Acta vet. scand. 1979, *20*, 469—476.

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COLOSTRAL TRYPSIN-INHIBITOR CAPACITY IN DIFFERENT ANIMAL SPECIES

By

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SANDHOLM, M. and T. HONKANEN-BUZALSKI: *Colostral trypsin-inhibitor capacity in different animal species.* Acta vet. scand. 1979, *20*, 469—476. — Colostral trypsin-inhibitor capacity was monitored during the first two weeks from parturition. The colostrum of the mare, sow, cow and ewe showed high antitrypsin activity at parturition, decreasing to about one hundredth during the first week. Canine milk remained on a relatively high antitrypsin level, and human milk was poor in antitrypsin from childbirth. The antitrypsin content seems to parallel the known changes in the colostral immunoglobulin levels of different species. The role of antitrypsin in protection of immunoglobulins from proteolytic damage during passive transfer of immunity to the newborn is obvious.

colostrum; trypsin inhibitor; protease inhibitors; colostral albumin.

Colostrum has been shown to contain protease-inhibitor activity; *Laskowski* and others described a trypsin inhibitor in bovine, sow and human colostrum (*Laskowski & Laskowski* 1951, *Laskowski et al.* 1957). The trypsin inhibitor in bovine colostrum is a glycoprotein with a molecular weight of about 12 000 (*Pineiro et al.* 1975, *Cechova* 1976). The distribution of proteins in colostrum differs from that in mature milk, thus the total protein in bovine colostrum is high, being about 150—200 g/l immediately after parturition, and some 60 % of this may be immunoglobulin (*Butler* 1974). The colostral immunoglobulin is important in the transfer of immunity to the offspring. Much of the lacteal immunoglobulin is derived from serum (central compartment) and some seems to be synthetized locally (secre-

tory compartment) (*Butler*). Animal species such as the sow, mare, cow and ewe that transfer little or no immunoglobulin to the foetus in utero contain large amounts of serum-derived IgG in their colostrum. On the other hand, e.g. human milk shows low levels of immunoglobulin.

The function of the colostral trypsin inhibitor might be to protect immunoglobulin molecules from proteolytic damage during the colostral-intestinal "transfusion" into the offspring. The antitrypsin activity and the colostral immunoglobulin of the sow have been seen to be transferred into the circulation of its offspring concomitantly, after which much of the antitrypsin is eliminated into the urine (*Carlsson et al.* 1974, *Jensen* 1978).

The present report describes species-specific variation of colostral trypsin-inhibitor capacity during the postparturient two-week period. The changes are comparable with the known differences in the immunoglobulin content of colostrum and the mechanism of immunoglobulin transfer to the offspring.

MATERIALS AND METHODS

Samples of colostrum from the mare, sow, ewe, cow, bitch and woman were collected immediately after parturition and 12 h thereafter followed by a sample taken daily over two weeks. In the cases where zero samples could not be obtained, the first sample was taken as soon as possible after parturition. A sample of a milk pool was obtained at a dairy to determine the average antitrypsin content in milk. Serum samples were collected from three animals of each species and 15 women in order to compare the antitrypsin capacity of colostrum with that of the serum.

Determination of antitrypsin activity

The determination was undertaken in accordance with the principle of *Sandholm et al.* by mixing colostrum and trypsin in different ratios followed by analysis of trypsin excess by enzyme diffusion in an agar-gel containing rennet-precipitated casein (*Sandholm et al.* 1976).

Preparation of plates. One g of purified agar (Difco) was dissolved in 50 ml of phosphate buffered saline (PBS)* pH 7.4, in a bath

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* Phosphate buffered saline (PBS), pH 7.4 (Dulbecco's solution): 8.0 g NaCl, 0.2 g KCl, 1.15 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.1 g $CaCl_2$, 0.1 g $MgCl_2 \cdot 6H_2O$, made up to 1000 ml with H_2O.

of boiling water. One g Ca-paracaseinate* and 0.1 g sodium azide were suspended in another 50 ml of PBS at 20°C. When the agar was dissolved, the mixtures were combined, mixed and poured on the glass plates to form a 2 mm layer. After solidification for 2 h in a humid chamber, holes of 6 mm diameter were punched out of the gel with a cork borer, and the resultant wells were emptied by suction.

