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Research Article

Zinc Uptake in Five Sectors of the Rat Gastrointestinal Tract: Kinetic Study in the Whole Colon

Sonia Luz Gisbert-González[1] and Francisca Torres-Molina[1,2]

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Purpose. The uptake of zinc as acexamic acid salt in the rat gastrointestinal tract, using an *in situ* static technique, was studied. Our aim was to investigate an absorption window for zinc and the uptake kinetics in the colon.

Methods. To detect selectivity phenomena in zinc absorption, buffered saline solutions of zinc (50 μg/ml) were perfused in stomach, whole colon and three 33-cm fractions of the small intestine (proximal, middle and distal segments). To characterize zinc uptake kinetics in whole colon, five different zinc concentrations (5, 25, 50, 150 y 250 μg/ml) were assayed. Zinc secreted into the gastrointestinal tract during the experiments was deducted from the uptake.

Results. Zinc secretion was characterized as an apparent zero-order process for all the studied segments (mean secretion rate = 0.10 ± 0.03 μg/(ml × min)). The stomach exhibited little ability to absorb zinc (apparent first order rate constant = 0.17 ± 0.07 h^{-1}), whereas the highest transport rates were found in the last two thirds of the small intestine and colon (first order constants: 0.66 ± 0.13 h^{-1}, 1.00 ± 0.06 h^{-1}, 0.97 ± 0.14 h^{-1}, 0.96 ± 0.19 h^{-1} for proximal, middle, distal and colon segments, respectively). Zinc uptake in the colon was characterized by means of a Michaelis-Menten and first-order combined kinetics, with the following parameters: Vm = 0.36 ± 0.02 μg/(ml × min), Km = 18.01 ± 0.40 μg/ml and Ka = 0.40 ± 0.01 h^{-1}.

Conclusions. Zinc is preferably absorbed in the middle and distal parts of the rat gastrointestinal tract. In the colon a saturable mechanism may be involved in apparent absorption.

KEY WORDS: absorption window; proximal, middle and distal intestinal segments; colonic zinc uptake kinetics; endogenous secretion.

INTRODUCTION

Because it is a trace element, zinc is essential for the development of all living beings. As it is involved in metabolism activation and in cell regeneration, zinc has various therapeutical applications, especially as an antiulcerogenic. Acexamic acid salt, the compound assayed in the present work, has been extensively used for this patology (1). In spite of the numerous studies that have examined zinc, many aspects, particularly those related to intestinal absorption, are not well understood.

A review of the literature revealed that a variey of experimental approaches and designs, used to study the site of intestinal zinc absorption in the rat have yielded conflicting data (2–9). A common conclusion is that zinc uptake from the intestinal lumen is saturable in nature (10–12).

Our first objetive was to investigate a possible absorption window for zinc in the rat's digestive tract, using a stable zinc (zinc acexamate) in buffered solutions. The experimental method used makes it possible to quantify the endogenous secretion of zinc into the gastrointestinal lumen, establish the kinetics of this process and separate it from that of absorption.

In this study, we have found an important rate of zinc uptake in colon. Although most authors have described a scanty absorption in colon (2,3), some of them have even suggested the involvement of a specialized mechanism for zinc absorption at this site (8,9). Only Ghishan & Sobo (9) have demonstrated the saturable nature of the process, but they used immature rats in their studies. Therefore, a second aim of the present work was to investigate zinc uptake in the whole colon of adult rats by means of an in situ static perfusion technique, using different concentrations of stable zinc to identify the saturable uptake mechanism and characterize its kinetics.

MATERIALS AND METHODS

Zinc Salt

The experiments were carried out with zinc acexamate (zinc e-acetamide caproate, $Zn(CH_3CONH(CH_2)COO)_2$), supplied by Viñas S.A. Laboratories, Barcelona, Spain.

Treatment of Material and Reagents

To prevent or minimize zinc contamination, glassware was avoided whenever possible (13) and only glass volumetric flasks

[1] Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Valencia, Avda. Vicente Andrés Estellés s/n, 46100-Burjassot, Valencia, Spain.

[2] To whom correspondence should be addressed.

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and pipettes were used; otherwise, polypropylene material was employed for laboratory operations.

All labware was first washed with soap and tap water, rinsed, maintained in a 20% nitric acid solution, then rinsed with deionized water and heated to 40°C (120°C for glassware) before use.

Reagents were of analytical grade and were free or practically free of zinc.

