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The Total Protein and Immunoglobulin Profile of Equine Colostrum and Milk

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Summary. Thirty-six samples of colostrum and milk were collected from ponies at various intervals after parturition. Levels of total protein and immunoglobulins IgG and IgG(T) were determined. In samples collected in the first 3 hours *post partum*, the total protein concentration was approximately twice that of serum protein and the immunoglobulins IgG and IgG(T) accounted for 30 per cent and 10 per cent respectively of this protein. The ratio of IgG to IgG(T) was similar to that in serum.

After suckling, a marked decline in both total protein and immunoglobulin concentration occurred. In addition, the relative concentration of immunoglobulins declined. Thus, IgG and IgG(T) made up 7 per cent and 2 per cent respectively of the total protein in samples collected 9–24 hours *post partum*. In all samples examined up to 33 days *post partum* the concentration of IgG (γ_2) was approximately three times the concentration of IgG(T) (γ_1). Thus no evidence was found for a selective secretion of a γ_1 immunoglobulin in equine colostrum or milk.

INTRODUCTION

The immunoglobulins of human colostrum have been studied extensively during the past few years and the literature has been reviewed by Heremans (1968), and Tomasi and Bienenstock (1968). As in other human external secretions, the predominant immunoglobulin in colostrum is IgA of the secretory type. Immunoglobulins M and G occur in colostrum but only as minor components. It has been suggested that the immunoglobulins of human colostrum are synthesized locally in the mammary gland and are not transferred from serum (Hochwald, Jacobson and Thorbecke, 1964).

With regard to the colostral immunoglobulins of lower animals, some species resemble man but most domestic species differ markedly. Thus the mammary secretions of the rabbit and dog are akin to man in that secretory IgA is the predominant immunoglobulin (Cebra and Robbins, 1966; Feinstein, 1963; Vaerman and Heremans, 1969). In the rabbit, colostral immunoglobulins are partially at least derived from serum and are not synthesized locally as occurs in man (Asofsy and Small, 1967). However, in ruminants, pigs and horses secretory IgA either does not occur in colostrum or makes only a minor contribution to the total immunoglobulin (Bourne, 1969; Genco, Yecies and Karush, 1969; Heimer, Jones and Maurer, 1969; Mach, Pahud and Isliker, 1969; Sullivan, Prendergast, Antunes, Silverstein and Tomasi, 1969). Immunoglobulin G is the predominant immunoglobulin in colostrum of these species. It is apparent from studies in the bovine that

colostral immunoglobulins are derived by selective transfer from serum and are not synthesized in the mammary gland (Blakemore and Garner, 1956; Dixon, Weigle and Vazquez, 1961; Pierce and Feinstein, 1965). That the transfer is selective is shown by the finding that IgG_1 may be present in colostrum at up to 100 times the concentration of IgG_2, but in serum the latter predominates (Murphy, Aalund, Osebold and Carroll, 1964). Recent studies in the pig have shown that whereas in early colostrum IgG accounts for 80 per cent of the total immunoglobulin, after 72 hours and throughout the subsequent lactation IgA predominates (Porter and Noakes, 1969).

With regard to equine colostral immunoglobulins Genco *et al.* (1969) have recently reported that the ratio of the three major serum immunoglobulins IgG, IgG(T) and IgM occur in the same ratio in colostrum as they do in serum. They found no evidence for the selective secretion of a γ_1 immunoglobulin that occurs in other species. However these workers only studied two colostral samples collected about 24 hours after parturition.

In this communication we record the concentration of total protein, IgG and IgG(T) (termed IgT in this paper) in many different samples of colostrum collected at various times after parturition.

MATERIALS AND METHODS

Collection of samples. Thirty-six samples of colostrum and milk were collected from Shetland type ponies at various intervals after parturition. The samples were cooled and then centrifuged at 20,000 ***g*** for 30 minutes at 4°. The clear fluid between the fatty supernatant and the sediment was stored at −20°. Serum samples were collected at the same time.

