemical properties of serum albumins Ahmed et al. (1976) showed that the C - terminal fragment (M.W. 12000) obtained by degradation of molecules in vitro, included 80% of the antigenic determinants. This is the fragment that reacts with monospecific antiserum against serum albumin.

On the basis of immunoelectrophoretic analysis with polyvalent anti-bovine antiserum (ABS), it was not possible to conclude whether IgG was present in the urine specimens or not. However, two partly similar precipitin lines were always found in the gamma-zone. Their morphological properties could signify the presence of protein components in urine samples with the antigen specificity of blood serum IgG (Figure 3).

The immunoelectrophoretic analysis of urinary proteins performed with monospecific antiserum against serum IgG indicated that proteins with antigenic properties of blood serum IgG were involved (Figure 5). Therefore, the two aforementioned precipitation lines in the gamma - zone possess the antigenic characteristics of IgG. Nevertheless, this antiserum uncovered a third fast migrating protein component whose precipitation arc was clearly positioned in the anode region. This was considerable in the findings of Stojić (1980) after analyzing normal adult bovine urine.

This indicates that urine of newborn calves after ingestion of colostrum contains a protein component with IgG specificity, which is lacking in calf serum as well as adult bovine serum under the same conditions. This is in agreement with the findings of Kickhofen et al (1971) and Stojić (1977, 1980). Therefore, the obtained results illustrate that three different molecular categories of proteins, which possess part of the antigenic structure of blood serum IgG exist in urine.

Immunoelectrophoretic analysis of Sephadex chromatography fractions of urine with monospecific antiserum, demonstrated that all fractions of the chromatographic peak II contained components with antigenic properties similar to IgG (Figure 6). This analysis showed the presence of three precipitin arcs. One arc of fast mobility was observed in the anode region and the other two precipitin arcs developed in the cathode region in the gamma globulin zone. There is evidence for the existence of at least three categories of IgG molecules (IgG - like

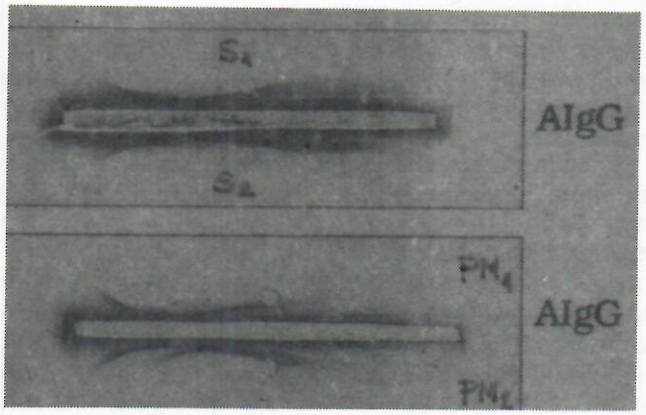

Figure 5. Immunoelectropherograms: S_1 - calf serum after colostrum intake; S_2 - calf serum 30 hours after birth; PM_1 - pooled urinary protein of the first sample; PM_2 - pooled urinary protein of the second sample developed with monospecific antiserum AlgG.

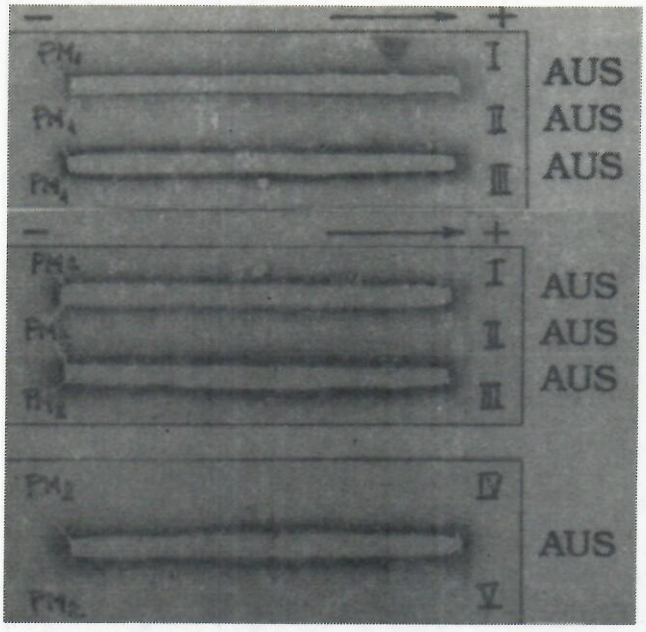

Figure 6. Immunoelectropherograms: I, II, III chromatogram protein fractions of the first sample; I, II, III, IV and V chromatogram protein fractions of the second sample developed with monospecific antiserum against serum IgG (AlgG).

