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Intestinal Absorption of Copper: Effect of Sodium (42545)

RAUL A. WAPNIR AND LILY STIEL

Department of Pediatrics, North Shore University Hospital, Manhasset, New York 11030; and Department of Pediatrics, Cornell University Medical College, New York, New York 10021

Abstract. The mechanisms of copper (Cu) absorption from the small intestinal lumen are poorly understood. In this study we investigated the role of sodium (Na) during the removal of Cu from the lumen of jejunal and ileal segments, using an *in situ* perfusion procedure in the anesthetized rat. Intestinal absorption of Cu from a 31 μM solution was highest in the presence of an isotonic concentration of NaCl, as compared to solutions containing either glycerol (GRL) or *N*-methyl-D-glucamine (NMG) as osmotic agents. In the jejunum, mean ± SEM Cu absorption rates in the presence of the following solutes were: with NaCl, 57.5 ± 10.5 pmole/min × cm; with GRL, 13.3 ± 14.7 ($P < 0.05$); with NMG, 18.4 ± 10.1 ($P < 0.05$). In the ileum, copper absorption in the presence of NaCl was 64.4 ± 9.6; with GRL, 24.3 ± 10.1 ($P < 0.01$); with NMG, 15.8 ± 3.7 ($P < 0.001$). Kinetic analysis of the carrier-mediated component of Cu absorption in rat jejunum yielded a V_{max} = 47.5 pmole/min × cm and an apparent K_t = 21 μM. The diffusion coefficient was calculated to be 1.4×10^{-5} cm^2/sec. The absorption of Cu was independent of net water absorption, which was highest in the presence of GRL and abolished and reversed into secretion by NMG. The data obtained are indicative of a significant role of Na in the small intestinal transport of Cu, *in vivo*, although not directly related to unidirectional water fluxes. The cation specificity of Na in this process remains to be elucidated, although the results support earlier studies which postulated that mediated transport may constitute a major component of Cu absorption in the mammalian small intestine. © 1987 Society for Experimental Biology and Medicine.

There is very limited information on the events occurring during the uptake of copper (Cu) from the intestinal lumen. In the rodent, mediated and nonmediated transport systems have been reported (1–3). Also, it is not clear whether the free metal or chelated forms of Cu are the actual substrates for absorption. Sodium (Na) may play a role in the quaternary conformation of metal-binding proteins, as well as be a participant in the transport of low-molecular-weight substances involved in the absorption of Cu. This phenomenon could also be pH dependent. In addition, Cu entry into the enterocyte could be associated with the bulk transfer of solutes, that is, with water movement across the mucosal brush border.

This study was undertaken to clarify the characteristics of Cu absorption in rat jejunum and ileum with respect to pH and the possible dependency on bidirectional water fluxes. Since water and electrolyte movements are closely linked (4), a better understanding of Cu transport would require an assessment of the role of Na in the intestinal absorption of Cu. This possible physiological association could have significant implications in determining the bioavailability of Cu in low Na foods, such as human breast milk and infant formulae.

Materials and Methods. The absorption of Cu was determined in intestinal segments of 20 to 30 cm in length, cannulated at both ends, and maintained vascularly intact. For the jejunum, the point of entry was immediately distal to the ligament of Treitz. For the ileum, the exit of the solution was located about 5 cm proximally to the ileocecal valve. The rats were Wistar-derived males (Crl:[WI]BR; Charles River Breeding Laboratories, Kingston, NY), weighing 125 to 150 g, and were anesthetized with an ip dose of urethane (1.3 g/kg). The solutions described below were perfused at 0.18–0.20 ml/min with a peristaltic pump (Harvard 1201). Following 1 hr of equilibration with the same solution to be tested, 15-min effluent fractions were collected for 2 hr. Each fraction was separately analyzed and averaged for each rat. The values from no fewer than six animals were required for each experimental condition.