Gel diffusion. A micro method was used with equipment supplied by the Gelman Instrument Co., Ann Arbor, Mich. (Ouchterlony, 1968).

Immunoelectrophoresis. A modification of the micromethod of Scheidegger (1955) was used with equipment supplied by the Gelman Instrument Co. The gel was Ionager No. 2 in barbital buffer ($\mu = 0{\cdot}025$). The chamber buffer was barbital buffer of ionic strength 0·1.

Preparation of antisera. (*a*) *Antihorse serum.* Five rabbits were injected subcutaneously in multiple sites with whole horse serum (10 mg of protein) emulsified in Freund's complete adjuvant (Difco). Subsequent injections in incomplete adjuvant were made at monthly intervals. Bleedings were taken at intervals and sera showing multiple antiprotein arcs by immunoelectrophoresis against horse serum were pooled.

(*b*) *Antiglobulin.* Purified IgG and IgM were prepared by the methods of Rockey (1967) and Zolla and Goodman (1968) respectively. IgT antigen was kindly supplied by Dr J. H. Rockey of the Pennsylvania Medical School, Philadelphia. Approximately 1 mg of purified antigen contained in 0·75 ml of phosphate buffer was emulsified with an equal volume of Freund's complete adjuvant. Rabbits were immunized by inoculation of the emulsion into the footpads (0·1 ml per pad), followed by a second injection of 1 mg of antigen emulsified in Freund's incomplete adjuvant into the same sites 3 weeks later. Antisera were collected after 3–4 weeks.

Each antiglobulin reagent was rendered monospecific by absorption with the appropriate concentrated serum fractions. All three antisera gave only one precipitation arc when tested against whole serum as antigen.

Measurement of immunoglobulins. The concentration of IgG and IgT was measured by the quantitative Oudin test in capillary tubes (Lovett-Mosely, 1968) and the results photo-

graphed. Standard curves were constructed using purified antigen preparations. Prior to the preparation of standard concentrations, the antigens were centrifuged at 105,000 ***g*** for 1 hour to remove aggregated protein. Protein concentrations were measured by the optical density at 277 mμ in 0·25 M acetic acid using assumed extinction coefficients ($E^{1\%}$ 1 cm) of 15 and 14·7 for IgG and IgT respectively.

Measurement of the total protein in colostrum and serum. The Biuret method was used.

RESULTS

(*a*) *Immunoelectrophoresis.* Fig. 1 shows the patterns of four colostra collected from the same pony at different times after parturition. Patterns from other colostra at the same stage were almost identical. Colostrum collected 1 hour after parturition contained a multitude of proteins including IgG, IgT and IgM. However after 14 hours only six protein arcs were observed (numbered 1–6 in Fig. 1). Of these six, IgG (No. 1), IgT (No. 3) and albumin (No. 5) were the most obvious. Immunoglobulin M was not detected by immunoelectrophoresis in this sample nor in those collected later. By the 9th day and 16th day, IgG, IgT and albumin were still demonstrable but the precipitation arcs were less distinct, suggesting lower antigen levels. In samples collected at 14 hours or later, the IgG was electrophoretically less heterogenous and more cathodal than serum IgG.

