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Zinc binding in cow's milk and human milk

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In both cow's milk and human milk, zinc was associated with proteins of high molecular weight (>100000), as judged by analysis with Sephadex G-75. Precipitation of the casein at pH 4.6 and filtration of the resultant acid whey on Sephadex G-25 led, however, to the recovery of about 90% of the zinc as a compound of low molecular weight, which was tentatively identified as zinc citrate. Over 95% of the zinc of cow's milk was sedimented with the casein micelles on ultracentrifugation. Filtration of these micellar caseins on Sephadex G-150 gave two peaks containing zinc, which corresponded to aggregates of α-casein–κ-casein and of α-casein–β-casein. Ultracentrifugation of human milk sedimented only approx. 40% of total zinc. Analysis of sediment and supernatant on Sephadex G-150, however, indicated that about 85% of the zinc was associated with a protein complex of molecular weight >150000. The major protein of this complex was identified as lactoferrin. A minor zinc-binding component of average molecular weight 30000 was also observed in the supernatant. The results indicated that zinc is bound to different macromolecules in cow's and human milk. This may be a factor affecting the bioavailability to the human infant of zinc from the two milks, and it is suggested that in human milk lactoferrin may be involved in the uptake of zinc.

Zinc is an essential trace element in human nutrition, playing a role in the structure or function of several enzymes (Underwood, 1977). Zinc deficiency can lead to anorexia, poor growth and wound healing, and skin problems (Sandstead, 1973). There is evidence that zinc in human milk is more efficiently utilized by the human infant than is zinc in cow's milk or milk formulas based on cow's milk. The plasma zinc concentration of breast-fed infants has been shown to be significantly higher than those fed on milk formulas, with or without supplementary zinc (Hambidge *et al.*, 1979). It has been suggested that human milk contains a specific zinc-binding compound, not present in cow's milk, that renders the zinc more available to the human infant by enhancing the zinc uptake across the small intestine (Eckhert *et al.*, 1977; Hurley *et al.*, 1977).

Parkash & Jenness (1967) analysed cow's milk in the ultracentrifuge and concluded that most of the zinc was in a bound form associated with casein, none being associated with whey proteins. However, more-recent studies have suggested that there is some uncertainty as to the identity of the zinc-binding compounds in human milk. Thus zinc was isolated in a citrate complex by gel filtration and ion-exchange chromatography (Hurley *et al.*, 1979; Lönnerdal *et al.*, 1980*a*). A higher concentration of zinc citrate was found in human milk than in cow's milk. Evans & Johnson (1980*a*) identified picolinic acid (a metabolite of tryptophan and a strong bidentate chelating ligand; Paul *et al.*, 1974) as a possible zinc-binding component in human milk, but not in cow's milk, by a modified gel-filtration technique. In tests with rats this compound facilitated zinc absorption (Evans & Johnson, 1980*b*) and stimulated growth (Evans & Johnson, 1980*c*). However, Rebello *et al.* (1982) detected picolinic acid in extremely low concentrations in human milk, insufficient to bind a significant proportion of the zinc. In addition proteins, including casein and serum albumin, have been implicated in zinc binding in human milk (Hoffman *et al.*, 1981).

Several zinc-binding compounds have been isolated from the small intestine, including amino acids (Hahn & Evans, 1973), a polypeptide (Schricker & Forbes, 1978), *NNN'*-trimethylethane-1,2-diamine (Hahn *et al.*, 1976) and prostaglandin E_2 (Song & Adham, 1978, 1979). However, when precautions were taken to minimize the action of proteinases, zinc was detected only in protein-bound forms in preparations of intestinal cell cytosol (Cousins *et al.*,

Abbreviation used: SDS, sodium dodecyl sulphate.

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1978) or in pancreatic secretions (Lönnerdal *et al.*, 1980*b*). The presence of a low-molecular-weight form of zinc in human milk that has a specific role in the uptake of zinc by the small intestine is therefore still uncertain.

The present study was designed to characterize the zinc-binding components of cow's milk and human milk. We found that most of the zinc in the two milks was associated with protein complexes of high molecular weight, though the individual proteins making up the complexes differed.