molecules) which differ in molecular weight. Their presence in the fractions of peak II of urinary proteins which eluted when serum protein elution is finished indicated that their molecular weight is less than the molecular weight of serum albumin. Furthermore, their presence in the protein fractions of peak II, which corresponds to chromatographic peak III for serum proteins, suggests that the molecular weight of urinary IgG is less than that of analogous serum proteins. Therefore, it may be concluded that normal urine does not contain whole IgG molecules, but rather various structural parts formed either as a result of fragmentation of complete molecules or by dissociation of the subunits.

In view of the finding of an electrophoretically fast IgG component, which is definitely a characteristic of the urinary immunoglobulin system, we believe that a component corresponding to the Fc fragment is originating from IgG. The other two categories of urinary proteins could represent parts of IgG molecules similar to Fab fragments. This is in agreement with the findings of Kichofen et al. (1971) and Martinson (1972). Bergarde and Bennich (1967) have identified Fc fragments in human serum and urine. Stojić (1980) reported the presence of IgG Fc fragments in bovine urine. He supposed that they originate from serum and are filtered through the glomeruli. Micogouchi et al. (1978) stated that receptors for Fc fragments exist in the glomerular wall. This indicates that when Fc fragments are found in the circulation the glomerulus is the spot where they are retained and filtered into the

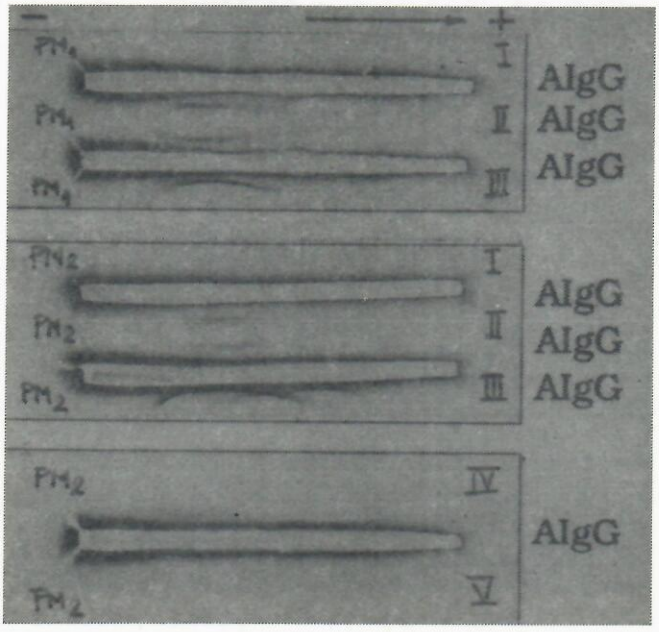

Figure 7. Immunoelectropherograms: I, II, III chromatogram protein fraction of the first sample; I, II, III, IV and V chromatogram protein fractions of the second sample developed with polyspecific antiserum against urinary proteins (AUS).

primary urine. Striker et al. (1987) reported the presence of receptors for the Fc portion of IgG on mesangial cells.

Urinary proteins of non - serum origin

Immunoelectrophoretic analysis of proteins constituting individual peaks in the chromatogram developed with polyspecific antiserum against urinary proteins (AUS) confirms the previous finding, that the majority of proteins elute in peak II on chromatograms obtained by gel filtration on Sephadex G-200 columns (Figure 7).

Immunoelectrophoresis of proteins eluted in peak II, with AUS antisera revealed 8 precipitation arcs. This indicates that the urinary protein system of calves consists of at least 8 protein components antigenically different.

When antibodies specific to serum proteins are removed from this antiserum (AUS), the presence of proteins is revealed in two precipitin arcs in fraction 3 of chromatographic peak II (Figure 8).

The results obtained in this analysis show that urine samples of calves after colostrum intake have at least 2 proteins which could not be detected in the blood sera. The results obtained by Stojić (1980) indicate that urine samples from cows have at least 5 proteins (derived from the urinary tract) with a lower molecular weight than serum albumins.