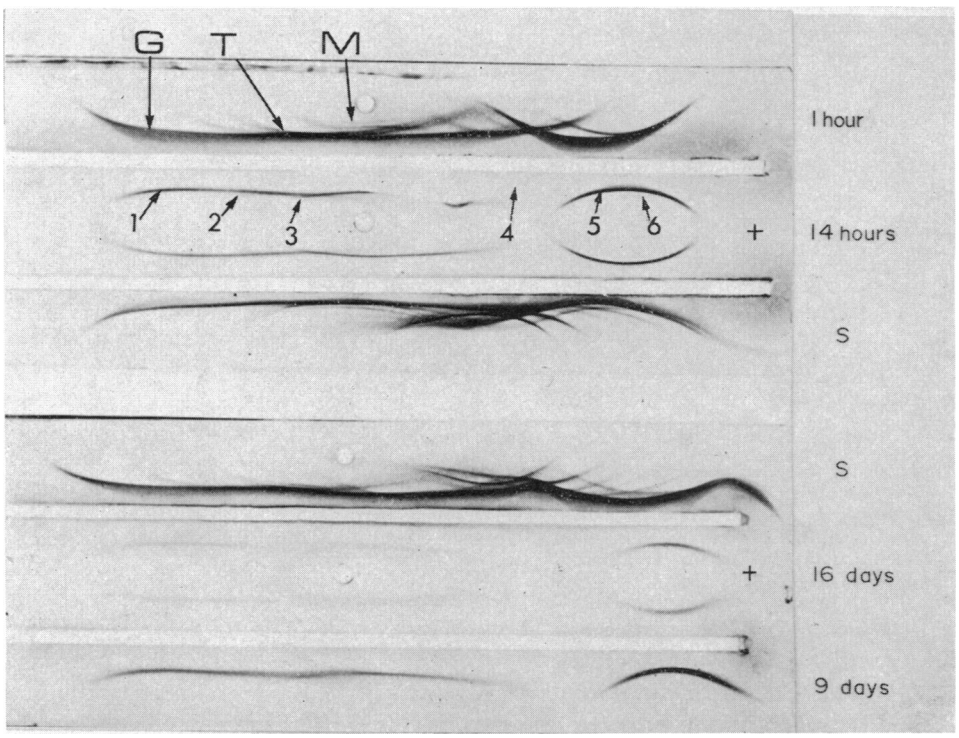

FIG. 1. Immunoelectrophoresis of colostrum collected from a pony at 1 hour, 14 hours, 9 days and 16 days *post partum* against antihorse serum. Two wells contain serum (S) collected from the same pony at 1 hour post partum. Immunoglobulins G, T and M are indicated in the 1 hour colostrum. The numbered arcs are referred to in the text.

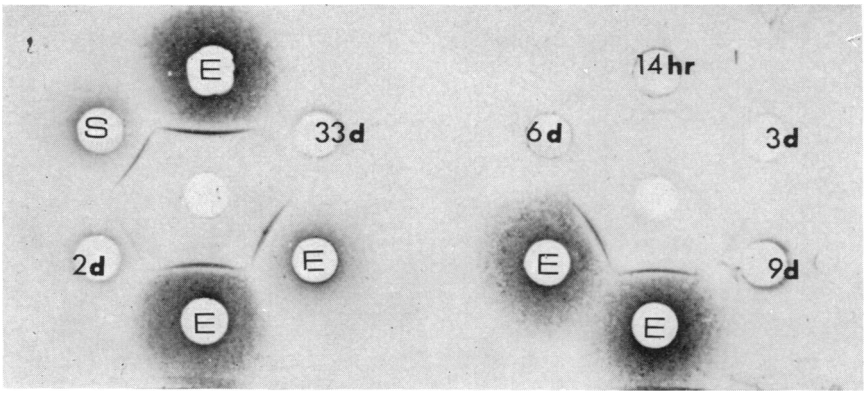

FIG. 2. Gel diffusion of eleven samples of colostrum collected at different intervals after parturition and 1 normal serum sample (S) against monospecific anti IgM serum. The time of collection (14 hours to 33 days) is indicated. Samples labelled E were collected between 0 and 3 hours after parturition.

(*b*) *Gel diffusion.* A number of samples of colostrum are shown reacting against specific anti IgM and anti IgT sera in Figs 2 and 3. All the samples, including one collected 33 days after parturition contained IgM. However, the precipitin lines in samples collected at 14 hours after parturition or later were very weak. Samples of colostrum and milk collected at intervals of up to 9 days after parturition all contained IgT (Fig. 3).