Experimental Technique and Test Solutions

Male Wistar rats weighing 250 to 350 g were used. They were housed in plastic cages with stainless steel tops and were fed a commercial diet (Letica) which contained an average of 85 ppm total zinc, and tap water ad libitum. All rats were deprived of food for 15 to 20 hours before experiments and anaesthetized 1 h prior to surgery with intraperitoneal ethylurethane (0.4 mg/Kg).

The in situ rat gut preparation recommended by Doluisio et al (14), modified as previously (15), was used. This technique has been validated and used extensively for studies of intestinal uptake of drugs the absorption mechanisms of which involve carrier-mediated (11) or passive (16) processes. In order to detect selectivity phenomena in apparent zinc absorption, five gastrointestinal sectors were investigated: stomach, whole colon (excluding the cecum) and three fractions of equal length (about 33 cm) of the small intestine, designated the proximal, middle and distal segments. The proximal segment was begun 1 cm distal to the pylorus, the middle segment was measured immediately after and the distal portion was measured in an ascending direction beginning 1 cm distally to the cecum. When the proximal segment of the small intestine was used, the common bile duct was ligated. In all cases, the sector to be perfused was gently washed with buffered 0.9% NaCl warmed to 37°C (as will be described later) to remove the luminal contents.

Selective Absorption Studies

Ten animals were used for each of the investigated gastrointestinal sectors (5 animals in the case of stomach). Perfusion was carried out in each segment at 37°C with solutions of the same zinc acexamate concentration (50 μg/ml of zinc), with a total volume of 4.5 ml for the three small intestine segments and 4 ml for stomach and colon. A zinc concentration of 50 μg/ml was considered suitable for avoiding saturation of the active absorption process, assessed previously in small intestine (11) and reported in the literature (2,10).

The perfusion solution was prepared from an isotonic solution (0.9% NaCl), buffered with 20 mM BISTRIS (bis[2-hydroxyethyl]imino-tris[hydroxymethyl]methane) and adjusted to pH values of 3.8 (stomach), 6.5 (proximal and middle segments) and 6.0 (distal segment and colon), using 11 N hydrochloric acid. The use of BISTRIS at the smallest effective concentration (20 mM) prevents any substantial variation in pH, which could lead to zinc precipitation in the perfusion fluids during the experiments. However, when the distal segment of the small intestine was assayed, the sample time had to be reduced from 30 to 18 min because of the greater capacity of this segment to maintain the physiological basic pH.

The zinc concentration in the perfusates was measured every 5 min for a total time of 30 min (every 3 min for a total of 18 min for the distal segment of the small intestine) in samples of 0.25 ml.

Apparent Absorption Kinetics in Colon

Solutions of five zinc acexamate concentrations (5, 25, 50, 150 y 250 μg/ml of zinc) with a total volume of 4 ml were assayed. Ten animals were used for each concentration set. The zinc concentration in perfusates was measured every 5 min for a total time of 30 min, except for the highest zinc concentration (250 μg/ml), for which the sample time was every 3 min, for a total of 18 min.

Secretion Studies

In order to assess whether appreciable amounts of zinc might be secreted through the intestinal wall into the lumen and thus alter the results of absorption studies, independent experiments were carried out in all the investigated gastrointestinal sectors (6 animals per set except for stomach, in which only 3 rats were studied). The secretion of endogenous zinc was assessed by means of the experimental procedure described above, but with the aforementioned zinc-free buffered saline as the perfusion solution.

Water Reabsorption Process

In both absorption and secretion experiments, some reduction in volume was seen at the end of the sampling period in most cases. Water reabsorption has been identified as an apparent zero-order process (11,15,17). In order to quantify it in our experimental conditions a method based on direct measurement of the volumes of the perfused test solutions remaining at the sample times was chosen (15). Due to the characteristics of the selected technique it was necessary to use a separate group of rats for water reabsorption assays. After perfusing zinc-free buffered saline (to correct luminal zinc concentrations in secretion studies) or the different zinc solutions (to correct zinc concentrations in absorption experiments), the remaining volume was determined at 0, 15 and 30 min for all cases except for the distal segment solutions and for the 250 μg/ml zinc colon concentration. For them the sample times were 0, 9 and 18 min.

Analytical Procedure

The zinc concentration in the samples was measured by atomic absorption spectrophotometry (detection limit: 50 ng/ml; coefficients of variation from 0.4 to 4.5 %). Biological samples were centrifuged to remove intestinal debris, and the supernatant was used for zinc assays. All samples were diluted with buffered saline to give solutions with concentrations ranging from 0.5 to 1 μg/ml.

