Identification of a Low Molecular Weight ^{65}Zn Complex in Rat Intestine[1] (37684)

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Although zinc is known to be an essential nutrient, very little is known regarding the process whereby zinc is transported from the intestinal lumen to the blood. Suso and Edwards (1, 2) have suggested that low-molecular-weight ligands are involved in zinc transport but, to date, no reports have appeared which describe the characteristics of a naturally occurring zinc-binding ligand in the intestinal lumen or mucosa. The present report describes the isolation and partial characterization of a zinc complex in the intestinal lumen and mucosa of the rat.

Methods. Male Sprague-Dawley rats age 70–90 days were used as a source of intestinal tissue. The animals had been housed in stainless steel cages and had been fed a diet of Purina Laboratory Chow[2] and tap water. All animals were fasted for 18 hr prior to being used in experiments.

Radioactive zinc solutions were prepared by diluting 1.0 μCi carrier-free zinc-65 (International Chemical and Nuclear Corp., Irvine, CA) in 1.0 ml distilled water which contained 0.065 μg Zn^{2+} in the form of $ZnSO_4$. The solutions were administered by gastric tube; 1 hr later, the animals were decapitated and a 15-cm segment of small intestine, beginning at the pylorus, was removed.

The small intestine was prepared by thoroughly rinsing the lumen with cold 0.85% NaCl. Thereafter, the mucosal cells were scraped from the serosal tissue by using a glass slide; the mucosa was then suspended in 10 ml of 0.85% NaCl, and the suspension was homogenized in a Potter-Elvehjem homogenizer equipped with a Teflon pestle. The homogenate was centrifuged at 105,000*g* for 1 hr in a refrigerated centrifuge (Beckman, Model L2-65B) after which the supernatant was removed and freeze-dried. The lumen contents were centrifuged at 4000*g* for 15 min after which the supernatant was removed and freeze-dried.

The freeze-dried lumen contents and intestinal supernatant were dissolved separately in 1.0 ml distilled water and applied to 0.9 × 60-cm columns packed with Bio-Gel P-10 (exclusion limit = 20,000 daltons) which had been equilibrated with 0.025 *M* phosphate buffer, (pH 7.3) and 0.05 *M* KCL. The same buffer was used to elute the samples. Fractions were collected in a refrigerated fraction collector and monitored for radioactivity in a gamma-well counter (Nuclear-Chicago, Model 4233).

The elution characteristics of the Bio-Gel P-10 columns used in these experiments were determined with blue dextran 2000 (elution volume (V_e) = 11 ml), cytochrome *c* (V_e = 22 ml) and a mixture of amino acids (V_e = 34 ml).

Following gel filtration chromatography on Biol-Gel P-10, the fractions comprising peak 4 from the intestinal mucosa (see Fig. 1) were combined and applied to a column packed with Biol-Gel P-2. The sample was eluted from Bio-Gel P-2 with distilled water and the radioactive fractions were combined and freeze-dried. The freeze-dried material

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[2] Trade names and company names are included for the benefit of the reader and do not imply any endorsement of preferential treatment of the product by the USDA.

was dissolved in 0.2 ml distilled water and 3 μl of this solution were analyzed by thin layer chromatography (tlc).

Labeled zinc-amino acid complexes were prepared for thin layer chromatography by adding 10 μCi of $^{65}ZnCl_2$ to a 1.0 ml solution which contained 10 mM amino acid. The solutions were left standing at room temperature for 24 hr after which 2 μl of solution were applied to the chromatogram. The migration of a labeled zinc salt on tlc was analyzed by applying 2 μl of a solution which contained 1.0 μCi $^{65}ZnCl_2$.

Thin layer chromatography was carried out on silica gel impregnated glass paper (Gelman Type ITLC-SA) with a solvent which contained butanol:acetic acid:water (4:1:1). Chromatogram development was repeated four times with the same solvent system. Following thin layer chromatography, the chromatograms were sprayed with Ninhydrin after which 1 $\times$ 1-cm sections were cut from the paper and monitored in a gamma-well counter.

Results and Discussion. As shown in Fig. 1, approximately 95% of the radioactivity in the intestinal lumen was contained within a single peak which eluted at the total volume of the column. In the intestinal mucosal supernatant, ^{65}Zn was eluted in four distinct peaks; approximately 40% of the ^{65}Zn in the eluted fractions was recovered in a peak (peak 4) which eluted at the total volume and the remainder of the isotope was recovered in fractions eluting with higher molecular weight material (peaks 1–3). We are currently investigating the properties of the zinc-binding components in peaks 1–3 in the mucosal supernatant but the remainder of this paper deals with the characteristics of the component represented by peak 4 in the supernatant and the major ^{65}Zn peak in the lumen.

