# Binding Capacity of Intestinal Mucosa and Blood Plasma for Zinc[1] (35566)

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Although a great deal of research has been conducted on quantitative and qualitative aspects of zinc nutrition, little attention has been given to the mechanism of zinc absorption. Whether an active transport of zinc across the intestinal wall and throughout the body is involved has not been clearly demonstrated. Experiments on human plasma proteins have indicated that plasma albumin binds zinc (1). This binding of zinc to plasma albumin takes place at the imidazole ring of histidine and has an intrinsic binding constant of 2.82. The ratio of moles of zinc: imidazole rings was found to be 1:1. The binding of copper by intestinal mucosa has been shown by Starcher (2). He also observed that the same protein which binds copper also binds zinc. The experiments reported here were conducted to determine how tightly zinc is bound to plasma proteins and to determine some general characteristics of the molecule that binds zinc in plasma and intestinal mucosa.

*Materials and Methods. Animals and dosing of the isotope.* Three groups of five mature single comb white leghorn males housed in metal growing batteries were used in these studies. They were fed a practical broiler diet and received water *ad libitum*. The birds were starved overnight and were then allowed to eat freely for a 45-min period before dosing with 2 ml of solution containing 100 $\mu$Ci of $^{65}$Zn (4.9 mCi/mg of Zn) orally, intramuscularly, or intravenously.

*Preparation of samples. Blood plasma.* Two hr after $^{65}$Zn dosing approximately 5 ml of blood was collected by heart puncture into a heparinized syringe, centrifuged, and the plasma was removed.

*Intestinal mucosa preparation.* After collection of blood, the chickens were sacrificed and the small intestine was removed, cut open longitudinally, washed with distilled water, and the mucosa was removed by scraping. The mucosal tissue was homogenized in a Potter Elvehjem homogenizer with 2 vol of 0.25 *M* sucrose at 0°. Cellular debris, nuclei, mitochondria, microsomes, and soluble materials were separated by the method of Shikita and Tamaoki (3).

The distribution of $^{65}$Zn in the cellular fractions obtained by centrifugation was expressed as a percentage of the $^{65}$Zn in the original homogenate. A comparison was made between mucosal preparations obtained when $^{65}$Zn was added to mucosal homogenate of nondosed birds and when the mucosal preparation was obtained from chickens orally dosed with $^{65}$Zn.

*Dialysis.* Dialysis of 2 ml of plasma or mucosal soluble fraction (MSF) was carried out for 72 hr with constant stirring at 5° against 4 liters of the appropriate solution maintained at pH 7. Dialyzing membrane and its contents were counted in a well-type scintillation counter at 0, 12, 24, 48, and 72 hr. Results were expressed as percentage of $^{65}$Zn retained in the dialysis membrane. All dialytic experiments involved the use of five samples, each one from a different animal, for each group.

Plasma from the $^{65}$Zn-dosed birds was dialyzed against distilled water and ethylenediaminetetraacetic acid (EDTA) solutions of $10^{-2}$ and $10^{-4}$ *M*. Mucosal soluble fraction from orally $^{65}$Zn-dosed chickens was dialyzed against distilled water at 5 different pH values (2.4, 4.3, 6.0, 8.2, 11.0) and against

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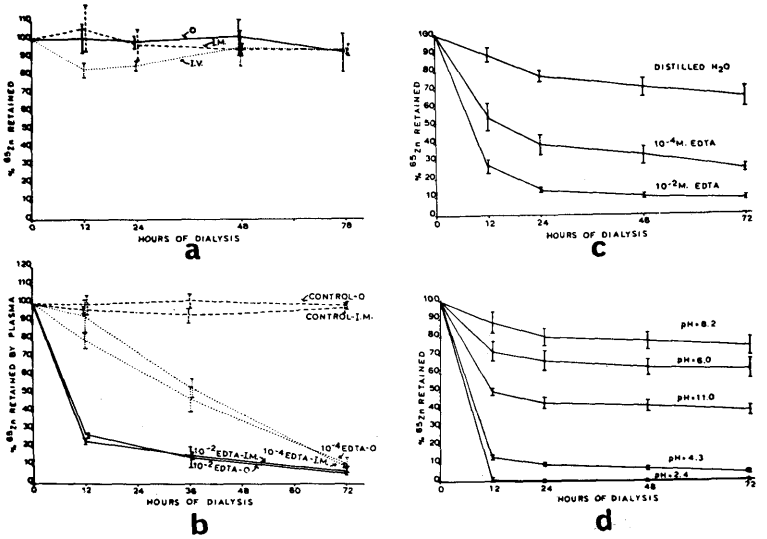


FIG. 1. Retention of $^{65}Zn$ by blood plasma and mucosal soluble fraction when dialyzed against different solutions: Vertical bars at each point indicate the standard error of the mean of 5 samples. (a) $^{65}Zn$ retention of blood plasma samples from orally (O), intramuscularly (IM) and intravenously (IV) dosed chickens dialyzed against distilled water. (b) Retention of $^{65}Zn$ by plasma from orally (O) and intramuscularly (IM) dosed chickens as affected by EDTA ($10^{-4}$ and $10^{-2}$ *M*) and distilled water dialysis. (c) Effect of EDTA ($10^{-4}$ and $10^{-2}$ *M*) and distilled water dialysis on $^{65}Zn$ retention by MSF. (d) Effects of dialysis at different pH on the retention of $^{65}Zn$ by MSF.