Data Fitting and Statistical Procedures

Water Reabsorption

In accordance with a zero-order kinetics, the remaining volumes V_t versus time t were fitted to the following equation:

$$V_t = V_0 - K_r \times t \quad (1)$$

Accordingly, in absorption experiments, the actual concentration A_t found at any time in the perfusing samples was corrected as follows:

$$A_t \times \frac{V_T}{V_0} = A_T \quad (2)$$

where A_T is the corrected concentration for the water reabsorption process. In order to correct the luminal zinc concentrations obtained in the secretion experiments, the corresponding theoretical volumes remaining at each time obtained with saline were used. The kinetic parameters V_0 and K_r for the all gastrointestinal segments are summarized in Table 1.

Secretion Data

To characterize the zinc secretion process, both first-order (eq 3) and zero-order (eq 4) kinetics were fitted to the secretion data:

$$\ln E = \ln E_0 + K_e' \times t \quad (3)$$

$$E = E_0 + K_e \times t \quad (4)$$

where E represents the zinc concentration found in the lumen at the sampling time t (corrected for water reabsorption), E_0 is the calculated intercept value at zero time, and K_e' and K_e represent the apparent first-order and zero-order rate constants respectively. The best fit provided the highest correlation coefficient value (r).

The selected secretion rate constants were compared by the one-way ANOVA test ($p < 0.05$).

Absorption Data: Individual Fittings

To detect selectivity phenomena in apparent zinc absorption, the first-order kinetics equation was fitted to data:

$$\ln A = \ln A_0 - K_a \times t \quad (5)$$

In equation 5, A values represent the remaining zinc found in luminal contents at the sampling times t (corrected for water reabsorption and zinc secretion). Initial non perfused samples (i.e concentrations at zero time, Ci) were not considered for regression to avoid membrane adsorption phenomena (considered an instantaneous process) and dilution effects (11,14,15). As a result, the A_0 values in equation 5 are extrapolated intercepts for each set of data and K_a is the apparent first-order rate constant. In the kinetic study in colon the amounts of zinc bound to the mucous was indirectly calculated as $Ci - A_0$.

To analyse significant differences between the absorption rates of the segments, K_a values were subjected to a Kruskal-Wallis test and to a subsequent Scheffé test. A p value of less than 0.05 was considered to indicate a statistically significant difference between two mean values. The same was done to compare the K_a values obtained in the colon for each concentration data set.

Table 1. Water Reabsorption Kinetics in the Gastrointestinal Tract of the Rat

	Equation parameters			
	Saline		Zn (50 μg/ml)	
GI Segment	V_0 (ml)	K_r (ml/min)	V_0 (ml)	K_r (ml/min)
Stomach	4.01	<0.01	4.01	0.01
Proximal SI	4.67	0.01	4.67	0.01
Middle SI	4.84	0.01	4.82	0.02
Distal SI	4.57	0.04	4.56	0.03
Colon	4.12	0.03	4.12	0.04

Note: Parameters V_0 and K_r corresponding to the apparent zero-order water reabsorption kinetics in the gastrointestinal tract of the rat (stomach, colon and three segments of the small intestine (SI)). The theoretical volumes at any time obtained with saline were used to correct the experimental zinc concentrations in secretion studies, whereas the corresponding volumes of the 50 μg/ml zinc solution were used to correct absorption data.

Global Fits in Whole Colon

Since a significant decrease in the K_a rate constants was observed as the initial solute concentration increased, a saturable kinetics was assayed. Therefore, a global fit of the integrated Michaelis-Menten equation (equation 6) to data (mean values for all concentrations tested) was performed (11,18):

$$t = \frac{1}{V_m} \times \left(A_0 - A + K_m \times \ln \frac{A_0}{A}\right) \quad (6)$$

In eq 6, A represents the mean concentration of zinc remaining in the luminal contents at the sampling time t, V_m is the greatest transport capacity and K_m is the Michaelis-Menten constant.

The fitting was done through a nonlinear least-squares procedure using the MULTI program (19) and the inverse of variance for each experimental value was used as a weighting factor. At the end of the fitting, the V_m and K_m values that globally satisfy all the mean concentrations, as well as the corrected A_0 intercepts for each perfusion set were found, so that a complete and continuous plot of A versus t could be obtained.

