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Zinc uptake and transcellular movement by CACO-2 cells: Studies with media containing fetal bovine serum

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A series of experiments using CACO-2 cells, have been conducted to describe the cellular events occurring during Zn uptake and transcellular movement in the presence of media containing fetal bovine serum (FBS). CACO-2 cells were grown for 21 days on either T-25 cell culture flasks or semipermeable membrane inserts maintained in six-well culture plates. Experiments were then conducted using normal growth medium containing FBS, to which ^{65}Zn was added. The rate of Zn transport in an apical to basolateral direction was much greater than the rate in the opposite direction. To study whether uptake and movement exhibited saturation kinetics, different concentrations of Zn (1–200 μM) were added with ^{65}Zn. Uptake at the basolateral membrane was saturable; apical to basolateral movement, basolateral to apical movement, and uptake at the apical membrane were not. ^{65}Zn moved apically to basolaterally at a small but constant rate regardless of the basolateral concentration of Zn. The binding ligands for Zn in the cytosol differed depending on the prior Zn status of the cell and the time postlabeling. Endogenous ^{65}Zn was released to the apical and basolateral sides at different rates. These data confirm a previous report that uptake and transcellular movement of Zn is different at the apical and basolateral membrane, and they further show that the presence of Zn-binding ligands alters these processes. (J. Nutr. Biochem. 6:137–144, 1995.)

Keywords: zinc; absorption; CACO-2; transport; cell culture

Introduction

Because Zn is essential for human health,[1,2] many studies have been directed toward determining the mechanism of Zn absorption by the gut. A variety of techniques have been used including isolated, perfused gut loops,[3–10] isolated membrane vesicles,[11–14] and Ussing chambers.[15] Additionally, cellular mechanisms of Zn uptake have been studied with cultured cells such as hepatocytes,[16,17] fibroblasts,[18,19] and endothelial cells.[20,21] Comparison between studies using different methodologies is difficult; isolated membrane vesicles reflect only what is occurring at one surface of the cell. Also, the method of isolated, perfused gut loops does not reflect a single event, rather a combination of many events such as uptake and release by the enterocyte and movement into and out of the vascular supply. However, the general consensus of most of these studies has been that Zn uptake and transport occurs both by diffusion and an unspecified facilitated process.

A human colon carcinoma cell line (CACO-2) has been used as a model of intestinal permeability for many substances including amino acids,[22–24] steroid hormones,[25] and trace elements.[26–29] Most recently this cell line was used by Raffaniello and co-workers[30] to characterize mechanisms of Zn uptake. These researchers found Zn uptake from the apical side was a saturable process, but uptake from the

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basolateral side and transport in either direction was not saturable.

In the present study, we have repeated several of the experiments of Raffaniello and coworkers,[30] but the experimental conditions are different. The former study used a HEPES-buffered, salt-based medium for the experiments, whereas cells were grown in Dulbecco's modified Eagle's medium (DME) with 10% fetal bovine serum (FBS). Certainly, the presence of FBS influences Zn uptake and transport[31] and it is also possible that an experimental buffer that differs from the growth medium could induce metabolic changes that could alter uptake and transport. As a result, we have conducted the present experiments in experimental medium that is the same as the growth medium, i.e., DME + 10% FBS. Comparison of the findings of these studies allows speculation about the way in which Zn ligands may influence Zn uptake and transport.

Another objective of the present report was to further the understanding of how CACO-2 cells take up and transport Zn by examining the internal movement and subsequent release of cellular Zn. Certainly the involvement of Zn-binding proteins in the intracellular retention and release of Zn in the gut is known,[32] but this study has examined the protein binding of Zn simultaneous with examining the release of internalized Zn.

Materials and methods

The CACO-2 cell line was purchased from American Type Tissue Collection. The cells were cultured in 75-cm^2 plastic flasks containing high-glucose (4.5 mg/L) Dulbecco's modified Eagle's medium (DME) with 10% fetal bovine serum (FBS, Gibco, NY USA). The medium also contained 1% nonessential amino acids and 50 μg/mL gentamicin. Cells were grown in 95% air, 5% CO_2, and 90% humidity. Stock cultures were split every 7 days, after detachment with 0.25% trypsin, and reseeded at a density of 15,000 cells/cm^2. All experiments were performed with cells between passage 20 and 50.

Experiments used cells cultured under two conditions: One type of experiment used cells grown on polyethylene terephthalate semipermeable membranes (PET; Falcon membrane filters, 4.65-cm^2 surface area, 0.45 μ pores, Becton-Dickinson Labware, Lincoln Park, NJ USA) and examined factors influencing the movement of ^{65}Zn from one side of the filter to the other, or factors influencing the uptake or release of Zn from the apical or basolateral membrane. Before seeding, membranes were coated with 50 μg of Type I rat tail collagen (Collaborative Biochem, Bedford, MA USA). Membranes were seeded at a density of 60,000 cells/cm^2 and placed in six-well plates with 2.5 mL of media in the well and 1.5 mL of media in the filter. Medium was changed on alternate days and cells were used for experiments after 21 days of culture.