Assay. The samples were diluted serially in PBS (1/2, 1/4, 1/8 . . .) after which an equal volume of trypsin solution (0.5 mg/100 ml) (Trypure, Novo, Diluted in 1 mM-HCl) was mixed with each dilution. Fifty µl of each mixture was pipetted into the respective wells in the agar plate and allowed to diffuse in a humid chamber at room temperature for 24 h. In the case of trypsin excess, a cleared zone around the wells can be seen (Fig. 1). The protease-inhibitor capacities are given as ml of each sample required to inactivate 1 mg of trypsin.

Determination of serum albumin (BSA) in bovine colostrum

Serial colostral samples from three cows were analyzed by radial gel diffusion in an antibody containing agar-gel (porcine anti-BSA) (*Mancini et al.* 1965).

RESULTS

The colostral antitrypsin content of different animal species is shown in Fig. 2, the total serum antitrypsin in Table 1, and correlation of the bovine colostral antitrypsin content with the colostral BSA in Fig. 3.

DISCUSSION

The determination of the trypsin-inhibitor capacity was performed simply by mixing a constant amount of trypsin with different sample dilutions and the eventual excess of trypsin detected by radial enzyme diffusion (Fig. 1). The use of rennet-precipitated casein is an improvement over the casein plate method (*Sandvik* 1962, *Fossum* 1970, *Carlsson & Karlsson* 1972). Ca-paracaseinate is completely digested by trypsin, therefore no staining is required for detection of the proteolysis.

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* Ca-paracaseinate: Skimmed milk was defatted by centrifugation for 15 min at 3000 × g. Casein was then precipitated by adding $CaCl_2$ to a 0.05 M concentration and incubating with rennin for 30 min at 30°C under magnetic stirring. The precipitate was washed by decanting the supernatant and resuspending the precipitate several times in PBS. The precipitate was then freeze-dried.

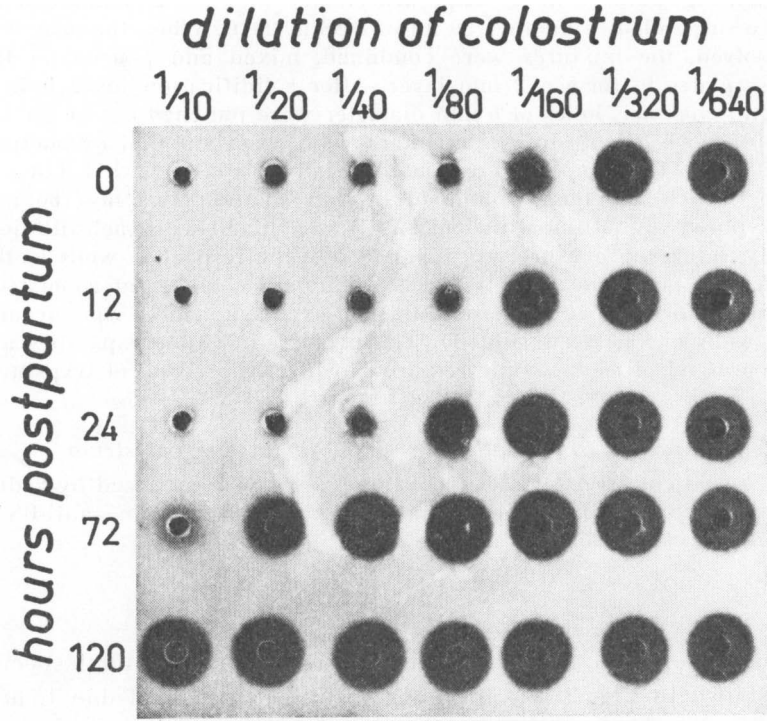

Figure 1. Determination of the antitrypsin in samples of bovine colostrum by the Ca-paracaseinate in agar-gel method. A dilution series of colostrum was prepared in PBS (1/10, 1/20, 1/40 ...) after which an equal volume of trypsin (5 μg/ml) was added. A sample of each mixture (50 μl) was transferred into the well on the agar plate.