Materials and methods

Materials

Sephadex G-25, Sephadex G-150 and Sepharose 6B were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Fresh cow's milk was obtained from the pedigree herd of British Friesian cows maintained at this Institute. Human milk samples were obtained from the John Radcliffe Maternity Hospital, Headington, Oxford, U.K., with the kind co-operation of Dr. A. Williams and his staff. Dithiothreitol, γ-globulins, haemocyanin, iodoacetamide, myoglobin, ovalbumin and trypsin inhibitor were purchased from Sigma Chemical Co., Poole, Dorset, U.K. Goat anti-(human lactoferrin) serum was purchased from Nordic Immunological Laboratories, Maidenhead, Berks., U.K. All other chemicals were purchased from BDH Chemicals, Poole, Dorset, U.K., Hopkin and Williams, Romford, Essex, U.K., or Aldrich Chemical Co., Gillingham, Dorset, U.K., and were of analytical grade where possible. Commercial baby-foods (Cow and Gate Premium and Glaxo Ostermilk Two) were purchased and reconstituted as described by the manufacturers. DEAE-cellulose (DE-52) was obtained from Whatman, Maidstone, Kent, U.K.

Preparative techniques

Defatted milks. Cow's milk and human milk samples were defatted by centrifugation at 3000 *g* for 10 min at 4°C. The solidified fat was removed with a spatula.

Samples of the resulting defatted milk were analysed on a column (55 cm × 2.5 cm) of Sephadex G-75, with 50 mM-Tris/HCl/0.02% NaN_3, pH 7.5, as eluting buffer.

Acid whey. Acid whey was prepared from defatted milk samples by adjusting the pH to 4.6 with 1 M-HCl. After incubation for 30 min at room temperature, the samples were centrifuged at 40000 *g* for 30 min at 4°C. Samples of the supernatant were analysed on a column (60 cm × 2.5 cm) of Sephadex G-25, with 50 mM-Tris/0.02% NaN_3, adjusted to pH 4.6 with 1 M-HCl, as eluent. The pH was monitored and remained constant at 4.6 (±0.1) throughout the analysis. Samples of the supernatant and sediment (resuspended in 1 M-NaOH) were analysed for zinc and protein.

Ultracentrifugation of milk samples. Samples of defatted milks were fractionated by ultracentrifugation at 178000 *g* ($r_{av.}$ 6.3 cm) for 2 h at 37°C in a Beckman Ti 60 rotor. The supernatants were decanted and sediments were resuspended in 50 mM-Tris/HCl/0.02% NaN_3, pH 7.5. Samples of sediments and supernatants were analysed on a column (50 cm × 2.5 cm) of Sephadex G-150, with 50 mM-Tris/HCl/0.02% NaN_3, pH 7.5, as eluting buffer.

Casein. The identification of the casein components of cow's milk was conducted by precipitating the casein of defatted milk with $CaCl_2$, followed by centrifugation to separate casein from whey proteins (Waugh *et al.*, 1962). The resultant whole casein was dialysed against 10 mM-imidazole/HCl/3.3 M-urea/0.1% (v/v) 2-mercaptoethanol, pH 7. A sample was loaded on a column (18 cm × 2.25 cm) of DEAE-cellulose, and the casein components were eluted with a linear gradient (500 ml) of 0–0.5 M-NaCl in the buffer (Thompson, 1966). Caseins were eluted in the order κ-casein, β-casein and α-casein, and were analysed on SDS/10%-polyacrylamide gels (Fig. 2, gels *g–i*). Subunit molecular weights obtained from this analysis were similar to previously published values (Waugh, 1971).