Another type of experiment used 25-cm^2 tissue culture flasks that were seeded at a density of 20,000 cells/cm^2. Five milliliters of medium was placed on the flask and changed on alternate days. Cells were used for experiments after 21 days of culture. Unless specified otherwise, all experiments were performed at 37°C.

Uptake experiments

The uptake of ^{65}Zn was studied with cells on PET membranes after 21 days of culture. Cells were washed once with low-glucose DME and new medium (normal growth medium) containing 18.5 kBq ^{65}Zn/mL (DuPont NEN Research Products, Boston, MA USA) was added to either the apical (1.5 mL) or basolateral (2.5 mL) compartment of the membranes (nonradioactive normal growth medium added to the other side) and allowed to incubate a set amount of time (see figure legends). Experimental media were always prepared at least 24 hours in advance and allowed to equilibrate overnight in the incubator. During the time that radioactive medium remained on cells, plates containing membranes were swirled gently by a rotating mixer (Cole-Parmer, Inc., Chicago, IL USA).

At the end of each time point, cells from multiple filters were harvested. Radioactive medium was removed and the cell layer was washed once with 5 mL of ice-cold HEPES buffer (10 mM HEPES, 140 mM NaCl, 7 mM KCl, 5.6 mM glucose, pH 7.4), incubated for 15 seconds in 5 mL of EDTA/HEPES buffer (10 mM EDTA, 10 mM HEPES, 150 mM NaCl, pH 7.4) and then rinsed twice with 5 mL of HEPES buffer.[31] Filters were then cut from the plastic insert and placed in 2 mL of a solution of 0.2% sodium dodecyl sulfate and 0.2% NaOH to solubilize the cells for subsequent gamma counting (Packard Instruments, Cobra Auto-Gamma, Meriden, CT USA).

Concentration dependence of Zn uptake was determined with media containing the same amount of ^{65}Zn as before but with nonradioactive Zn concentrations of 1 to 200 μM. (Concentration dependence of Zn uptake and transport were initially evaluated in media containing Zn concentration between 200 and 1200 μM. However, uptake and transport both increased linearly between the Zn concentrations of 200 to 1200 μM [data not shown], so further experiments only used Zn concentrations of up to 200 μM.) This medium was added to either to apical or basolateral side and normal growth medium was added to the other side. Media containing 10% normal FBS contain 6 μM Zn; FBS was dialyzed extensively[33] to prepare media with Zn concentrations of less than 6 μM. Because this procedure also decreased the protein concentration of the FBS, media were prepared containing 14% dialyzed FBS. Cells were incubated with the prepared media for either 10 minutes or 5 hours on a rotating shaker and harvested as described previously.

Transcellular movement experiments

The transcellular movement of Zn in different directions and under different experimental conditions was also studied with cells grown on membranes. Specifics for the different experiments are given in the figures and results.

Cells (after 21 days of culture) were examined for confluence, and membranes that showed areas of no cell growth, tears in the membrane, or did not exclude phenol red (PR) from the basolateral compartment were not used. (DME containing PR was placed in the apical compartment; DME without PR in the basal compartment. After overnight incubation, basolateral media were collected and analyzed spectrophotometrically for PR.) Old growth medium was removed and experimental media containing ^{65}Zn and differing concentrations of nonradioactive Zn were added to one side and normal growth medium to the other. Plates were incubated on a rotating shaker for specified times (see figures), then media from the side that received the nonradioactive medium was removed for gamma counting. In some experiments, it was possible to study transport and uptake simultaneously by collecting the membrane after collecting medium.

Subcellular distribution and release studies

The subcellular distribution and release of endogenous Zn were studied in experiments in which cells were pulsed with ^{65}Zn-

labeled media followed by a chase of unlabeled media for defined lengths of time. Additionally, some cells were grown in different concentrations of Zn to determine whether cellular Zn status affected the distribution and release of Zn.

In the first experiment, cells grown on PET membranes were incubated with 4.62 kBq ^{65}Zn/mL for days 18 to 21. After cells were loaded with ^{65}Zn, apical and basolateral media of all cells were replaced with normal growth medium (6 μM Zn) containing no ^{65}Zn. At defined times, thereafter, cells, apical medium, and basolateral medium were harvested and counted for gamma energies. To correct for ^{65}Zn loosely bound to the cell surface, radioactivity in the media removed from both compartments after 10 minutes was subtracted as a blank.