On the other hand, the possible existence of a parallel pathway for zinc absorption by means a passive process was assessed by fitting the following equation (11,18) to data:

$$t = \frac{1}{K_a \times K_m + V_m} \times \left[K_m \times \ln \frac{A_0}{A} + \frac{V_m}{K_a} \times \ln \frac{(A_0 + K_m) \times K_a + V_m}{(A + K_m) \times K_a + V_m}\right] \quad (7)$$

which combines the Michaelis-Menten and first-order kinetics. Finally, for comparison the global fit of the integrated first-order equation (eq. 8) to data was also performed:

$$t = \frac{\ln A_0 - \ln A}{K_a} \quad (8)$$

In principle, this kinetics is not suitable when there is evidence of nonlinearites, but by comparing it with the remaining fits (Michaelis-Menten and combined kinetics) more information for selecting the best absorption model is obtained. To evaluate the aforementioned kinetic models, four features of the fits were compared: the weighted residual plots, the AIC value found after application of the Akaike information criterion (20), the correlation coefficient between experimental and model-predicted values (r) and the Snedecor F-test.

RESULTS

Water Reabsorption

The experimental volumes remaining at each time and the theoretical parameters (V_0 and K_r) used to correct the zinc concentrations in the samples (both in the absorption and secretion experiments) are given in Table 1.

Zinc Secretion

The mean experimental zinc concentrations at each time together with the mean apparent secretion rate constants (K_e) found in each segment are summarized in Table 2. A zero-order secretion model provided the best fit for experimental zinc concentrations in all the investigated gastrointestinal segments. No statistical differences were found between the K_e values.

According to the assessed secretion kinetics, the mean theoretical amounts of secreted zinc for each segment were subtracted from the corresponding experimental zinc concentrations determined in absorption studies.

Selective Zinc Absorption

The mean experimental zinc concentrations (corrected for the water reabsorption and zinc secretion process) together with the mean apparent absorption rate constants (K_a) for each investigated segment are summarized in Table 3. The plot of mean zinc percentages remaining in the lumen versus time are represented in Fig. 1.

As can be seen in Table 3, the statistical comparison between the K_a values (shown by letter superscripts) clearly indicates that apparent zinc absorption occurs throughout the gastrointestinal tract, but there is a wide preferential absorption area formed by the last two-thirds of the rat small intestine and the colon.

Zinc Colonic Absorption

The mean experimental zinc concentrations (corrected for the water reabsorption and zinc secretion process) and standard deviations (SD) for each set of data, together with the parameters corresponding to linear regression K_a, A_0 and the correlation coefficient r, are summarized in Table 4. The mean absorption rate constants ($K_a \pm$ SD) and the dose-normalized areas under curves (AUCn $\pm$ SD) are included. As can be seen in Table 4, zinc concentrations were substantially higher than those determined in secretion experiments (Table 2) except for the smallest zinc concentration perfused (5 μg/ml), so it is only in this case that the correction of data appreciably modifies the experimental absorption concentrations.

The statistical comparison between K_a and AUCn values (Table 4) indicates the existence of non-linearities in apparent zinc absorption. Accordingly, fittings of Michaelis-Menten and combined integrated equations to data were performed (Table 5). Statistical AIC and r values corresponding to these equations were very similar and the same conclusion was obtained from the corresponding residual plots. However, the above criteria clearly rule out the first order kinetics (eq. 8). On the other hand, as a result of the F Snedecor test, there is no statistical justification for the choice of the equation with the largest number of parameters (eq. 7). Nevertheless, physiological criteria such as the prevalence of passive processes in the colon suggest that the combined kinetics is closest to the realities of the studied phenomenon. The representative continuous plot of the time course of zinc absorption based on the combined equation (eq. 7) is reproduced in Figure 2.

DISCUSSION

Zinc Secretion

Zinc secretion into the intestine via biliary and pancreatic secretions and from the mucosal surface of the epithelial cells has been demonstrated (21). In our case, since the bile duct was ligated at the junction with the small intestine, biliary and pancreatic secretions were avoided and no factor related to these secretions (excreted zinc (5) or low molecular weight ligands (22)) can have affected the results. On the other hand, since zinc secretion through the intestinal wall can not be avoided, experiments were carried out to quantify it and separate it from the apparent absorption process. Although low zinc concentrations were found in these experiments (Table 2), the absorption data were duly corrected. In all the assayed segments,

