5. A. D. Steinberg, T. Pincus, N. Talal, *J. Immunol.* **102**, 788 (1969).
6. ———, *Immunology* **20**, 523 (1971).
7. R. W. Sidwell, L. B. Allen, J. H. Huffman, J. T. Witkowski, L. N. Simon, *Proc. Soc. Exp. Biol. Med.* **148**, 854 (1975).
8. K. P. Schofield, C. W. Potter, D. Edey, R. Jennings, J. S. Oxford, *J. Antimicrob. Chemother.* **1** (Suppl), 63 (1975).
9. N. Talal and A. D. Steinberg, *Curr. Top. Microbiol. Immunol.* **74**, 79 (1974).
10. R. W. Sidwell, J. H. Huffman, G. P. Kharl, L. B. Allen, J. T. Witkowski, R. K. Robins, *Science* **177**, 705 (1972); G. P. Kharl, R. W. Sidwell, J. T. Witkowski, L. N. Simon, R. K. Robins, *Antimicrob. Agents Chemother.* **3**, 517 (1973).
11. We thank Drs. L. N. Simon and R. K. Robins for supplying ribavirin; Dr. H. E. Machamer for supplying ara-A; Dr. M. A. Chirigos for advice and for supplying levamisole; and Dr. A. O. Geiszler for supplying phosphonoacetic acid.

22 June 1976; revised 24 August 1976

Zinc Binding: A Difference Between Human and Bovine Milk

Abstract. *Gel chromatography indicated that most of the zinc in cow's milk was associated with high-molecular-weight fractions, whereas zinc in human milk was associated with low-molecular-weight fractions. A species difference in zinc-binding ligands may explain why symptoms of the genetic disorder of zinc metabolism, acrodermatitis enteropathica, can be alleviated by feeding human but not cow's milk.*

Acrodermatitis enteropathica (AE) is an autosomal recessive inherited disorder characterized by severe skin lesions on the extremities and around body openings, alopecia, and diarrhea (*1*). The onset of symptoms of AE usually occurs when such infants are weaned from human breast milk to cow's milk (*1–3*). The therapeutic value of human milk in this disorder has been known for a long time (*2*). Moynahan and Barnes (*4*) have reported low levels of zinc in the plasma of AE patients and the successful treatment of the disorder with oral zinc, resulting in an increase in the levels of zinc in the plasma and subsequent clearing of epidermal lesions.

Human milk generally contains less zinc than bovine milk, with the zinc concentrations of both decreasing progressively throughout lactation (*5, 6*). Since the zinc concentration of human milk is generally lower than that of cow's milk we postulated that the zinc in human milk must be present in a form different from that found in cow's milk, and predicted that it contains a specific zinc-binding ligand not present in the bovine milk. To test this hypothesis we have separated both human and bovine milk by gel filtration to determine the association of zinc with protein fractions in the two species.

Fresh samples of human (N = 5) and Holstein cow's (N = 5) milk were cooled at 4°C and centrifuged at 1000*g* for 5 minutes to separate the fat. The zinc content of the fat-free cow's milk was 4.22 ± 0.37 μg/ml (S.E.M.) with a range of 3.56 to 4.80 μg/ml compared to 0.97 ± 0.29 μg/ml with a range of 0.27 to 2.05 μg/ml for the human samples.

Fat-free samples were diluted with an equal volume of 13 m*M* tris buffer, *p*H 7.4, and chromatographed on a Sepharose 2B column (50 by 2.6 cm). Fractions obtained by gel filtration were assayed for protein by the method of Warburg and Christian (*6*) and for zinc by means of a Unicam SP 90 atomic absorption spectrophotometer.

Representative elution patterns from gel filtration of bovine and human milk on Sepharose 2B are shown in Fig. 1, a and b. In cow's milk, zinc was associated with the fractions of higher molecular weight, whereas zinc in human milk was associated with the fractions of lower molecular weight. In order to observe whether the major zinc-binding peak of human milk was present in cow's milk but was masked by the large zinc-containing peak of the fractions of higher molecular weight, zinc-containing fractions (Fig. 1) were concentrated and chromatographed again on a Sephadex G-200 column (50 by 2.0 cm). The pooled gel-filtration fractions of cow's milk were eluted, resulting in a large zinc peak at void volume and four small zinc peaks (Fig. 1c). The major zinc-containing fraction of human samples eluted from Sepharose 2B were similarly chromatographed again on Sephadex G-200. Zinc was eluted in two broad peaks (Fig. 1d), but the second peak (fractions 130 to 150) varied directly with the amount of zinc in the original fat-free sample.