The molecular localization of ^{65}Zn in cell cytosol was studied with cells grown on 25-cm^2 cell culture flasks cultured with either normal growth medium the entire time or normal medium the first 10 days and medium containing 100 μM Zn the last 11 days. After 21 days of culture, growth medium containing 37 kBq/mL of ^{65}Zn (total of 0.185 MBq/flasks) was added and cells were incubated for 4 hours. After that the radioactive medium was removed and replaced with the respective nonradioactive media (6 or 100 μM Zn) for defined periods of time (see figure 7).

At the end of the specified periods, cells were harvested and sonicated after mixing 1:4 in 0.3 M Tris (pH 7.5) buffer containing 154 mM NaCl, 0.2 g/L Na azide, 35 mg/L phenylmethylsulfonyl fluoride, 0.6 mg/L leupeptin, and 0.9 mg/L pepstatin. Cellular cytosol was prepared by centrifugation at 40,000*g* for 1 hour. One hundred microliters of cytosol were chromatographed on a 1.5 × 32 cm Superose 12 column.[34] Column void was determined with DNA, total volume with deuterium oxide, and the column was calibrated with standards of known molecular weight. The sample was eluted with the sonication buffer using an Isco high-pressure liquid chromatography (HPLC) system (Isco Inc., Lincoln, NE USA) at a flow rate of 0.25 mL/m; fractions were collected and analyzed for ^{65}Zn.

Statistical analysis

Uptake and transport data were modeled by linear and nonlinear regression models. Saturation kinetics was modeled with or without a diffusional component. All models were fit by using the SAS software package (SAS, SAS Institute Inc., Cary, NC USA). The mean square error (MSE) from each model and the plot of the residuals (residual = predicted value − actual value) versus predicted values were examined to determine which model provided the best fit.[35] Only the models that best described the data are reported here.

Most experiments were replicated several times (see figures). Results presented are from representative experiments.

Results

Time course of Zn uptake and transport

Cellular uptake of Zn (*Figure 1*) increased with time with no sign of a plateau (except perhaps at the last time point). When ^{65}Zn was placed on the basolateral side of membrane, it was taken up by cells at a decreasing rate (*Figure 2*) which began to plateau after 30 hours. Total cellular accumulation of Zn from the basolateral side, after 25 hours, was similar to the total accumulation from the apical side, but transcellular movement of Zn from the basolateral to apical side was negligible.

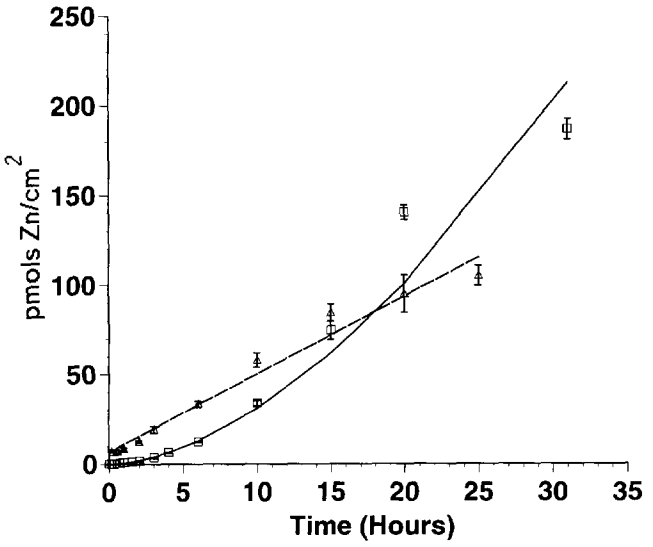

Figure 1 Time course of cellular uptake of Zn by the apical membrane of CACO-2 cells (open triangles, dashed line) or apical to basolateral movement of Zn by CACO-2 cells grown on semipermeable membranes (open squares, solid line). Wells incubated with ^{65}Zn in the apical compartment for the specified times, then membranes and basolateral medium collected and counted for gamma energies. Five or six wells used per time point per experiment; experiment repeated twice. Data expressed as picomoles of Zn per cm^2 cell surface.