In experiments other than those conducted to determine absorption kinetics, Cu was perfused at 31 μM (2 mg/liter), in isotonic solutions containing 140 meq/liter NaCl, 280 mM

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glycerol (GRL), or 140 meq/liter *N*-methyl-D-glucamine (NMG), adjusted to the desired pH with HCl. All were buffered with 5 m*M* Tris–HCl. The solutions also contained tracer amounts of ^3H_2O (5 μCi/liter; ICN Radiochemicals, Irvine, CA) and 20 mg/liter of phenol red, a nonabsorbable marker, to allow for the calculation of unidirectional water fluxes and net water absorption.

Cu was assayed by atomic absorption spectrophotometry (Perkin–Elmer 305A). The calculations took into account net water absorption, estimated from phenol red concentrations (5). The data were expressed as pmole per min × cm. The lumen-to-mucosa water influx was determined by isotope dilution (Beckman LS 3800) and gravimetric measurements of the incoming and outgoing solutions.

The determination of Cu absorption rates at various pH was done by adjusting the pH between 5.8 and 7.8 with either HCl or NaOH prior to the perfusion. To avoid introducing another variable, the buffer base (Tris) was maintained, in spite of the pH range being extended beyond its optimal effectiveness. The kinetics of jejunal Cu absorption were calculated from perfusions carried out in NaCl-containing solutions where Cu concentration ranged between 6.3 and 63 μM. The absorption rates were adjusted for net water fluxes. To discriminate between mediated and nonmediated components of intestinal Cu absorption, the graphic method of Neame and Richards, as described in Ref. (6), was applied. Briefly, it is based on extrapolating, to the origin of abscissae, the linear portion of the plot obtained at high substrate concentrations and transposing the line to the origin of ordinates. The values thus obtained are attributable to diffusion, or nonmediated transport. The mediated transport curve is obtained by subtraction of diffusion from the overall absorption rate. The points representing the mediated or saturable Cu transport rates were plotted in a graph relating the rates of absorption (V) and the ratio V/S, S being the initial concentration of Cu. The kinetic parameters V_{max} and K_t were obtained, respectively, from extrapolation of the regression line to the ordinate and from the slope (6).

In intergroup comparisons, the data were statistically evaluated by one-way analysis of variance, and the critical differences between means were determined by application of a multiple t test based on the analysis of variance. Pearson's product–moment correlation coefficient r was calculated between net water and copper absorption rates (7).

Results. The rates of Cu intestinal absorption in the jejunum and ileum of rats varied with the presence of Na in the perfusing solutions. Cu absorption was several times higher from the solution containing NaCl than from those formulated with equiosmolar GRL or NMG. This effect was seen both in the jejunum and in the ileum (Fig. 1, upper panel). There were no differences between the results obtained with GRL and with NMG. In contrast, maximum rates of net water absorption were obtained when GRL was the osmotic agent, approximately doubling the amount of water absorbed when Na was included in the solution (Fig. 1, lower panel). When NMG was present, there was consistent net water secretion. These findings occurred both in the jejunum and in the ileum. The r values were

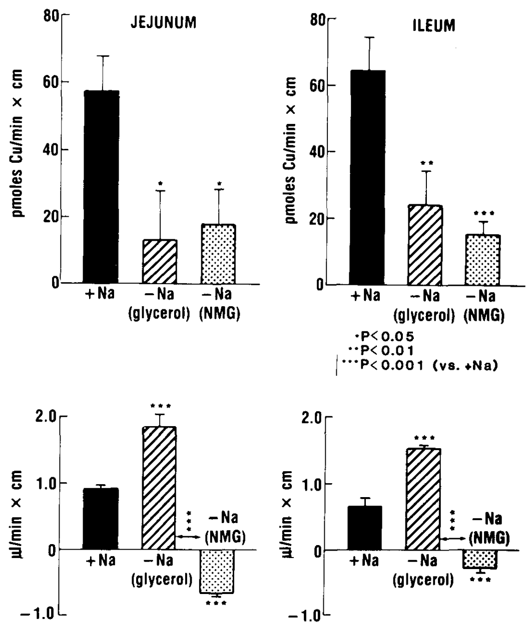

FIG. 1. Effect of Na on Cu absorption. Absorption rates were determined at 31 μM Cu in isotonic solutions containing NaCl, GRL, or NMG, buffered at pH 6.8. Upper panel: Cu absorption rates. Lower panel: net water absorption. N = 6 rats per group. Operational details are given under Materials and Methods.

higher for the NaCl solutions in the two portions of the gut. For the jejunum, in the presence of NaCl, GRL, or NMG, the correlation coefficient r was 0.294, 0.003, and 0.077, respectively. For the ileum, r was 0.371, 0.142, and 0.155, respectively. Since these values were computed taking the average rates for each rat, which reduced the degrees of freedom, the correlations did not reach the $P < 0.05$ threshold of significance.