Lactoferrin. Lactoferrin was purified from human milk by a method similar to that described by Bläckberg & Hernell (1980). Sepharose 6B (50 ml) was activated with CNBr (7 g) and coupled to heparin (50000 units) as described by David & Reisfeld (1974). A column of heparin–Sepharose 6B was poured and equilibrated with 10 mM-sodium phosphate/0.1 M-NaCl, pH 7.5. The supernatant obtained from the ultracentrifugation of defatted human milk was loaded on the column and washed overnight with the buffer at 4°C. Lactoferrin was eluted from the column with a linear gradient (200 ml) of 0.1–1.5 M-NaCl in the same buffer. Lactoferrin appeared at approx. 1 M-NaCl, and exhibited a single subunit, of approximate molecular weight 75000 when analysed by electrophoresis on SDS/10%-polyacrylamide gels (Fig. 4, gel *f*). This is in good agreement with previously published values (Querinjean *et al.*, 1971).

Analytical techniques

Assays. Protein was assayed as described by Lowry *et al.* (1951), with bovine serum albumin as standard. Zinc was assayed by atomic-absorption spectrophotometry, at 213.9 nm, with a Pye Unicam SP. 2900 atomic-absorption spectrophotometer. Citrate was assayed spectrophotometrically as described by Lowenstein (1969).

Calibration of gel-filtration columns for molecular weight. The Sephadex G-150 column was calibrated with myoglobin, trypsin inhibitor, ovalbumin, bovine

serum albumin and γ-globulins; haemocyanin was used to measure the exclusion volume. A linear relationship was obtained between mobility and log (molecular weight) in the molecular-weight range 16000–155000.

SDS/polyacrylamide-gel electrophoresis. Protein samples (1–5 mg) were solubilized in 1% (w/v) SDS, and reduced and carboxyamidomethylated as described by Louis & Shooter (1972). Samples were analysed on discontinuous 10% (w/v) polyacrylamide disc gels in the buffer system of Ugel *et al.* (1971). Gels were stained with Coomassie Blue (for protein) or Schiff reagent (for carbohydrate) as described by Fairbanks *et al.* (1971). The molecular weights of the different components of the stained gels were determined by using calibration proteins of known molecular weight (Louis & Shooter, 1972).

Spectrophotometric procedures were performed with a Pye Unicam SP.8-100 u.v. spectrophotometer.

Immunodiffusion. Goat anti-(human lactoferrin) serum was reconstituted in 1 ml of water, and the immunoglobulins were precipitated twice with 40% (w/v) (final concn.) $(NH_4)_2SO_4$ before use. Immunodiffusions against various samples derived from human milk were performed as described by Ouchterlony (1958).

Results

Analysis of defatted milks

When samples of defatted cow's milk and human milk were analysed on a column of Sephadex G-75, zinc was eluted together with protein components of high molecular weight immediately after the exclusion volume of the column. There was no detectable amount of zinc associated with non-protein components of low molecular weight in cow's milk. Human milk exhibited a diffuse minor species (11.6% of the total zinc; mean of six experiments) of low molecular weight that was eluted after all protein components.

Analysis of acid whey

The distribution of protein between supernatant (acid whey) and sediment fractions after the production of the acid wheys of cow's milk and human milk and two commercial baby-foods differed widely between the samples (Table 1). However, the distribution of zinc and citrate was similar in all samples. About 90% of the zinc and 95% of the citrate were released into the supernatant fraction. When the supernatants of cow's milk and human milk were analysed on a column of Sephadex G-25, zinc was observed as a single peak coincident with citrate emerging at the same relative elution volume in both cases. The zinc/citrate ratios in the peak fractions were similar in the two milks, and indicated a large molar excess of citrate (Table 1). Likewise, analysis by absorption spectrophotometry showed that zinc peaks derived from cow's milk and human milk exhibited a major peak at 215 nm and a relatively minor one at 265 nm. A mixture of zinc (4 μg/ml) and citric acid (2 mg/ml), adjusted so that the concentrations of the two nutrients were similar to those found in milk, gave results very similar to those described above for milk samples when analysed in the same way. It appears therefore that treatment of both cow's milk and human milk to produce an acid-whey fraction resulted in the release of most of the zinc as a low-molecular-weight form associated with citrate. Similar results were obtained when the commercial baby-foods were analysed in the same way.