Zn concentration dependence of Zn uptake and transport

Data from studies examining the Zn concentration dependence of Zn uptake and transcellular movement were mod-

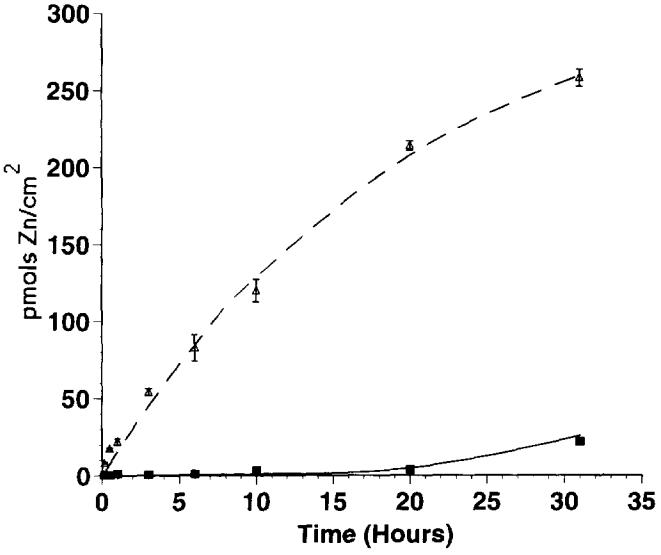

Figure 2 Time course of cellular uptake of Zn by the basolateral membrane of CACO-2 cells (open triangles, dashed line) or basolateral to apical movement of Zn by CACO-2 cells grown on semipermeable membranes (open squares, solid line). Wells incubated with ^{65}Zn in the basolateral compartment for the specified times, then membranes and apical medium collected and counted for gamma energies. Five or six wells used per time point per experiment; experiment repeated twice. Data expressed as picomoles of Zn per cm^2 cell surface.

eled with linear models (diffusion type kinetics) or with nonlinear models (saturation type kinetics) with or without a diffusional component. Uptake experiments were conducted for 10 minutes (instantaneous kinetics) or 5 hours (steady-state kinetics). Nonlinear models, with and without a diffusion component, did not fit apical Zn uptake data from cells incubated 10 minutes (*Figure 3A*) or apical Zn uptake and apical to basolateral movement data from cells incubated 5 hours (*Figure 3B*) better than linear models. In contrast, Zn uptake from the basolateral side after 10 minutes (*Figure 4A*) and after 5 hours (*Figure 4B*) was saturable and the half-maximal rate of uptake for cells incubated 5 hours was 49 ± 11 μM. Transcellular movement from the basolateral to apical side, however, occurred in linear fashion with respect to increasing concentrations of Zn.

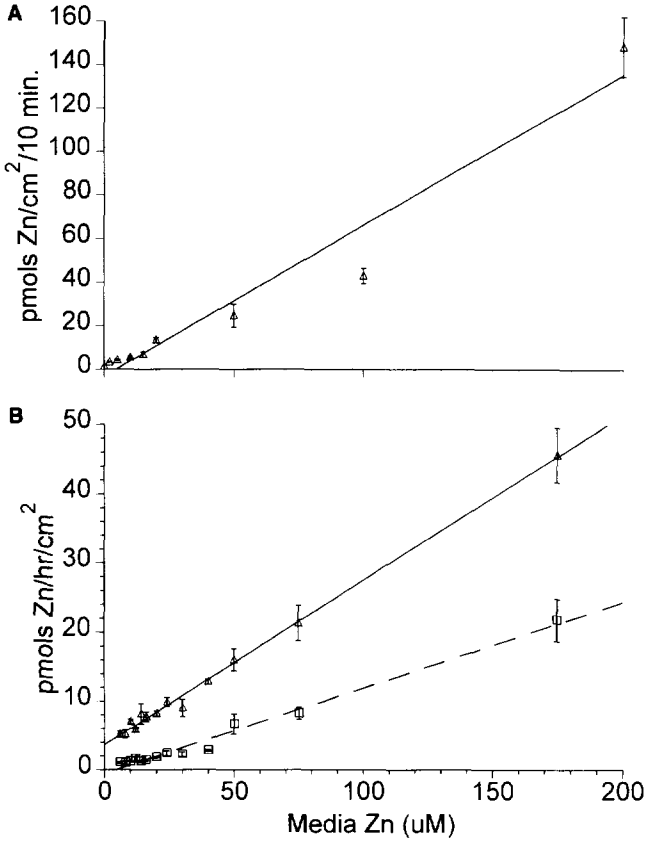

Figure 3 Concentration dependence of Zn uptake by the apical membrane of CACO-2 cells and/or apical to basolateral movement of Zn by CACO-2 cells grown on semipermeable membranes. Radioactive Zn and varying concentrations of nonradioactive Zn added to the apical compartment and normal growth medium added to the basolateral compartment. Cells incubated for the specified times and then basolateral medium and/or membranes collected and counted for gamma energies. All data expressed as picomoles of Zn/cm^2 cell surface/hr. (A) Concentration dependence of apical uptake of Zn by CACO-2 cells incubated with ^{65}Zn for 10 min. Six membranes collected per concentration selected; experiment repeated twice. (B) Concentration dependence of apical uptake of Zn (solid line) or apical to basolateral movement of Zn (dashed line) by CACO-2 cells incubated with ^{65}Zn for 5 hr. Six membranes and wells of basolateral medium collected per concentration selected; experiment repeated twice.