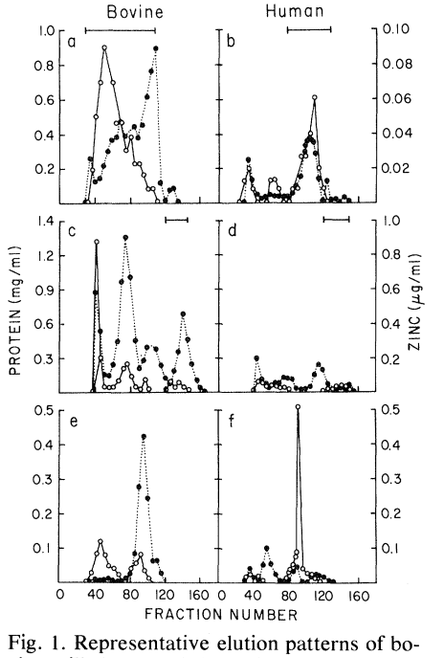

Fig. 1. Representative elution patterns of bovine milk (a, c, and e) and human milk (b, d, and f) separated by gel filtration. Fat-free samples were applied to a Sepharose 2B column, and fractions containing zinc (bracketed) (a and b) were pooled, concentrated by lyophilizing, and applied to a Sephadex G-200 column. Fractions containing zinc eluted from the Sephadex G-200 column (bracketed) (c and d) were pooled, concentrated by lyophilizing, and applied to a Sephadex G-75 column. (a and b) Sepharose 2B column (2.6 by 50 cm), 13 m*M* tris buffer, *p*H 7.4, 2 ml per fraction; (c and d) Sephadex G-200 column (2.0 by 50 cm), 13 m*M* tris buffer, *p*H 7.4, 1 ml per fraction; (e and f) Sephadex G-75 column (1.5 by 50 cm), 13 m*M* tris buffer, *p*H 7.4, 1 ml per fraction. Symbols: ○——, zinc; ●....., protein.

These zinc-containing fractions from human samples and the corresponding cow's milk fractions (Fig. 1, c and d) were further purified by gel filtration on a Sephadex G-75 column (50 by 1.5 cm). The zinc in both bovine and human samples separated into two peaks (Fig. 1, e and f). The elution patterns showed large peaks in both samples, but, surprisingly, they were of different composition. The large protein peak in cow's milk samples was associated with a small quantity of zinc, whereas, conversely, the large zinc peak in human samples, with a slightly smaller elution volume, was associated with only a small protein peak. To ensure that the zinc was tightly bound in samples from both human and bovine peaks, fractions were dialyzed for 22 hours against 13 m*M* tris buffer, *p*H 7.4, containing 10 m*M* EDTA and rechromatographed on Sephadex G-75. The zinc remained bound to both ligands, and elution volumes were nearly identical to those obtained prior to dialysis. After additional purification of the low-molecular-weight zinc-binding ligand on DEAE-cellulose, the molecular weight was estimated by gel filtration on Bio-Gel A-5m (6*M* guanidium chloride, *p*H 7.0) to be 8700. Insulin (chains A and B), trypsin inhibitor, ribonuclease, myoglobin, chymotrypsinogen, and ovalbumin were used as marker proteins (*7*).

The demonstration of a difference in zinc binding between human and cow's milk supports our hypothesis that there is a difference in the association of zinc to milk components in the two species.

In contrast to the binding of zinc to large-molecular-weight fractions in cow's milk, we have demonstrated that human milk contains a small ligand that binds a large proportion of the total zinc. Analysis of human colostrum indicated that the low-molecular-weight zinc-binding ligand was present in greater quantities than in milk obtained later in lactation.

Almost 40 years ago, Brandt (*2*) suggested that the symptoms of AE resulted from deficiency of a nutrient in breast milk, which children with this condition required but could not obtain from other food sources because of a gastrointestinal disorder. Other investigators, however, believed that the disease was caused by infection of the gastrointestinal tract (*8*), and Dillaha *et al.* (*9*) treated an AE patient with diiodohydroxyquinoline for a yeast infection. The drug controlled the symptoms of AE and subsequently became the treatment of choice although its mechanism of action is still unknown (*4, 10, 11*). Moynahan (*12*) has proposed that in the intestine of AE patients there is a missing or defective enzyme which normally hydrolyzes a small peptide, a breakdown product of all dietary proteins except human milk (*11, 12*). According to this hypothesis the noxious oligopeptide chelates dietary zinc, reducing its availability, while the therapeutic action of diiodohydroxyquinoline results from a greater affinity for the peptide than for zinc, thereby freeing the metal for the metabolic needs of the host (*11*).

A simpler explanation not requiring the presence of a noxious factor is that the metabolic lesion in AE patients is a defect in the normal intestinal mechanism of zinc absorption. Lombech *et al.* (*13*) have reported that a 5-year-old male AE patient absorbed only 15 percent of a tracer dose of ^{65}Zn (control, 58 to 77 percent), while another 5-year-old male AE patient fed human milk absorbed 45 percent of the tracer dose.

In our study, the small-molecular-weight binding ligand isolated from human milk may enhance absorption of zinc in AE patients. It is possible that this zinc-binding ligand protects zinc from chelation with other dietary components thereby increasing the absorption of zinc, or that it actually transports zinc across the intestine prior to the development of specific mechanisms for intestinal absorption of the element. Thus, feeding either human milk or readily available forms of zinc could improve a defective zinc-absorption system.