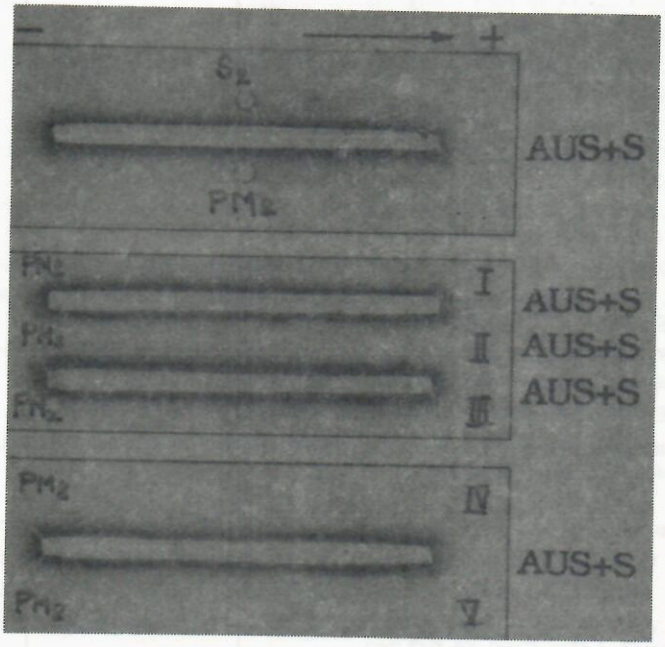

Figure 8. Immunoelectropherograms: S_2 - calf serum 30 hours after birth; PM_2 - pooled urinary protein of the second sample; I, II, III, IV and V chromatogram protein fractions of the second sample developed with polyspecific antiserum against urinary proteins previously absorbed from serum proteins (AUS + S).

The results of this investigation show that the urinary proteins excreted under physiological conditions by calves represent a complex system of protein components originating from the blood serum and partially from the urinary tract. Serum derived urinary proteins are significantly different from the respective blood plasma proteins which is most pronounced in the immunoglobulin G class and albumins. Immunoglobulins G and some albumin fractions in the urine of new born calves are found not as complete molecules but as fragments.

Values obtained for the molecular weight of calf urinary proteins after colostrum intake, indicate that the permeability of the glomerular capillary wall in calves is similar to that in adult cattle. However, these findings do not exclude the possibility of trace quantities of complete IgG molecules existing in the primary urine and their degradation in the urine tract.

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KONCENTRACIJA I NEKE KARAKTERISTIKE URINARNIH PROTEINA TELADI POSLE UZIMANJA KOLOSTRUMA

FRATRIĆ NATALIJA i STOJIĆ V.

SADRŽAJ

Proučavane su kvantitativne i kvalitativne karakteristike urinarnih proteina koje tele izlučuje pod fiziološkim uslovima posle uzimanja kolostruma. Ispitivanja su vršena na sedmoro muške teladi crno-bele rase krava od rođenja do 38 časova.

Prosečna koncentracija proteina u urinu novorođene teladi posle uzimanja kolostruma do 16 časova posle rođenja iznosila je 2,7±2,4 g/l, a 30 časova 2,8± 3,5 g/l. Visoke vrednosti standardne devijacije ukazuju na velika individualna variranja u nivoima urinarnih proteina kod ispitivane teladi. Koncentracija urinarnih proteina u teladi posle uzimanja kolostruma ne može se prikazivati merama varijacije proizvedenim iz normalne distribucije frekvencija.

Proučavanja fizičko-hemijskih osobina urinarnih proteina, pokazala su da se u mokraći novorođene teladi nalaze dve kategorije proteina različitog porekla: jednu čine proteini krvne plazme, a drugu proteini koji se nalaze u urinu ali ne i u serumu. Od proteina serumskog porekla, IgG klase i albumini su najviše zastupljeni.

Zapaženo je da su albumini izlučeni u mokraći posle uzimanja kolostruma, heterogeni po molekulskoj masi i da imaju veću elektroforetsku pokretljivost od albumina krvnog seruma.

Utvrđene su tri proteinske frakcije sa antigenskim karakteristikama sličnim serumskim IgG. Sobzirom da se ove frakcije razlikuju po elektroforetskoj pokretljivosti i molekulskoj masi od kompletnih molekula IgG, zaključili smo da se ovde radi o fragmentima i subjedinicama.

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Fratrić Natalija et al.: Concencration and some characteristics of proteins in calf urine after colostrum intake

Rezultati postignuti u ovom radu pokazuju da mokraća novorođene teladi posle uzimanja kolostruma sadrži najmanje dve proteinske komponente koje se nisu mogle dokazati u krvnom serumu.