EDTA solution of $10^{-2}$ and $10^{-4}$ *M*.

*Column chromatography.* Dialyzed plasma or mucosal soluble fraction were chromatographed on a 2.5 × 55-cm Sephadex G-150 column with distilled water used for elution at 5°. Five-ml fractions were collected from the column and 1 ml was removed to estimate its protein content by measuring the optical density at 280 mμ in a spectrophotometer. $^{65}Zn$ content was determined in a well scintillation counter and total zinc by atomic absorption spectrometry.

*Results.* There was no detectable loss of $^{65}Zn$ activity throughout the 72-hr period when whole plasma was dialyzed against distilled water (Fig. la). The different routes of administration did not seem to affect the Zn binding capability of the plasma. EDTA at either $10^{-2}$ or $10^{-4}$ *M* resulted in almost complete zinc removal from plasma after 72 hr of dialysis (Fig. lb). EDTA at the higher concentration caused a rapid decline in $^{65}Zn$ bound to plasma during the first 12 hr of dialysis; thereafter, the rate of removal was very slow. When plasma was dialyzed for 24 to 48 hr and then centrifuged, there was less than 5% $^{65}Zn$ activity in the precipitate, while the rest of the isotope was present in the soluble fraction.

Dialysis of MSF against water at different pH (Fig. ld) removed less than 40% of the $^{65}Zn$ at pH 6 or 8.3; however, more than 50% of the original $^{65}Zn$ was removed at extreme acidic or basic pH. The removal of $^{65}Zn$ by water at extreme pH appeared to be caused by protein denaturation. As in the case of plasma, EDTA at both concentrations removed most of $^{65}Zn$ after 72 hr of dialysis. There was a fast decline of $^{65}Zn$ in the dialysis bag during the early hours of dialysis followed by a slower rate of removal during the rest of the experiment (Fig. 1c). Distilled water at neutral pH showed the least capacity for removal of zinc from the MSF. When the $^{65}Zn$ was added to the intestinal mucosa at the time of homogenization and then dialyzed against distilled water, $10^{-2}$ *M* EDTA, and $10^{-4}$ *M* EDTA solution, the results were the same as those obtained for dialysis of MSF from birds that were given

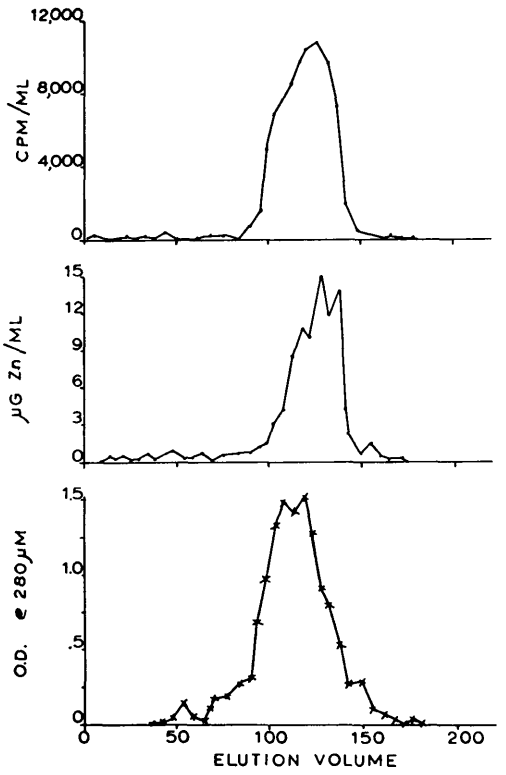


FIG. 2. Elution pattern obtained by Sephadex G-150 gel filtration chromatography of blood plasma from chickens dosed orally with $^{65}$Zn. The data, obtained from one chicken, are representative of those of the group of five used in this experiment.

$^{65}$Zn orally.

$^{65}$Zn was associated with one major group of plasma proteins after gel filtration (Fig. 2). Analysis of the column fraction for total zinc content by atomic absorption revealed the same distribution for the element as for the $^{65}$Zn. The electrophoretic properties of the protein peak that contained the largest $^{65}$Zn concentration were identical to those of plasma albumin. The elution pattern for $^{65}$Zn and blood plasma proteins from gel filtration chromatography were identical to those shown in Fig. 2 when the $^{65}$Zn was administered orally or intramuscularly.