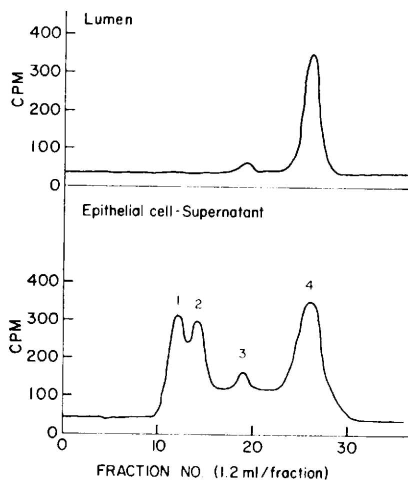

FIG. 1. Elution of ^{65}Zn from intestinal lumen and intestinal mucosa supernatant on Bio-Gel P-10. Each graph is representative of at least six assays.

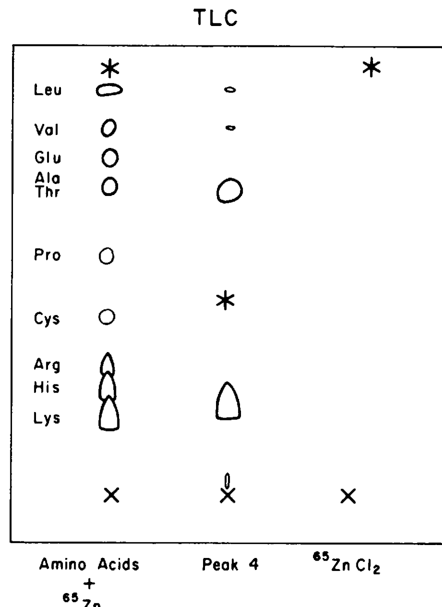

FIG. 2. Thin layer chromatography of the low-molecular-weight ^{65}Zn complex obtained from rat intestinal mucosa. (X) The point of application; (*) the area in which ^{65}Zn radioactivity was detected; and the symbols depict the position and approximate shape of Ninhydrin-positive areas. The figure represents the results observed in three tlc assays.

Since both the large ^{65}Zn peak from the lumen and peak 4 from the mucosal supernatant were eluted at the total volume of the column, we first suspected that these peaks might represent unbound ionic zinc. However, when a solution which contained 10 μg ^{65}Zn-$ZnCl_2$ was applied to the column, less than 1% of the radioactivity was recovered. These results indicated that uncomplexed ^{65}Zn is adsorbed on Bio-Gel P-10 and suggested that the intestinal ^{65}Zn eluted at the total volume

of the column may be associated with a specific ligand.

As shown in Fig. 2, the migration of ^{65}Zn obtained from peak 4 of the intestinal mucosa was markedly different from that of labeled zinc salts and amino acid complexes. The salt solution of ^{65}Zn migrated with the solvent front ($R_f = 1.0$) whereas the ^{65}Zn from peak 4 migrated approximately one-half the distance ($R_f = 0.47$) of the solvent front. When the labeled zinc-amino acid solutions were chromatographed, the isotope migrated with the solvent front which suggests that zinc-amino acid complexes dissociate in the solvent used in these experiments. Thus, a large proportion of orally administered ^{65}Zn in the intestine of the rat is bound to a low-molecular-weight compound which possesses chemical properties differing markedly from those of zinc salts and simple zinc-amino acid complexes. Moreover, infrared spectroscopy data indicate that the ^{65}Zn-binding component from the lumen is identical to that obtained from peak 4 in the intestinal mucosa (unpublished data). We are currently attempting to fully characterize the zinc-binding ligand by the use of nuclear magnetic resonance spectroscopy and infrared spectroscopy.

A low-molecular-weight complex from intestinal mucosa has not been reported in previous investigations. This discrepancy probably results from differences in experimental techniques. Although Van Campen and Kowalski (3) examined intestinal zinc binding in rats, these authors fractionated the mucosal homogenate with ammonium sulfate and chromatographed the fraction which precipitated between 45 and 80% saturation. Using this method, the low-molecular-weight fraction of intestinal zinc was probably discarded in the unprecipitated supernatant fraction. Neither Starcher (4) nor Suso and Edwards (1, 5) identified a low-molecular-weight form of zinc following gel filtration chromatography of the intestinal mucosa from chicks. However, both of these groups of investigators observed that 25–30% of the zinc in the mucosal supernatant is dialyzable in solutions at or near neutral pH. Since both Starcher and Suso and Edwards used high-exclusion-limit gels, the identification of a low-molecular-weight zinc complex may have been precluded. Suso and Edwards (1) did separate a low-molecular-weight ^{65}Zn complex from intestinal digests when the material was applied to gels with a lower exclusion limit.

To suggest that the ^{65}Zn-complex described above is responsible for zinc transport from the lumen to the blood would be speculative at this point. Further experimentation will be necessary in order to delineate the exact physiological role of this metallo-ligand. However, several investigations have demonstrated that low-molecular-weight chelators function in transporting essential elements across biological membranes (6–8).

Summary. A low-molecular-weight ^{65}Zn complex was identified in the intestinal lumen and mucosa following oral administration of ^{65}Zn to rats. Analysis of the ^{65}Zn complex by thin layer chromatography indicates that the complex possesses properties markedly different from those of zinc salts and simple zinc-amino acid complexes. These experiments demonstrate that zinc is bound to a low-molecular-weight ligand as well as a variety of macromolecules in the intestine of the rat.

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