Table 1. *Distribution of some nutrients in fractions of milks and commercial baby-foods after the preparation of acid wheys*

Samples of milks or commercial baby-foods (a, cow's milk; b, human milk; c, Cow & Gate commercial baby-food; d, Glaxo commercial baby-food) were defatted by centrifugation. They were fractionated to produce acid-whey preparations, and samples of the different fractions were assayed for protein, zinc and citrate as described in the Materials and methods section. Results are means of three determinations.

	Protein (%, w/v)				Zinc (%, w/v)				Citrate (%, w/v)				Zn/citrate molar ratio			
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
Defatted milk	100	100	100	100	100	100	100	100	100	100	100	100	1:115	1:61	1:120	1:196
Acid whey (supernatant)	9.9	76.1	34.6	11.4	86.7	86.3	90.6	93.3	98.6	99.6	84.3	92	1:154	1:60	1:124	1:124
Sediment	90.1	23.9	65.4	88.6	13.3	13.7	9.4	6.7	1.4	0.4	15.7	8	1:13.7	1:1.4	1:223	1:150
Zinc-containing peak of acid whey analysed on Sephadex G-25													1:42	1:42	1:64	1:43

Analysis by ultracentrifugation

Table 2 shows the distribution of zinc and protein between fractions of cow's milk and human milk after they had been submitted to ultracentrifugation. More than 95% of the zinc was sedimented from cow's milk by this process, but rather less protein was sedimented (approx. 85%). In contrast, in human milk, in which the distribution of zinc and protein closely paralleled each other, only approx. 40% of the total zinc was sedimentable. Rather more zinc and protein were sedimented from pasteurized than from fresh human milk samples when analysed in this way.

Cow's milk

Fig. 1(*a*) shows the analysis of the sediment fraction of cow's milk, fractionated by ultracentrifugation, on a column of Sephadex G-150. One zinc-containing peak was detected, which appeared immediately after the exclusion volume of the column. This was coincident with a broad protein peak shown by SDS/polyacrylamide-gel electrophoresis to consist of casein micelles (Fig. 2, gel *d*). Re-analysis of the zinc-containing peak on the same column of Sephadex G-150 resulted in the partial disaggregation of the casein complex to form two protein peaks, both of which contained significant amounts of zinc (Fig. 1*b*). The first appeared immediately after the exclusion volume of the column, and electrophoretic analysis indicated that this consisted primarily of an aggregate of α- and κ-caseins (Fig. 2, gel *e*). The second had an apparent molecular weight of 110000, electrophoresis showing that this consisted mainly of an aggregate of α- and β-caseins (Fig. 2, gel *f*). The individual caseins were identified as described in the Materials and methods section, and photographs of SDS/polyacrylamide-gel electrophoretograms of the purified caseins are shown in Fig. 2 (gels *g*–*i*).

Human milk

Fig. 3(*a*) shows the analysis of the sediment fraction of human milk, prepared by ultracentrifugation, on a column of Sephadex G-150. A single zinc-containing peak was observed, which was coincident with a protein complex present immediately after the exclusion volume of the column. Analysis of the supernatant fraction produced by ultracentrifugation similarly showed that most of the zinc was associated with a protein complex that emerged close to the void volume (Fig. 3*b*). However, there was also a minor diffuse zinc-containing region, with an average molecular weight of 30000. This contained only 13.4% (mean of three results) of total milk zinc. No non-protein-bound zinc of low molecular weight was detected.

Fig. 4 shows human milk proteins separated by ultracentrifugation and filtration on Sephadex G-150