Table 2. Gastrointestinal Zinc Concentrations in Secretion Experiments

	Zinc concentrations in luminal perfusates (μg/ml)				
Time (min)	Stomach (n = 3)	Proximal (n = 6)	Middle (n = 6)	Distal[a] (n = 6)	Colon (n = 6)
5	0.90 ± 0.61	0.74 ± 0.32	0.88 ± 0.55	0.77 ± 0.20	2.04 ± 1.22
10	1.22 ± 0.62	1.17 ± 0.42	1.10 ± 0.29	1.23 ± 0.40	2.72 ± 1.20
15	1.81 ± 0.81	1.69 ± 0.35	1.99 ± 0.68	1.41 ± 0.41	3.26 ± 1.05
20	2.27 ± 0.89	2.11 ± 0.74	2.27 ± 0.38	1.65 ± 0.55	4.01 ± 1.49
25	2.67 ± 1.11	2.73 ± 0.41	3.05 ± 0.88	1.77 ± 0.54	4.48 ± 1.62
30	3.02 ± 1.18	3.01 ± 0.88	3.69 ± 0.39	1.95 ± 0.67	4.90 ± 1.60
Ke	0.09	0.09	0.12	0.07	0.12
E_o	0.44	0.26	0.15	0.68	1.54
r	0.997	0.997	0.990	0.975	0.996
Mean Ke	0.09 ± 0.02	0.09 ± 0.02	0.12 ± 0.02	0.08 ± 0.04	0.12 ± 0.04

Note: Values ± standard deviations (SD) and parameters corresponding to linear regression of data for each segment: K_e, E_0 (expressed as μg/(ml × min) and μg/ml respectively) and r; n is the number of rats. The mean Ke ± SD has also been included.

[a] Sampling time was every three minutes.

Table 3. Apparent Zinc Absorption: Zinc Concentrations in the Gastrointestinal Tract

	Zinc concentrations in luminal perfusates (μg/ml)				
Time (min)	Stomach (n = 5)	Proximal (n = 10)	Middle (n = 10)	Distal[a] (n = 10)	Colon (n = 10)
5	43.68 ± 3.26	39.84 ± 1.74	36.02 ± 1.80	38.83 ± 1.23	37.16 ± 1.68
10	43.38 ± 3.51	37.86 ± 1.64	32.96 ± 1.90	37.27 ± 1.27	34.37 ± 1.64
15	42.93 ± 3.90	35.85 ± 1.65	30.53 ± 1.64	35.52 ± 1.24	32.16 ± 2.01
20	42.34 ± 3.97	33.92 ± 1.70	28.16 ± 1.90	33.89 ± 1.27	29.67 ± 2.24
25	41.52 ± 3.97	32.06 ± 1.60	25.90 ± 1.48	32.03 ± 1.24	27.17 ± 2.32
30	40.75 ± 4.10	30.20 ± 1.70	23.62 ± 1.23	30.50 ± 1.50	24.92 ± 2.62
K_a	0.17	0.66	1.00	0.98	0.95
A_0	44.56	42.25	39.12	40.99	40.46
r	0.984	0.999	0.999	0.999	0.999
Mean Ka	0.17 ± 0.07[a]	0.66 ± 0.13[b]	1.00 ± 0.06[c]	0.97 ± 0.14[c]	0.96 ± 0.19[c]

Note: Values ± standard deviations (SD) corrected for water reabsorption and zinc secretion processes, and parameters corresponding to linear regression of data for each segment: K_a, A_0 (expressed as h^{-1} and μg/ml respectively) and r; n is the number of rats. The mean Ka ± SD has also been included.

[a]Sampling time was every three minutes.

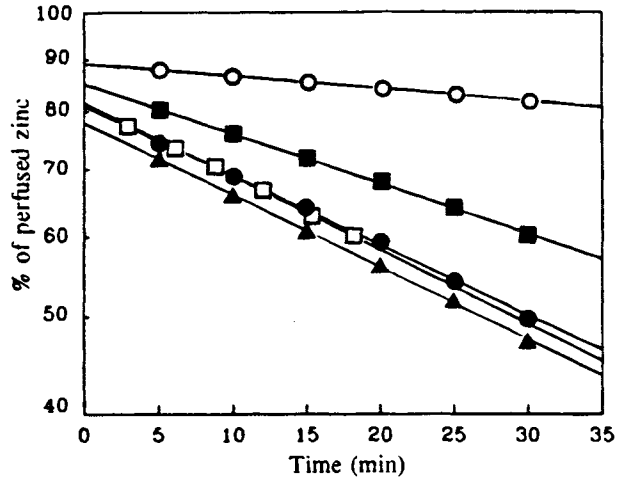

Fig. 1. Time course of zinc uptake from the different gastrointestinal segments: stomach (○), colon (●) and proximal (■), middle (▲) and distal (□) intestinal segments; zinc uptake was determined as disappearance of zinc from luminal perfusate and expressed as percent of perfused zinc.

the zinc secreted was directly proportional to time (apparent zero order process) and no significant differences were found in the apparent secretion rates (K_e).