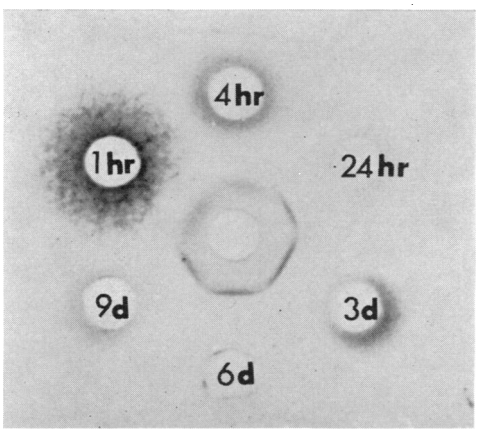

FIG. 3. Gel diffusion of six samples of colostrum collected at different intervals after parturition against monospecific anti IgT serum. The time of collection (1 hour to 9 days) is indicated.

(*c*) *Total protein and immunoglobulin.* The concentration of total protein and immunoglobulin G and T in colostrum collected during the first 3 hours after parturition is shown in Table 1. The amount of total protein in colostrum was approximately twice that in serum collected at the same time. In one sample (A13), taken prior to suckling, the total protein concentration was × 25g/100 ml. Levels of IgG ranged from 3·1 to 6·0g/100 ml and accounted for 21·8–43·5 per cent of the total protein. However, levels of IgT were lower and ranged from 0·6 to 3·0 g/100 ml.

TABLE 1

TOTAL PROTEIN AND IMMUNOGLOBULIN G AND T IN COLOSTRUM COLLECTED 0–3 HOURS AFTER PARTURITION

Pony	TP* in Colostrum (g/100 ml)	TP in serum (g/100 ml)	IgG (mg/100 ml)	IgT (mg/100 ml)	IgG (per cent of TP)	IgT (per cent of TP)	Colostrum IgG : IgT	Serum IgG : IgT
8	13·2	7·4	5750	2820	43·5	21·5	2	
A5	15·6	7·2	5700	1800	36·5	11·6	3	1·5
10	11·9	8·0	3120	875	26·0	7·4	3·5	2
26	15·6	7·2	3400	3000	21·8	19·2	1	1·3
A13	25·0	7·7	6000	1900	24·0	7·6	3	3·5
A14	11·5	7·7	3400	880	29·5	7·6	3·5	2
49	15·1	7·5	5400	600	34·5	3·8	9	7
A19	15·6	6·3	5200	725	33·5	4·6	7	4
A17	10·6	7·2	3450	700	32·5	6·7	7	2

* TP—total protein concentration.

The ratio of IgG to IgT in colostrum ranged from 1 : 1 to 1 : 9. Except in one sample (A17), the ratio of the two globulins in serum collected at the same time was of the same order of magnitude.

A dramatic decline in the concentration of total protein and immunoglobulin in colostrum occurred between 0–3 hours and 4–8 hours after parturition (Table 2 and Fig. 4). Thus, the mean IgG concentration in the 0–3 hr samples was 4500 mg/100 ml (representing 30 per cent of the total protein), but by 4–8 hours had fallen to 726 mg/100 ml. By the 2nd day, the secretion resembled milk and there was no further decline in the total protein, IgG, or IgT after this time. In all the samples the ratio of IgT to IgG remained approximately the same.

Although a decline in total protein level occurred with the fall in the concentration of immunoglobulin, the decrease in immunoglobulin was far more striking. Thus, in the 0–3 hour samples, IgG and IgT accounted for 30 per cent and 10 per cent of the total protein respectively, but by 9–24 hours was only 6·7 per cent and 2·1 per cent, and by 1–5 days had fallen to 1·8 and 0·33 respectively.