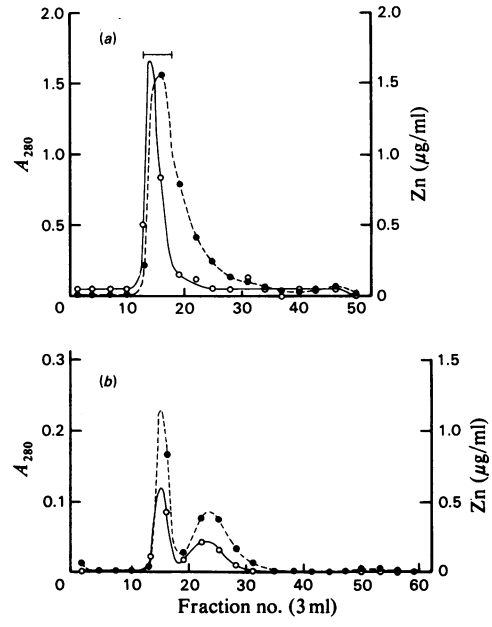

Fig. 1. *Analysis of the sediment obtained from the ultracentrifugation of cow's milk on a column of Sephadex G-150*

(*a*) Cow's milk was defatted by centrifugation and fractionated by ultracentrifugation as described in the Materials and methods section. The resulting sediment was resuspended in 50mM-Tris/HCl/0.02% NaN_3, pH7.5; a sample (5ml) was loaded on a column of Sephadex G-150 (50cm × 2.5cm) and the column was eluted with the same buffer. Samples (3ml) were collected, analysed spectrophotometrically at 280nm and assayed for zinc. (*b*) The samples containing the peak zinc values of the analysis of (*a*) above were pooled (indicated by the bar in *a*) and a sample (5ml) was analysed on the same column of Sephadex G-150, with identical elution conditions. Samples (3ml) were collected, analysed spectrophotometrically at 280nm and assayed for zinc. ●, A_{280}; ○, zinc.

and analysed by SDS/polyacrylamide-gel electrophoresis. The major zinc-binding components of both sediment and supernatant exhibited a protein of subunit molecular weight 75000 as their largest constituent (Fig. 4, gels *d* and *e*). A positive staining reaction with Schiff reagent (Fairbanks *et al.*, 1971) indicated that this protein contained carbohydrate. Lactoferrin purified from human milk exhibited a single subunit of molecular weight 75000 containing carbohydrate on SDS/polyacrylamide-gel electrophoresis (Fig. 4, gel *f*). This is similar to results obtained in previously published work (Querinjean *et al.*, 1971; Spik *et al.*, 1974). In

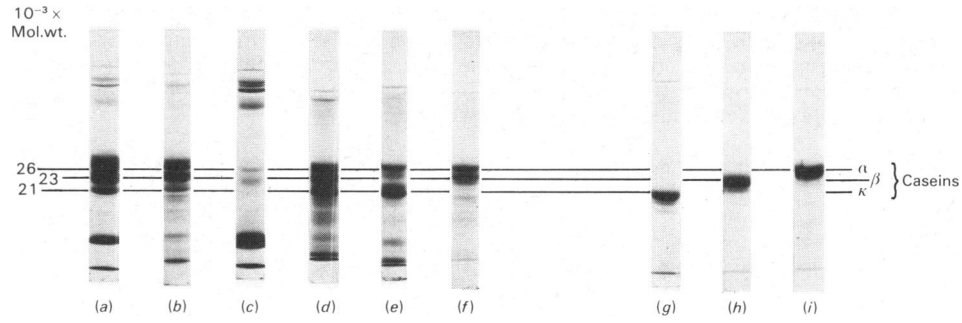

Fig. 2. *Analysis of the zinc-binding proteins of cow's milk by electrophoresis on SDS/10%-polyacrylamide gels*
Samples of protein (1–5 mg) were solubilized in SDS, reduced and carboxyamidomethylated, and analysed on SDS/10%-polyacrylamide gels, as described in the Materials and methods section. Gels were stained with Coomassie Blue and photographed. Gel (*a*), cow's milk, defatted by centrifugation; gel (*b*), sediment obtained by the ultracentrifugation of defatted cow's milk; gel (*c*), supernatant obtained by the ultracentrifugation of defatted cow's milk; gel (*d*), material from the zinc-containing peak obtained by the analysis of (*b*) on a column of Sephadex G-150; gel (*e*), high-molecular-weight material from the zinc-containing peak obtained by the analysis of (*d*) on a column of Sephadex G-150; gel (*f*), lower-molecular-weight material from the zinc-containing peak obtained by the analysis of (*d*) on a column of Sephadex G-150; gels (*g*)–(*i*), casein components of cow's milk as identified by the analysis of cow's-milk samples on a column of DEAE-cellulose (*g*, κ-casein; *h*, β-casein; *i*, α-casein).