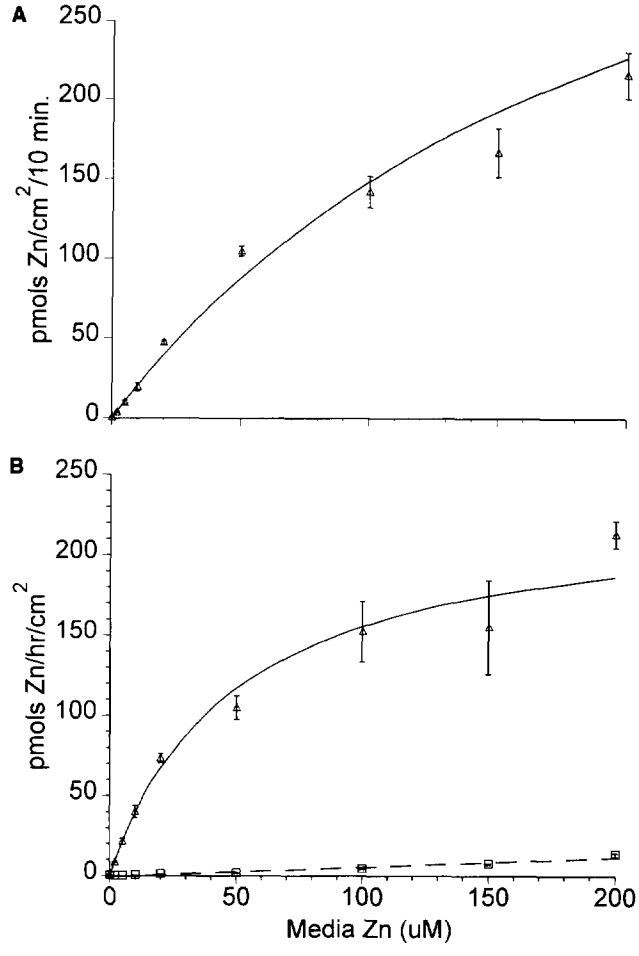

Figure 4 Concentration dependence of Zn uptake by the basolateral membrane of CACO-2 cells and/or basolateral to apical movement of Zn by CACO-2 cells grown on semipermeable membranes. Radioactive Zn and varying concentrations of nonradioactive Zn added to the basolateral compartment and normal growth medium added to the apical compartment. Cells incubated for the specified times and then apical medium and/or membranes collected and counted for gamma energies. All data expressed as picomoles of Zn/cm^2 cell surface/hr. (A) Concentration dependence of basolateral uptake of Zn by CACO-2 cells incubated with ^{65}Zn for 10 min. Six membranes collected per concentration selected; experiment repeated twice. (B) Concentration dependence of basolateral uptake of Zn (solid line) or basolateral to apical movement of Zn (dashed line) by CACO-2 cells incubated with ^{65}Zn for 5 hr. Six membranes and wells of apical medium collected per concentration selected; experiment repeated twice.

Effect of zinc concentration in the basolateral compartment on the apical uptake and apical to basolateral transcellular movement of Zn

Normal growth medium containing ^{65}Zn was placed in the apical compartment of cells and growth medium containing no ^{65}Zn, but varying concentrations of nonradioactive Zn, was placed in the basolateral compartment. After 5 hours, membranes and basolateral media were collected. Uptake of Zn from the apical side (*Figure 5*) was inhibited to an increasing extent as the basolateral concentration of Zn increased. Apical to basolateral transport of Zn, however,