It is not inconceivable that more or less species-specific binding ligands for a number of nutrients could exist in various milks. Such a mechanism to aid in the absorption of essential nutrients in newborn infants would be of obvious advantage for survival of the species. Future investigations of the effect of the zinc-binding ligand from human milk on zinc absorption as well as of intestinal zinc-binding ligands in infants should provide a better understanding of the primary defect in AE and enhance our basic knowledge of zinc absorption.

CURTIS D. ECKHERT
MARTIN V. SLOAN
JOHN R. DUNCAN, LUCILLE S. HURLEY
Department of Nutrition,
University of California, Davis 95616

References and Notes

1. N. Danbolt and K. Closs, *Acta Derm. Venereol.* **23**, 127 (1942).
2. T. Brandt, *ibid.* **17**, 513 (1937).
3. N. Danbolt, *ibid.* **28**, 532 (1948).
4. E. J. Moynahan and P. M. Barnes, *Lancet* **1973-I**, 676 (1973).
5. M. Sato and K. Murata, *J. Dairy Sci.* **15**, 451 (1932).
6. M. F. Picciano and H. A. Guthrie, *Am. J. Clin. Nutr.* **29**, 242 (1976); L. Ekman and M. Palm, in *Symposium om Zink*, B. Zederfeldt, Ed. (AB Tika, Lund, 1974), p. 49.
7. E. Layne, *Methods Enzymol.* **3**, 451 (1957).
8. J. G. Hopkins, *Arch. Dermatol. Syphilol.* **25**, 599 (1932).
9. C. J. Dillaha, A. L. Lorincz, O. R. Aavik, *J. Am. Med. Assoc.* **152**, 509 (1953).
10. F. E. Pittman and M. C. Westphal, *Pediatrics* **54**, 81 (1974); K. H. Neldner and K. M. Hambidge, *N. Engl. J. Med.* **292**, 879 (1975); A. F. Robertson and J. Sotos, *Pediatrics* **55**, 738 (1975); M. Chvapil, J. N. Ryan, C. F. Zukoski, *Proc. Soc. Exp. Biol. Med.* **140**, 642 (1972).
11. E. J. Moynahan, *Lancet* **1974-II**, 399 (1974).
12. ———, *Proc. R. Soc. London* **55**, 240 (1962); ———, F. R. Johnson, R. M. H. McMinn, *ibid.* **56**, 300 (1963).
13. I. Lombeck, H. G. Schnippering, F. Ritzl, L. E. Feinendigen, H. J. Bremer, *Lancet* **1975-I**, 855 (1975).
14. We thank Mothers' Milk Bank, Inc., Childrens Hospital, 3700 California Street, San Francisco, Calif., for supplying us with human milk samples, and the Department of Dairy Science, University of California, Davis, for supplying us with unpasteurized cow's milk. Supported in part by funds provided from National Institutes of Health postdoctoral fellowship 1-F32 HD-05219-01 and National Institutes of Health research grant HD-01743.

23 August 1976

Presynaptic Electrical Coupling in *Aplysia*: Effects on Postsynaptic Chemical Transmission

Abstract. *The large cholinergic interneuron L_{10} in the abdominal ganglion of* Aplysia *mediates both chemical and electrical synaptic transmission. The amplitudes of postsynaptic potentials produced by different branches of L_{10} are differentially affected when the electrically coupled neuron L_{20} is depolarized or hyperpolarized. Polarizations applied to L_{20} are transmitted to L_{10} branches by the "presynaptic" electrical synapse. Depolarization increases the amplitude of the postsynaptic potential, while hyperpolarization has the opposite effect. The differential effects occur because current supplied through the electrical synapse undergoes more electrotonic decrement for the distant branches than for branches closer to the electrical synapse. These findings indicate that the presynaptic electrically coupled neuron may have an integrative role in the modulation of chemical synaptic efficacy mediated by L_{10}.*

In the nervous system, transfer of information from one nerve cell to another occurs through the synapse, which may mediate either chemical or electrical transmission (*1*). In the case of chemical synaptic transmission, the action potential arriving at the presynaptic terminal of a nerve cell causes the release of a chemical (transmitter substance) which reacts with a postsynaptic receptor and changes the conductance of the membrane for certain ions; this results in postsynaptic potentials. The conductance changes may cause depolarizing (excitatory) or hyperpolarizing (inhibitory) potentials. The electrical impulse itself is shunted into the low-resistance gap located between the pre- and postsynaptic neurons. In electrically mediating synapses, the gap between the pre- and postsynaptic membranes is very narrow, and hence the resistance between the gap and the extracellular space is relatively high, while the resistance of the electrical pathway between the two neurons is relatively low. An action potential arriving at the terminal crosses the gap and is transmitted electronically with attenuation into the postsynaptic neuron.

Although chemical and electrical synaptic transmission have quite different mechanisms, they have a number of similar functional characteristics (*2*). Presynaptic inhibition, a functional characteristic observed in some chemical synapses, has not been reported in electrical synapses. In this report I show that in the abdominal ganglion of *Aplysia* presynaptic modulation of chemical transmission can be mediated by electrical synaptic transmission.

In the vertebrate nervous system "presynaptic" effects are observed when a test postsynaptic potential (PSP) pro-