addition, human milk, purified lactoferrin and the zinc-binding components revealed by the analysis of the supernatant and pellet fractions of ultracentrifuged human milk all gave rise to strong precipitates on reaction against goat anti-(human lactoferrin) by immunodiffusion (Fig. 5). By these criteria lactoferrin was identified as a major component of the protein complex that binds zinc in human milk.

Discussion

It has been suggested that the analysis of zinc-binding compounds on gel-filtration media is complicated by the binding of zinc to the gel (Johnson & Evans, 1980), unless the gel is first equilibrated with $Zn(NO_3)_2$ (Evans *et al.*, 1979). However, we found that a thorough pre-equilibration of the gels with buffer was sufficient to ensure complete recovery of zinc. Similar observations have been made by Cousins & Smith (1980) and Lönnerdal *et al.* (1980*a*).

We observed that most of the zinc in both cow's milk and human milk was associated with protein complexes of high molecular weight when samples were analysed on a column of Sephadex G-75. These results, together with the absence of a non-protein zinc complex of low molecular weight from cow's milk, and the presence of a minor non-protein component of low molecular weight that bound approx. 10% of the zinc in human milk, are in good agreement with the findings reported by other workers (Cousins & Smith, 1980; Lönnerdal *et al.*, 1980*a*).

The resultant zinc compounds produced by the mild acid treatment (pH 4.6) used to prepare whey from cow's milk and human milk and from commercial baby-foods were very similar. In all samples approx. 90% of the zinc was released as a non-protein complex of low molecular weight, which was eluted as a single peak, coincident with citrate, from a column of Sephadex G-25. A mixture of zinc and citric acid, in similar relative concentration to those found in the milks, filtered in the same position, as a single peak of zinc and citrate. The zinc component was similar in molecular weight, zinc/citrate molar ratio and u.v.-absorption spectrum whether isolated from either of the milks or the baby-foods. Clearly these results could not be used to explain the apparent differences in availability to the human infant of zinc from the two milks. A more detailed analysis of the zinc–protein relationships of cow's milk and human milk was therefore undertaken.

The ultracentrifugation of cow's milk and subsequent analysis on a column of Sephadex G-150 showed that more than 95% of the zinc was bound to casein micelles. These are known to be of very high molecular weight and composed of subunits of approximate molecular weight 20000–30000 (Waugh, 1971). Further resolution showed that fractions composed predominantly of mixtures of α-casein–κ-casein and α-casein–β-casein complexes

Table 2. *Recoveries of protein and zinc after the ultracentrifugation of milk samples*

Samples of (a) fresh cow's milk, (b) pasteurized and frozen human milk and (c) fresh human milk were defatted by centrifugation. The defatted milks were ultracentrifuged as described in the Materials and methods section, and the subsequent sediments and supernatants were analysed for protein and zinc. Results for (b) are the means of two experiments; those for (a) and (c) are the means of three experiments.

	Protein (%, w/v)			Zinc (%, w/v)			Zinc/protein (μg/mg)		
	a	b	c	a	b	c	a	b	c
Defatted milk	100	100	100	100	100	100	1:9.8	1:4.5	1:5.8
Supernatant	16.2	47.2	57.3	3.1	39.4	58.4	1:68.9	1:5.1	1:4.8
Sediment	83.8	52.8	42.7	96.9	60.6	41.4	1:6.1	1:3.3	1:3.1