Unidirectional fluid movement, in the jejunum and the ileum, was the greatest when GRL was present (Fig. 2). In the jejunum, although not in the ileum, water influx with GRL was higher than that with NaCl, and both solutes induced greater water influx than that with NMG. On the contrary, the osmotic agent of the solutions made no difference on the water efflux, either in the jejunum or in the ileum. The magnitude of the unidirectional fluid movement was comparable in both areas of the intestine.

Although the intestinal absorption experiments described above were conducted at a physiological pH (6.8), the possibility that either the degree of Cu ionization or the presence at the site of absorption of low-molecular-weight ligands secreted by the mucosa and present in the microenvironment was further explored by altering the pH of the perfusing solutions and evaluating, simultaneously, the relationship between Cu and water net absorption.

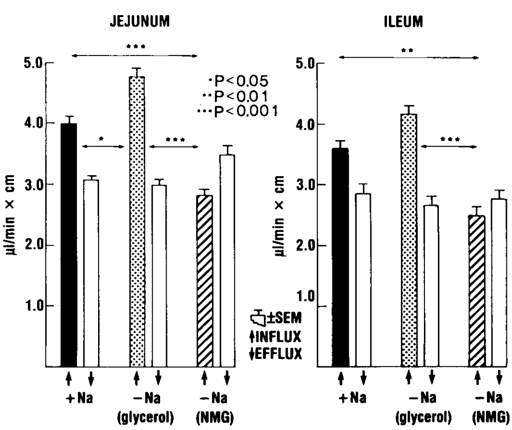

FIG. 2. Unidirectional water fluxes during Cu absorption under conditions described in the legend to Fig. 1. Significant differences among the three solutes occurred only for water influx. Water efflux rates were indistinguishable, both in the jejunum and in the ileum.

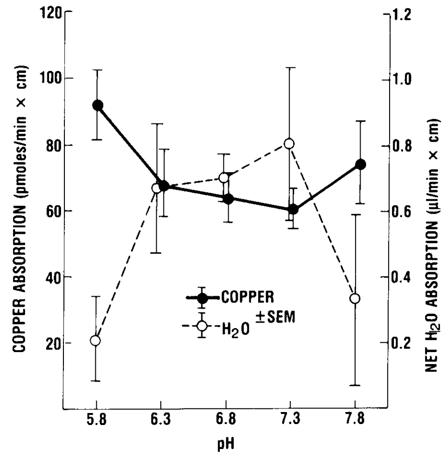

FIG. 3. Effect of pH on Cu and net water absorption in rat jejunum. The perfusing solutions were buffered at pH's ranging between 5.8 and 7.8, as detailed in the text. No differences were observed for either Cu or water absorption across the pH range.

sorption. As shown in Fig. 3, Cu absorption in the jejunum, in the presence of NaCl, was not significantly altered by changes in the pH. The data for water absorption had a variance too large to allow for a significant discrimination of optimum pH. In contrast to the jejunum, Cu absorption in the ileum was greatest at both the lowest pH (5.8) and the highest pH (7.8) (Fig. 4). Conversely, net water absorption was at a minimum at the extreme ranges of pH tested, confirming the dissociation between Cu removal from the lumen and fluid uptake by the small intestine. Unidirectional water fluxes were independent of pH in the jejunum (not shown). In the ileum, however, water influx at pH 7.3 was significantly higher than that at the other pH considered.