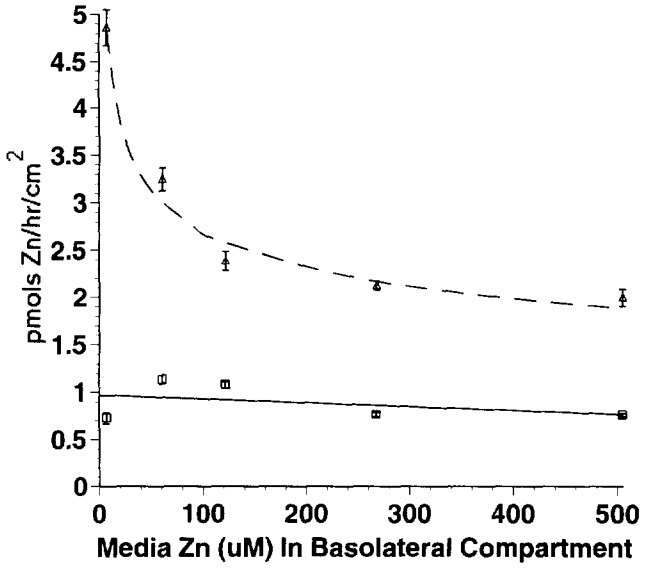

Figure 5 The effect of increasing concentrations of Zn in the basolateral compartment on the apical to basolateral movement (solid line) and uptake (dashed line) of Zn by the apical membrane of CACO-2 cells grown on semipermeable membrane filters. Cells incubated with experimental media containing ^{65}Zn in the apical compartment and nonradioactive medium containing varying concentrations of Zn in the basolateral compartment for 5 hr. Basolateral medium and membranes then collected and analyzed for gamma energies. Each point at selected concentrations is mean of six wells and membranes. Data expressed as picomoles of Zn/cm^2 cell surface/hr.

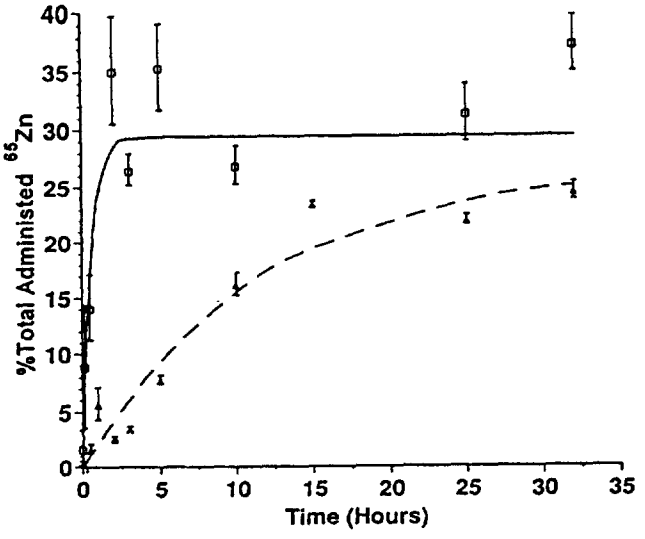

Figure 6 Time course of release of endogenous ^{65}Zn to the apical (open squares, solid line) or basolateral (open triangles, dashed line) compartments of CACO-2 cells. Cells were incubated with ^{65}Zn in the apical and basolateral compartments for days 18 to 21 of culture, then radioactive media were replaced with nonradioactive media. Apical and basolateral media and membranes were collected at various time points and counted for gamma energies. Each time point is the mean of six wells and membranes. Data expressed as percent of total counts (sum of radioactivity in apical and basolateral compartments plus radioactivity incorporated into cells on the membrane) appearing in the apical or basolateral media.

was about 1 pmol/cm^2 cell surface/hr at all concentrations of basolateral Zn. This is similar to the rate of transport observed in other experiments when normal growth medium containing ^{65}Zn was placed in the basolateral compartment and incubated for 5 hours.

Molecular distribution and release of internalized ^{65}Zn

For the first 2 hours, release of endogenous ^{65}Zn to the apical side occurred at a faster rate than release to the basolateral side (*Figure 6*). After 2 hours, release to the apical side had plateaued, but release to the basolateral side continued at the initial rate. At this time 25 to 35% of the endogenous ^{65}Zn was in the apical compartment, but only 2 to 5% was in the basolateral compartment and 60 to 70% remained inside the cell. Release of ^{65}Zn to the basolateral side did not begin to plateau until after 15 hours. By the end of the experiment, approximately 37% had been released to the apical side, 25% released to the basolateral side, and 35 to 40% remained inside the cell.

Cytosol of cells grown in 100 μM Zn had two major Zn-binding peaks (*Figure 7*). One peak was at the beginning of the elution and corresponded to a molecular weight of 200,000 to 400,000 daltons. The second ^{65}Zn peak corresponded to a molecular weight of 5 to 15 kD and metallothionein (Mt) co-eluted with a portion of this peak. In addition to these distinct peaks, there was random labeling of many proteins in the intermediate weight range. Maximum labeling of both the low-molecular-weight (LMW) and high-molecular-weight (HMW) peaks occurred between 6 and 12 hours; thereafter, counts in both peaks decreased.

There was much less total ^{65}Zn in the cytosol of cells grown in normal growth media and the ^{65}Zn was more randomly distributed, although the HMW peak was consistently labeled. Maximum labeling occurred at 12 hours and decreased thereafter. The major observed difference in Zn-binding ligands as the result of previous Zn status was that the LMW ^{65}Zn peak (the major peak in cells grown in 100 μM Zn) was not observed in the cytosol of cells grown in normal media.