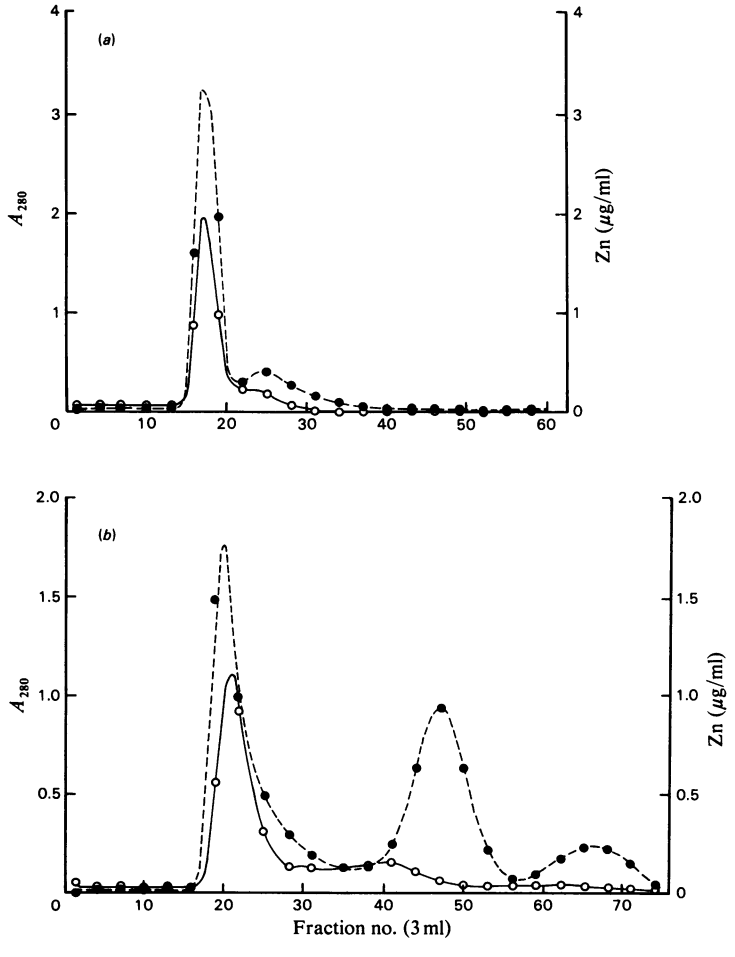

Fig. 3. *Analysis of the sediment and supernatant obtained from the ultracentrifugation of human milk on a column of Sephadex G-150*

Fresh human milk was defatted by centrifugation and fractionated by ultracentrifugation as described in the Materials and methods section. The resulting sediment was resuspended in 50 mM-Tris/HCl/0.02% NaN_3, pH 7.5. Samples (5 ml) of (*a*) resuspended sediment or (*b*) supernatant were loaded on a column of Sephadex G-150 (50 cm × 2.5 cm) and the column was eluted with the above buffer. Samples (3 ml) were collected, analysed spectrophotometrically at 280 nm (●) and assayed for zinc (○).

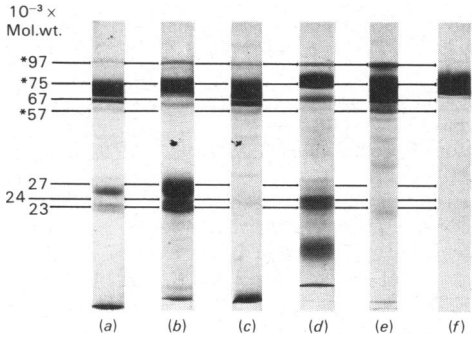

Fig. 4. *Analysis of the zinc-binding proteins of human milk by SDS/10%-polyacrylamide-gel electrophoresis*
Samples of protein (1–5 mg) were solubilized in SDS, reduced and carboxyamidomethylated, and analysed by SDS/10%-polyacrylamide gel electrophoresis, as described in the Materials and methods section. Gels were stained with Coomassie Blue and photographed. Gel (*a*), human milk, defatted by centrifugation; gel (*b*), sediment obtained from the ultracentrifugation of human milk; gel (*c*), supernatant obtained from the ultracentrifugation of human milk; gel (*d*), material from the zinc-containing peak obtained from the analysis of (*b*) on a column of Sephadex G-150; gel (*e*), material from the major zinc-containing peak obtained from the analysis of (*c*) on a column of Sephadex G-150; gel (*f*), lactoferrin, purified by affinity chromatography on heparin–Sepharose 6B. Components marked (*) were stained with Schiff reagent.