A curvilinear response characteristic of overlapping mediated and nonmediated transport mechanisms was obtained when Cu absorption rates in the jejunum were plotted as a function of Cu concentration (Fig. 5). Applying the graphic discriminatory procedure described under Materials and Methods, a nonmediated (diffusive) and a mediated component were defined. The regression obtained from the mediated transport data represented in a V vs V/S graph yielded a value for V_{max} of 47.5 pmole/min × cm, and a K_t of 21 μM. The nonmediated diffusion coefficient was

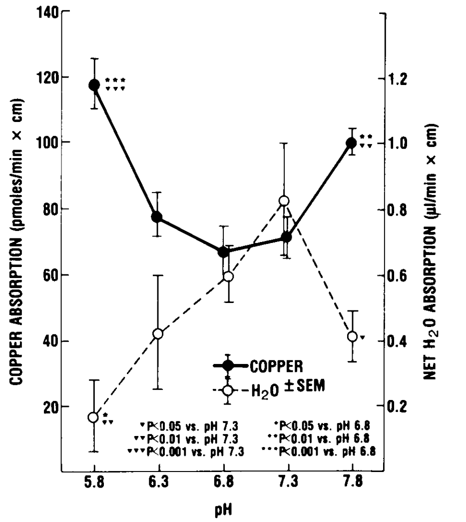

FIG. 4. Effect of pH on Cu and net water absorption in rat ileum. Conditions were as listed in Fig. 3 and under Materials and Methods. The extreme pH ranges tested were significantly different from the results obtained at physiologic pH (6.8 and 7.3), for both Cu and net water absorption.

calculated, from the slope of the straight line at higher Cu concentrations, to be 1.4×10^{-5} cm^2/min.

Discussion. The most remarkable finding of this study was the large difference observed between Cu absorption rates in the presence and in the absence of Na (Fig. 1). Even under conditions which maintain a net steady water inflow, as with a nonelectrolyte actively transported by the small intestinal mucosa such as GRL (8), Cu absorption was essentially indistinguishable from zero, in the jejunum, and very modest in the ileum. The limited contribution of bulk flow to Cu transport, when present at 31 μM in the gut, was demonstrated even more graphically when NMG was the osmotic agent substituting for NaCl. In this case, net water secretion prevailed, but some net Cu removal from the lumen also occurred, especially in the ileum.

Since water efflux was similar in both areas of the small intestine, regardless of the solute present in the Cu solutions (Fig. 2), the large discrepancy in Cu absorption was not commensurate with the differences in water influx when either Na or GRL was present, which did not exceed 20%, both in the jejunum and in the ileum. This finding indirectly supports the hypothesis that a mediated transfer of Cu between the lumen of the intestine and the enterocyte is responsible for a significant part of the overall removal of Cu from the luminal phase.

Another point which may influence Cu absorption rates is the molecular species which actually may be involved in the intestinal absorptive process. Many potential chelating substances form tightly bound complexes with transition elements. This has been well documented for zinc (9–12), as well as for Cu (9, 13–17), among trace metals of nutritional significance. The pH-dependent stability of organometallic complexes makes it important to assess the relationship between Cu absorption and proton concentration. At pH below 6, free metallic ions predominate. In serum, free Cu^{2+} is only 0.34 pM (9). The isoelectric point of chelates obtained with low-molecular-weight substances of natural origin is close to neutrality (18). At higher pH, positively charged complexes constitute the major chemical species. In our experiments, pH had no effect on jejunal Cu absorption (Fig. 3), demonstrating again independence from net water absorption rates. In contrast, the data from the ileum (Fig. 4) were indicative that mediated absorption mechanisms operate, apparently, for both free Cu^{2+} and positively charged Cu complexes. This dissociation between net water inflow was evident, since Cu absorption maxima were coincidental with net water absorption minima.

The absence of added putative luminal ligands does not exclude the possibility of *in situ* chelation of Cu by intestinal secretion products or the turnover of mucosal proteins (11, 19). The value obtained for the K_t in our experiments was on the same order of magnitude, but higher than the 4.3 μM published value for mouse duodenum with a different technique (3). These values are also comparable to the affinity of the hepatocyte for Cu transport, reported to be 11–15 μM, in the absence or in the presence of histidine (20). The estimate for the diffusion coefficient, 1.4×10^{-5} cm^2/sec, falls close to those of other solutes of comparable molecular weight, such as NaCl and KCl (1.39 and 1.68×10^{-5} cm^2/sec, respectively) (21), a result compatible with a diffusion of the free cation, and not of larger copper chelates. By graphical interpolation