TABLE 2

MEAN VALUES FOR TOTAL PROTEIN AND IMMUNOGLOBULIN G AND T IN COLOSTRUM COLLECTED AT INTERVALS OF 0 HOURS TO 33 DAYS AFTER PARTURITION

Stage of Lactation	No. of samples	TP* (g/100 ml)	IgG (mg/100 ml)	IgT (mg/100 ml)	IgG : IgT	IgG (per cent) of TP)	IgT (per cent) of TP)
0–3 hours	9	14·9	4500	1500	3	30	10
4–8 hours	6	5·05	726	187	4	14·4	3·7
9–24 hours	7	3·85	250	81	3	6·7	2·1
1–5 days	6	4·05	72	13·5	5	1·8	0·33
6–33 days	7	4·1	45	17	3	1·1	0·41

* TP—total protein concentration

DISCUSSION

The most striking feature of these results was the dramatic change in total protein and immunoglobulin levels that occurred in colostrum collected only a few hours after suckling. Thus, the concentration of total protein was about twice that of serum in early colostrum, but by 9–24 hours had fallen to approximately one half of the serum level.

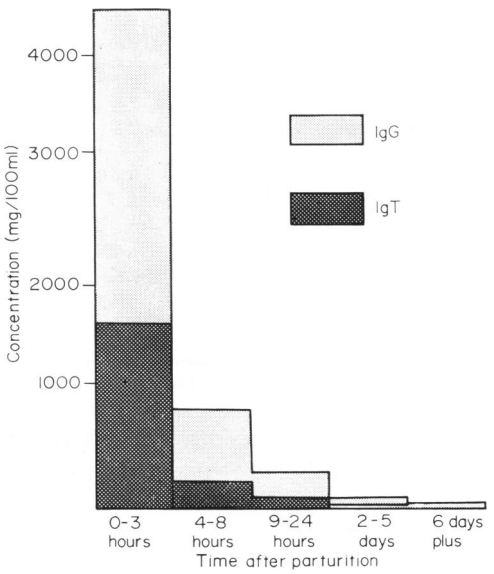

FIG. 4. The concentration of immunoglobulins G and T in colostrum collected at different intervals after parturition.

Similar observations were made with cows (Dixon *et al.*, 1961; Nordbring, 1957a) and sows (Bourne, 1969; Karlsson, 1964). In the sow, Bourne (1969) demonstrated a fall in the concentration of the mean total protein in colostrum from 19·6 g/100 ml to 4·1 g/100 ml in 24 hours. These values resemble our results. However, it is probable that prior to suckling, equine colostrum has an even higher concentration of total protein because some samples are so viscous that it is impossible to pour them from a beaker. In the present investigation only one such secretion (A13) approached that consistency, the total protein concentration being 25 g/100 ml. In man, a fall in total protein concentration also occurs, but the decline is more gradual (Nordbring, 1957b).

More impressive than changes in total protein concentration were those of the immunoglobulin levels. In early colostrum these proteins represented more than 40 per cent of the total protein, but by one day they accounted for only a minor portion. Similar results have been recorded for woman (Lundsford and Deutsch, 1957; Nordbring, 1957b), the cow (Dixon *et al.*, 1961; Larson, 1958) and the sow (Karlsson, 1964). Changes in immunoglobulin concentration have also been noted indirectly by a fall in antibody titre as colostrum was replaced by milk. Bruner, Edwards and Doll (1948) found a lower level of red cell and anti-bacterial agglutinins in mare milk compared with colostrum. They also observed that the udder secretion changed from colostrum to milk in less than 12 hours when suckled by active foals. The present studies support this observation.

Although the decline of immunoglobulin levels was pronounced the ratio of IgG to IgT varied only slightly in early colostrum and 'mature' milk. In both, the concentration of IgG was always higher than IgT and the ratio of these two immunoglobulins was similar to that in serum. In this respect these studies support the view of Genco *et al.* (1969) who, from investigations on two 24–26 hour samples of colostrum, came to the same conclusion. In addition, in those samples collected at 14 hours at later, the population of IgG molecules

was electrophoretically less heterogenous than serum IgG with only the slow-moving (γ_2) molecules being present. It appears, therefore, that the horse differs from other animals studied in that a γ_1 immunoglobulin does not predominate in colostrum or milk. This observation supports the possibility that no division exists between the serum and secretory immunoglobulin systems in the horse.

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