Discussion

Numerous investigators have examined Zn absorption by many different methods.[3–10,15] There is not a clear consensus as to how Zn is removed from the lumen at the brush border of the enterocyte and delivered to the mucosal side of the gut; however, most researchers seem to agree that absorption is a combination of mediated and diffusional processes.[9,11,12,30,36,37] Because of the diversity and complexity of the tissues in the gut, it is difficult to identify the processes at work and to determine where the point(s) of control may be.

In a previous report, Raffaniello and co-workers used CACO-2 cells to examine the basic mechanisms of Zn uptake and transport.[30] They used a defined salt medium to conduct their transport experiments, which simplifies explanation of the uptake and transport data. In the present

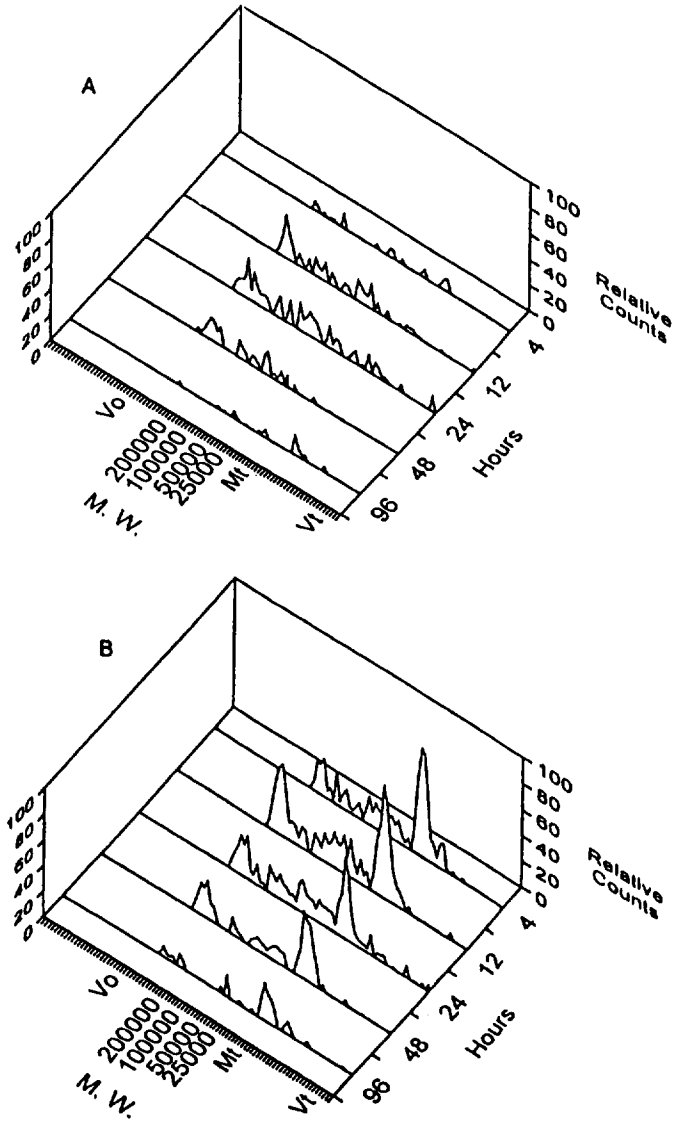

Figure 7 The effect of medium Zn concentration and time postlabeling on the distribution of ^{65}Zn among Zn-binding ligands in the cytosol of cells grown on flasks with normal growth media (A) or medium containing 100 μM Zn (B) by HPLC size exclusion chromatography. Twenty-one-day-old cells were pulsed for 4 hr with ^{65}Zn, which was followed by incubation with nonradioactive medium. Cells were harvested at times shown, cytosol prepared and chromatographed by HPLC. Cytosol from three flasks pooled for each time point. The distribution of ^{65}Zn among cytosolic ligands is shown at ties from 4 to 96 hr after labeling.

study, we have also used CACO-2 cells as an in vitro model of the intestinal epithelia to study Zn uptake into and movement across the enterocyte. However, in the body, very little zinc would exist as the free ion in either the lumen of the gut or the vasculature, as it would be complexed to some ligand. Thus, we have conducted our studies in medium containing FBS in which the Zn present would be complexed to various physiological ligands. Whereas serum is present in the basolateral environment of the enterocyte, the apical (luminal) environment does not normally contain serum. However, Zn in the luminal environment would also be complexed, and many of the ligands would be amino acids, peptides, and proteins.[38] As such, FBS also simulates the luminal environment to a greater degree than does a salt buffer.