There seems to be a significant species-specific behaviour in the antitrypsin activity of the colostrum during the first few days after parturition. Animal species transferring passive immunity by the colostral route show high colostral antitrypsin activity at parturition followed by a rapid decrease during the first few days. For example, in the cow the trypsin-inhibitory capacity decreases during the first week to one hundredth of what it was at parturition (Fig. 2). On the other hand, human milk was poor in antitrypsin from childbirth and did not show any significant change during the first week (Fig. 2). The bitch seems to differ from other species; the colostrum remained rich in antitrypsin during the whole two-week observation period (Fig. 2).

Table 1. Total serum antitrypsin activity of healthy control sera (ml serum required to inactivate 1 mg trypsin).

	Serum trypsin-inhibitor capacity (ml serum required to inactivate 1 mg trypsin)
Mare	0.5
	0.5
	0.5
Sow	1.0
	1.0
	1.0
Cow	0.5
	0.5
	0.5
Ewe	1.0
	1.0
	1.0
Bitch	2.0
	2.0
	2.0
Woman n = 13	1.0
n = 2	2.0

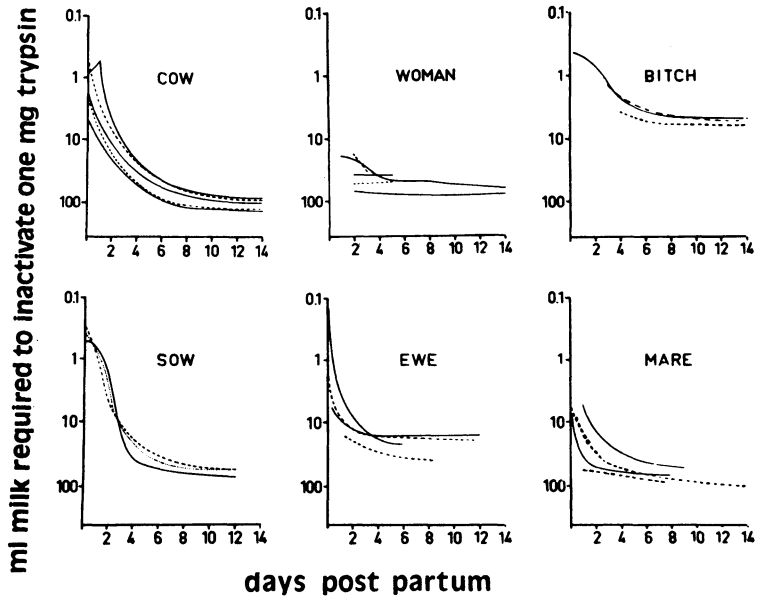

Figure 2. Decrease of colostral antitrypsin content in different animal species after parturition. The antitrypsin capacity is given as ml of colostrum required to inactivate 1 mg trypsin. To neutralize this amount of trypsin 250 ml of unprocessed pooled milk was required.