Previous secretion experiments similar to those described here but with only one sample at 30 min provided zinc concentrations of less than 1 μg/ml. This suggests that gastrointestinal handling during the sampling procedure might be the cause of the observed increase (Table 2) given the well-known protective activity of zinc on mucous membranes (1). This behaviour could be considered a protective response of the gastrointestinal epithelium.

Selectivity Phenomena in Apparent Absorption

A Kruskal-Wallis test revealed significant differences between the K_a values for the five gastrointestinal sectors (Table 3). A subsequent Scheffé test allowed us to conclude that the stomach exhibits a more limited ability to absorb zinc than the other segments. In spite of the possible inaccuracy due to determination of water flux and zinc secretion on different groups of animals, the K_a value assessed for the proximal segment of the small intestine was significantly lower than those found in the other segments, including the colon. These differences in apparent absorption rates can be graphically observed in Figure 1. The explained features suggest that if there is an absorption window for zinc in the rat, it should be identified for practical purposes as a wide region formed by the last two thirds of the small intestine and the colon.

Although the pH values of the working solutions ranged from 6.5 (proximal and middle segments) to 6.0 (distal segment and colon), it was verified that in all the intestinal sectors the pH of the solutions tended to rise and a few minutes after the start of perfusion became stable within a narrow range (6.5–7) and remained so throughout the assays. It is our opinion that the pH-factor is not strong enough to explain the differences observed in the apparent absorptive capacities of the investigated intestinal segments.

A review of the literature revealed that a variety of experimental approaches and designs applied to study the site of intestinal zinc absorption in the rat have yielded conflicting data. Little research has been done on the colon. Higher rates of zinc transport or absorption by the distal segments of the small intestine in rats have been reported by Kowarski et al (6) and Antonson et al (5). In contrast, Van Campen (4), Methfessel & Spencer (3), Davies (2), and Seal & Heaton (23) found higher rates of zinc absorption in the duodenum. It is difficult to reconcile these contradictory findings or to draw conclusions because of the different experimental techniques used, and even the strain of rats and the diet greatly influence the results. In addition, many authors do not include the whole intestinal tract in their studies but select a small portion of it as representative of the duodenum, jejunum or ileum. This approach seems questionable for the location of the zinc carriers, homogeneous or not, throughout the small intestine is still unknown.

At any rate, results similar to those obtained here have been reported by Meneely & Ghishan (7), Ghishan & Sobo (9), Wapnir et al (24) and Seal & Mathers (8), who attribute an important absorbent capacity to the colon, as opposed to

Table 4. Zinc Uptake Kinetics in Colon

	Luminal zinc concentrations (μg/ml)				
Time (min)	5 (n = 10)	25 (n = 10)	50 (n = 10)	150 (n = 10)	250* (n = 10)
5	2.66 ± 0.80	17.58 ± 1.09	37.16 ± 1.68	117.5 ± 6.2	202.6 ± 13.3
10	2.20 ± 0.71	16.41 ± 0.98	34.37 ± 1.64	112.2 ± 5.7	196.7 ± 13.0
15	1.93 ± 0.79	15.25 ± 1.04	32.16 ± 2.01	106.7 ± 4.8	192.5 ± 12.3
20	1.60 ± 0.81	14.04 ± 1.04	29.67 ± 2.24	101.6 ± 4.4	187.5 ± 13.2
25	1.37 ± 0.80	12.99 ± 1.14	27.17 ± 2.32	96.8 ± 4.7	184.1 ± 12.6
30	1.07 ± 0.74	11.85 ± 1.29	24.92 ± 2.62	92.1 ± 4.1	180.1 ± 12.0
K_a	2.12	0.94	0.96	0.59	0.46
A_0	3.20	19.17	40.46	123.5	206.5
r	0.997	0.999	0.999	0.999	0.997
AUCn	0.34 ± 0.28[a]	0.84 ± 0.18[b]	0.87 ± 0.16[b]	1.41 ± 0.10[c]	1.80 ± 0.25[d]
Mean K_a	2.51 ± 1.01[a]	0.95 ± 0.21[b]	0.96 ± 0.19[b]	0.58 ± 0.05[b]	0.47 ± 0.06[b]

Note: Values ± standard deviations (SD) corrected for water reabsorption and zinc secretion processes, and parameters corresponding to linear regression of data: K_a, A_0 (expressed as h^{-1} and μg/ml respectively) and r; n is the number of rats; the mean Ka ± SD and the normalized areas under curves (AUCn ± SD) have also been included.
[a]Sampling time was every three minutes.