Contrary to the prior report,[30] we did not see saturation of Zn uptake from the apical side in the presence of increasing concentrations of Zn. The previous study calculated a V_{max} of 0.3 nmol/cm^2/10 min or 1.8 nmol/cm^2/hr for uptake of Zn by CACO-2 cells in a serum-free media. They also calculated a K_t of 41 μM which gave an uptake rate of approximately 0.8 nmol/cm^2/hr. In this study, the maximal apical uptake rate (at 200 μM Zn) was only 50 pmol/cm^2/hr. Thus, if Zn is taken up as the free ion,[18] this study may indicate that, in the presence of serum, saturation of a Zn carrier at the apical surface does not occur. In support of this hypothesis, we observed that a small, but constant, amount of Zn moved apically to basolaterally regardless of the concentration of Zn in the basolateral compartment.

Also contrary to what was reported previously for CACO-2 cells,[30] uptake of Zn from the basolateral membrane was saturable. However, the prior study also showed that uptake was inhibited by ouabain and vanadate, suggesting the involvement of (Na-K)-ATPase. A study using isolated basolateral membrane vesicles from rat intestine did find evidence for saturable uptake of Zn[14] and the concentration at which uptake was half-maximal (24 μM) was similar to the concentration at which basolateral uptake was half-maximal in this study (49 μM). Again, perhaps the reason for the discrepancy between this study and Raffaniello et al.[31] has to do with the amount of free ion present. Free ionic Zn may be able to diffuse across the cell, whereas ligand-complexed Zn may not be able to. Thus, it is possible that when large amounts of the free ion are present, basolateral uptake of Zn by a facilitated process is negligible compared with uptake due to diffusion. However, if the affinity for Zn by a carrier is greater than the affinity for Zn by ligands present in the medium, then even though the total amount of carrier-mediated transport of Zn is small, it represents the majority of Zn taken into the cell from the basolateral side.

Such a hypothesis could also explain why basolateral to apical transcellular movement in this study occurred at a much lower rate than apical to basolateral movement, whereas the reverse was true for the study of Raffaniello et al.[30] The previous investigators showed an approximately 1.8-fold greater rate of basolateral to apical transport for the first 50 minutes. The rate of apical to basolateral transport through 6 hours in this study, however, was 15-fold greater than the rate of basolateral to apical transport. Serosal to luminal transfer of Zn has been reported,[9,39] but Steel and Cousins[9] concluded that, whereas much Zn may enter the enterocyte from the vascular system, relatively little is transferred to the lumen. Results of this study support this in that Zn moved into the cell from the basolateral side but very little Zn moved out of the cell on the apical side, resulting in a primarily apical to basolateral transfer of Zn.

Previous studies have examined the subcellular distribution of Zn and the association of Zn with proteins in animal gut tissue. CACO-2 cells synthesize Mt,[40] but there have not been reports of the kinetics of protein bound Zn or of the efflux of endogenous Zn. The movement of endogenous ^{65}Zn out of CACO-2 cells was different depending on

whether it moved across the apical or basolateral membrane. Endogenous radioactive Zn quickly equilibrated with medium in the apical environment, but ^{65}Zn was released at a slower rate to the basolateral compartment and the ^{65}Zn in the basolateral compartment never did equilibrate with that inside the cell. Although such kinetics does not show the mechanism of Zn release, it does suggest that the basolateral membrane is a greater barrier to Zn efflux than the apical membrane. This is consistent with the finding of saturable uptake by the basolateral membrane but concentration-dependent uptake by the apical membrane. The involvement of the basolateral membrane in controlling Zn release from the enterocyte has been noted previously.[41]

When cytosol was subjected to HPLC chromatography, the major change seen with time was a decrease in the amount of ^{65}Zn associated with the HMW peak and random proteins, especially for cells grown in the normal media. CACO-2 cells grown in 100 μM Zn have a large portion of intracellular Zn associated with HMW proteins and about 50% of the intracellular Zn associated with Mt.[41] The present data show that over time Zn is removed from HMW proteins, either to outside the cell or to LMW proteins. Hoadley et al.[8] suggested that the association of Zn with Mt was a major determinant of the amount of Zn free to move to the vascular system. Hempe and Cousins[32] have postulated that the cysteine-rich intestinal protein (CRIP) competes with Mt for Zn, and thus modulates the amount of Zn free to move across the cell and out across the basolateral membrane.

In conclusion, this study has found that, similar to Raffaniello et al.,[30] CACO-2 cells have distinct mechanisms of uptake and transport at the basolateral and apical membranes. However, we have shown that in the presence of serum, which contains many Zn ligands, Zn movement in an apical to basolateral direction does not exhibit saturation kinetics. Uptake of Zn from the basolateral side does saturate under the same conditions, suggesting a greater affinity for Zn by the carrier present on that side than for Zn ligands in the serum. Further we have found that the efflux of endogenous Zn occurs differently at the apical and basolateral membranes.

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