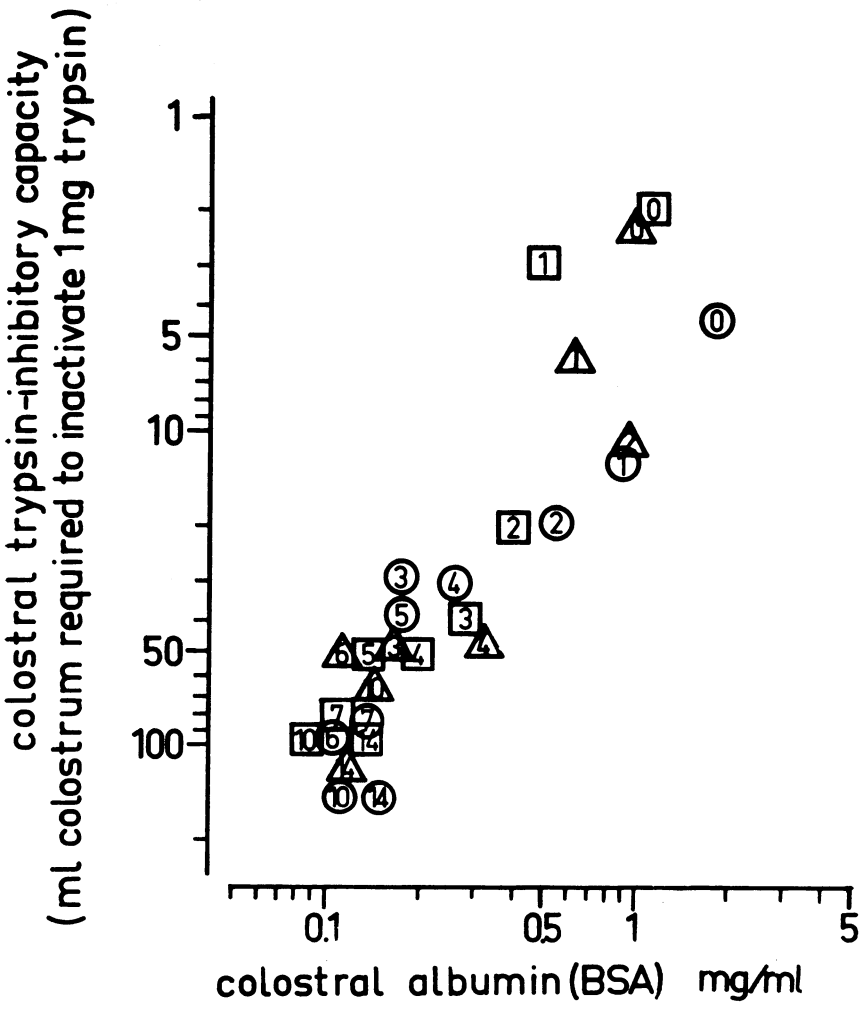

Figure 3. Correlation between bovine colostral trypsin-inhibitor capacity and colostral albumin (BSA) in three cows. The times of taking the samples (hours post partum) are given for each specimen.

The transient-trypsin-inhibitor-rich period in colostrum of the mare, cow, ewe and sow seems to correlate with the known decrease in the immunoglobulin levels during the first few days after parturition (*Butler* 1974). This indicates that colostral immunoglobulin and trypsin inhibitor are secreted concurrently. This observation is confirmed by the fact that species such as

man, which has a low immunoglobulin content in the milk from childbirth, have a low antitrypsin activity too.

There is some disagreement as to whether the colostral antitrypsin is derived from blood or produced locally in the mammary gland. The principal trypsin-inhibitor activity in the colostrum has been located to one distinct molecular species, different from the many protease inhibitors in serum. A colostral trypsin inhibitor with a molecular weight of about 12 000 has been identified in the human, cow and sow (*Laskowski et al.* 1957, *Chamberlain & Perry* 1965, *Pineiro et al.* 1975, *Cechova* 1976). No antigenic relationship has been seen between the colostral trypsin inhibitor and serum inhibitors. On the other hand the primary structure of the colostral trypsin inhibitor does show a homology with the basic pancreatic trypsin inhibitor (Kuniz-inhibitor) (*Cechova*).

The antitrypsin capacities measured during the present investigation show that the highest colostral antitrypsin activity (at parturition) is comparable to the total antitrypsin activity of serum. The colostral antitrypsin of the cow decreases in a direct ratio with serum-derived albumin (BSA). These findings would support a model where the colostral antitrypsin or its precursor is diffused like albumin from blood into mammary gland.

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SAMMANFATTNING

Trypsin-inhiberande kapacitet av kolostrum hos olika djurslag.

Kolostrum av sto, sugga, ko och tacka innehöll en hög trypsinhämmande kapacitet efter förlossningen. Aktiviteten sjönk till cirka en hundradedel under första veckan. Kolostrum från tikar innehöll en längre tid en högre antitrypsinaktivitet, men däremot är aktiviteten i modersmjölken låg redan från början.

Antitrypsinaktiviteten tycks följa immunoglobulinmängden. Antitrypsinet kan tänkas skydda immunoglobulinerna från proteolys under den passiva intestinala transporten av immunoglobulinerna till det nyfödda djuret.

(Received February 2, 1979).

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