Table 5. Global Fitting of the Selected Equations to the Data Obtained in Colon

Parameters	Michaelis-Menten (eq. 6)	Combined (eq. 7)	First-order (eq. 8)
Vm ± SD (μg/ml × min)	2.15 ± 0.02	0.36 ± 0.02	—
Km ± SD (μg/ml)	112.0 ± 0.9	18.01 ± 0.40	—
Ka ± SD (h^{-1})	—	0.40 ± 0.01	0.69 ± 0.01
A_0 ± SD (μg/ml)	205.0 ± 0.1	208.0 ± 0.2	215.9 ± 0.3
AIC	26.41	25.60	62.45
r	0.99998	0.99998	0.99963

Note: Parameter values and standard deviations (SD) after fitting the integrated equations to the data. The statistical AIC and r values have also been included.

what is reported by other authors (2,3). One of the most striking features of the present work is the absorption rate for zinc determined in the large bowel, with an absorption half-life of about 45 min, very close to those found in the middle and distal segments of the small intestine. As early as 1982, Meneely & Ghishan (7), using an in vivo recirculating technique in 2-week-old (suckling) and 6-week-old (adolescent) rats, found that the net absorption of zinc was significantly greater in all the perfused segments (jejunum, ileum and colon) of the suckling than in the corresponding segments of the adolescent rats. Moreover, the zinc transport rates of the adolescent rats were similar in all intestinal segments, whereas transport rates in colonic segments of the suckling rats were significantly greater than those corresponding to the small intestinal segments. The authors explained these findings by arguing that the permeability for ions of the intestinal membranes in the suckling rats was greater than in mature rats. However, later Wapnir et al. (24) by means of a similar experimental technique, found that the absorption of zinc across the colon was consistently more effective than in two regions of the small intestine (jejunum and ileum) in normal adult rats regardless of the ligand present in the solution (L-proline, L-histidine, Glycylsarcosine or citrate). The authors emphasize that the physiological importance that colonic absorption could have in salvaging zinc from the intestinal tract has not yet been determined.

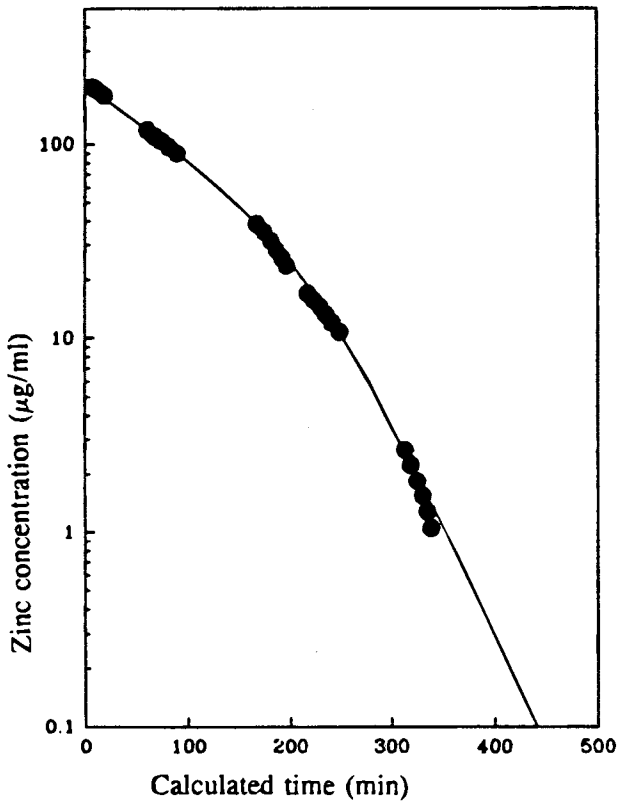

Fig. 2. Semilogarithmic continuous plot representing zinc absorption from the colon according to combined Michaelis-Menten and first-order kinetics (Eq. 7.). Parameter values are shown in Table 5.