The distribution of $^{65}$Zn in the different cellular fractions of intestinal mucosa from orally dosed chickens and from mucosa samples in which $^{65}$ Zn was added to the homogenate is presented in Table I. The major part of the zinc, in both cases, appeared as part of the MSF. There were some differences in the amount of isotope present in the mitochondria, cell debris and nuclei, and microsomal fractions (Table I) when $^{65}$Zn was either dosed orally or added to the homogenate. Gel filtration of the MSF dialyzate resulted in a different pattern than that of blood plasma (Fig. 3), where $^{65}$Zn and nonradioactive zinc were present in a fraction containing a protein of smaller molecular weight than the one binding zinc in blood plasma. Electrophoretic studies of the MSF and of the fractions collected from gel filtration disclosed nine bands, three of which had a negative migration, five had positive, and one stayed at the point of application. One of the bands which had a positive migration was identified as plasma albumin but it did not correspond to the protein group that carried most of the $^{65}$Zn. The zinc binding protein had a motility of about one-third the distance traveled by plasma albumin.

*Discussion.* Dialysis of MSF and blood plasma against distilled water indicated binding of $^{65}$Zn to the mucosal and plasma proteins. Since less $^{65}$Zn was dialyzable from plasma than from MSF, it is suggested that plasma proteins bind zinc to form stronger complexes than mucosal proteins. This proba-

TABLE I. $^{65}$Zn Content of Cell Fractions from Chicken Intestinal Mucosa Obtained by Centrifugation.

| Fractions | $^{65}$Zn | |
|---|---|---|
| | Added to mucosa during homogenization (%) | Dosed orally to the chicken (%) |
| Nuclei and cell debris | 17.4 | 10.3 |
| Mitochondria | 4.4 | 2.4 |
| Microsome | 16.1 | 21.1 |
| Mucosal soluble fractions (MSF) | 62.1 | 66.2 |

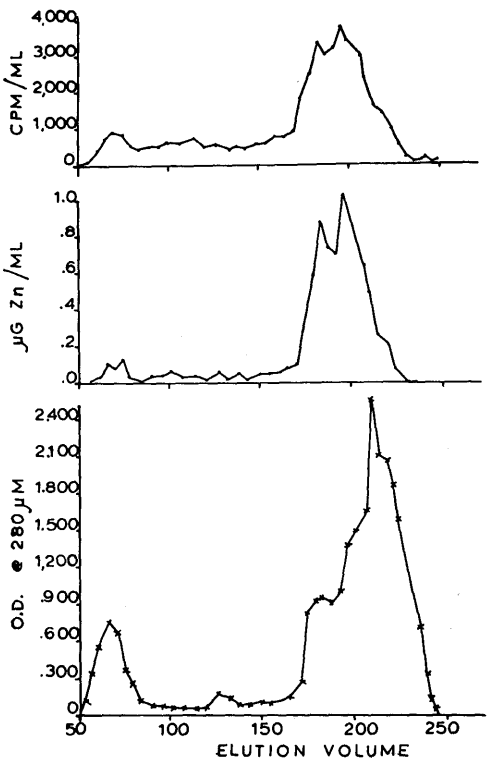


FIG. 3. Protein and $^{65}Zn$ elution pattern of chicken MSF obtained with a 2.5 × 55-cm Sephadex gel G-150 column. The data, obtained from one chicken, are representative of those of the group of five used in this experiment.

bly facilitates the removal of the zinc from the mucosal lining into the blood capillary system, thus completing the process of absorption. During the early dialysis of plasma by $10^{-2}$ *M* EDTA, 90% of the zinc was removed. The removal of the remaining 10% of zinc required a longer time. This is in agreement with previous findings (4). It would appear that two types of zinc were present at the plasma level, one easily removed by $10^{-2}$ *M* EDTA, while the other one required longer periods of time for complete removal. This suggests that there may be two binding sites for zinc, one having a greater affinity for zinc than the other. According to Laurell (4) this loosely bound zinc present in plasma albumin represents its form of transport.

Gel filtration and electrophoretic studies indicated that the main peak of zinc binding protein in blood plasma is albumin. Gurd and Goodman (1) reported that in humans zinc binds with plasma albumin by means of the imidazole ring of histidine.

When $^{65}Zn$ was added to the mucosa prior to homogenization and compared with mucosa that had bound zinc *in vivo*, differences were observed in the nuclei and cell debris, mitochondria, and microsome fractions. These differences may have resulted because sufficient time was not alloted for incubation and homogenization was done at a cold temperature, thus reducing the possibilities for the isotope to be freely transported to the different parts of the cell.

Chromatography of the MSF by gel filtration resulted in a different elution pattern from the column than those reported by Starcher (2). This difference may be attributed to differences in the method of centrifugation and preparation of the sample that was chromatographed. Although one of the protein fractions from MSF migrated like plasma albumin during electrophoresis, it did not carry the highest concentration of the isotope. The protein from MSF which bound the zinc had a greater elution time than plasma albumin, thus indicating a smaller size. However, the solubility properties seemed to indicate that the zinc binding protein belonged to the albumin group.

*Summary.* Studies conducted on plasma and mucosal soluble fractions indicated that zinc is bound to both plasma and mucosal proteins.

The binding of zinc at the plasma level was associated with the plasma albumin fraction and the complex formed appeared to be stronger than that of the mucosa.

The protein binding zinc in the intestinal mucosa was not identified; however, it appeared to be an albumin-like protein of smaller molecular weight than plasma albumin.

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