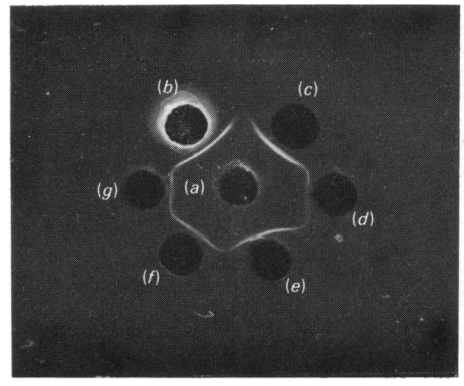

Fig. 5. *Identification of lactoferrin in samples of human milk*
An Ouchterlony plate was set up as described in the Materials and methods section, with samples of (*a*) anti-(human lactoferrin) serum, (*b*) human milk, (*c*) lactoferrin purified from human milk, (*d*) and (*e*) material from the major zinc-containing peak from the analysis of the pellet derived from ultracentrifuging human milk on Sephadex G-150, and (*f*) and (*g*) material from the major zinc-containing peak from the analysis of the supernatant derived from ultracentrifuging human milk on Sephadex G-150. The plates were incubated for 24 h at room temterature.

bound significant amounts of zinc. Human milk exhibited a different pattern of zinc-binding protein components when subjected to the same analysis. Zinc was again associated with protein complexes of high molecular weight, but the major protein component was identified as lactoferrin. Similar results were obtained when pasteurized or fresh milk samples were analysed in this way. It seemed that the pasteurization of human milk did not markedly affect its zinc–protein interactions.

Lactoferrin is a major glycoprotein component in human milk, of subunit molecular weight 75 000, and is structurally distinct from caseins. It acts as an iron-binding protein (Querinjean *et al.*, 1971) and has been reported to be a bacteriostatic agent (Bullen *et al.*, 1972). An unequivocal characterization of lactoferrin as the primary zinc-binding protein of human milk was not obtained, as there was little zinc associated with the protein as purified by affinity chromatography on heparin–Sepharose 6B. However, a preliminary report has indicated that lactoferrin, isolated from human milk by chromatography on CM-Sephadex C-50, contained approx. 0.33 mol of zinc/mol of lactoferrin (Ainscough *et al.*, 1980). Also, we have shown in equilibrium-dialysis experiments that lactoferrin purified from human milk has the capacity to bind large amounts of added zinc (P. Blakeborough & D. N. Salter, unpublished work).

These results can be interpreted to explain the apparent differences in availability of zinc from cow's milk and human milk to the human infant. Casein, present in cow's milk at about 10 times its concentration in human milk (Hambraeus, 1977), forms hard curds in the stomach of the human infant, and some may pass through the gastrointestinal tract in an undigested form (Fomon, 1974). In this way a significant proportion of the zinc in cow's milk may be inaccessible to the digestive and absorptive processes of the human infant.

The fact that cow's milk contains little lactoferrin compared with human milk may be of greater significance (Masson & Heremans, 1971). Immunohistochemical data have shown that the mucosal surfaces of the human gastrointestinal tract are coated with a thin layer of lactoferrin (Masson *et al.*, 1969). The protein may act to donate zinc to the human small intestine, and lactoferrin in milk may serve a similar function during the development of this process in the infant. There is evidence that lactoferrin plays such a role in the absorption of iron (Cox *et al.*, 1979).

Most of the zinc in pancreatic secretions (Lönnerdal *et al.*, 1980*b*), intestine (Cousins *et al.*, 1978; Lönnerdal *et al.*, 1980*c*) and plasma (Smith *et al.*, 1978) appears to be bound to protein. Zinc absorption may hence be regulated both by zinc–protein complexes and by non-protein compounds of low molecular weight.

It will be useful to isolate the zinc-binding components of the stomach and intestine of animals fed on cow's and human milk to find out whether they alter as milk passes down the gastrointestinal tract.

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