Zinc Colonic Adsorption

In Figure 3 it can be observed that the percentage of zinc bound to the mucosa decreased as the starting concentration increased. This feature suggests that a saturable mechanism may be involved in the zinc binding process. Similar behaviour was described by Davies, who determined directly the amount of zinc bound to the mucous using Zn^{65} in duodenal loops (2). Moreover, the use of three different zinc concentrations lower than 50 μg/ml made it possible characterize the adsorption process more accurately. The author found that concentrations from 1 to 50 μg/ml of Zn^{65} exhibited saturation kinetics, probably due to binding to specific sites of the mucosa. Between 50–200 μg/ml the amount of zinc bound increased in an approximately linear fashion as a result of non-specific adsorption on the mucosa.

The non-specific adsorption sites seem to be related to phosphate or other ionic groups on the membrane (12), whereas the specific sites are believed to be related in the small intestine to carrier brush-border proteins linked to the Na^+-substrate transport system (25). The adsorption process has even been considered a previous step to zinc absorption (2,26). However, the exact mechanism in the small intestine is still not clear and nothing is known about the process in the colon.

Zinc Absorption in Whole Colon

The present work shows that zinc uptake in colon is saturable in nature. In a similar way, Ghishan & Sobo (9), using an in vivo single pass perfusion technique in adolescent rats, found that the relationship between the luminal zinc concentration and net absorption and lumen-to-mucosa flux was curvilinear in the colon. A lineweaver double reciprocal plot of the transport data indicates that the curve fits a Michaelis-Menten kinetics with a Km of 50 ± 6 μM and a Jmax of 1.2 ± 0.1 μmol/h/g dry weight. The authors concluded that the intestinal zinc transport is characterized by a maturational pattern which evolves with age.

The results of the present study suggest the involvement of a passive mechanism together with a saturable one in zinc colonic absorption. We reached a similar conclusion in a previous work in whole rat small intestine using the same experimental technique (11). In that study, the saturable component was characterized by Vm and Km values of 10.85 ± 0.05 μg/(ml × min) and 295 ± 10 μg/ml respectively, whereas the passive absorption rate constant was 0.31 ± 0.06 h^{-1}. If we compare those parameters with the ones determined now in colon (Table 5, eq. 7), it can be concluded that the saturable component is more heavily involved in the small intestine than in the colon. These differences could be explained by the smaller number of carriers in the colon or by a different type of transport system at this site. However, until now, no organic nutrient carriers have been described in the colon (27). Nonetheless, Wapnir et al. (24), using an in vivo recirculating technique in juvenile rats, found that zinc was taken up in the colon but that amino acids (L-proline, L-histidine, glycylsarcosine and citrate) were not. The authors saw that oligopeptides could have a positive role in zinc absorption by the colon and a much larger one than in the small intestine. On the other hand, Seal & Mathers (8), using everted gut sacs from duodenum, ileum and colon, was the first to demonstrate that the rate of mucosal/serosal transfer and tissue accumulation of zinc were reduced by the inclusion of 0.25 mM ouabain in the incubation medium (the reduction was greater for the duodenum and colon than for the ileum). This indicates the presence of active energy-demanding Na^+, K^+ATPase-dependent transport mechanisms in the gut preparation used.

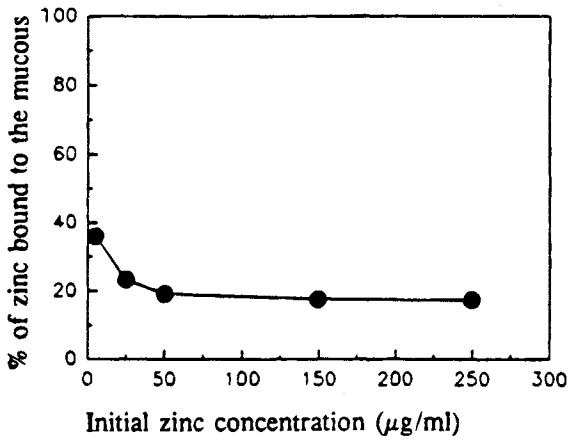

Fig. 3. Plot of mucosal-bound zinc versus zinc in solution.

In the small intestine it has sometimes been reported that zinc causes competitive inhibition of active absorption of substances that use the Na^+-substratum transport system, such as glucose (28), D-galactose (29) and L-treonine (25), and this means that the same transport is used by zinc to enter the cell. With regard to the large intestine, nothing is known about the aforementioned absorption mechanism; only Seal & Mathers (8) have reported the use of the Na^+, K^+-ATPase system for zinc transport in the colon of the rat.

In conclusion, the results of the present work indicate that rat colonic zinc uptake is a saturable process. Further investigation is needed to confirm and characterize the possible colonic zinc carriers.

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