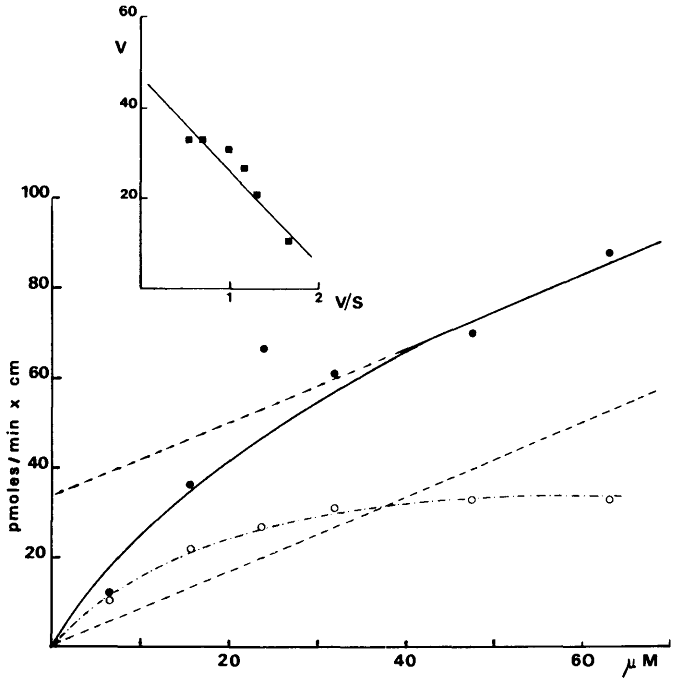

FIG. 5. Changes in Cu absorption rates as a function of Cu concentration in rat jejunum determined in NaCl-containing solutions at pH 6.8 (—). Extrapolation of the linear part of the curve to the ordinate and shift to the origin indicate the diffusion contribution (---). The mediated component (···) is drawn from the difference between the overall absorption rates and the diffusion. See details under Materials and Methods. The insert depicts the V vs V/S transformation of the mediated component data. The intercept and slope yield V_{max} and K_t, with values given in the text.

(Fig. 5, lower panel), it can be estimated that in the 31 μM perfusions, in the presence of NaCl, and at physiologic pH slightly less than half the total absorption rate was contributed by the nonmediated transport.

Although in some recent work with brush border membrane vesicles the investigators could not demonstrate a control mechanism for Cu uptake, this failure to discriminate a mediated transport could possibly be related to the absence of Na in the medium used (22). It should be noted that earlier investigators concerned with Cu intestinal absorption studies concluded that diffusion alone could not account for the overall metal absorption, and that a rate-limiting component had to play a significant role. The conclusion was the same whether *in vitro* or *in vivo* techniques were used (1, 2, 23).

Although Na is known to participate in amino acid and glucose absorption (24), its role in divalent cation transport remains poorly explored. Sodium has been shown to be required for optimal magnesium absorption in the ileum and the colon (25). An earlier study reported a sodium requirement for calcium extrusion from the cell (26), although no recent substantiation has been published. However, similar requirements for transition elements have not been described. A further assessment of the role of Na will require a kinetic evaluation of Cu absorption in the duodenum and the ileum, as well as experiments in the presence of GRL and with the use of inhibitors of Na transport. The near total suppression of Cu removal from the lumen, in the presence of NMG, supports the view that water fluxes, rather than Na transport, regulate nonmediated Cu absorption.

There has been an increased awareness about the importance that low-molecular-weight ligands may have in the stability of divalent cations in physiological fluids and as vehicles for their passage across biological membranes. The known low dissociation constants of Cu–amino acid complexes determine that over 98% of serum Cu is chelated to three molecular combinations of the metal with cystine and histidine (9, 14). The presence in bile of high free amino acid levels and of small peptides with Cu-binding properties has been suggested as an explanation for the positive role the biliary secretion may have on the absorption of Cu (16, 17, 19). In addition, there has been a growing awareness that pancreatic secretion, ingested proteins, and the turnover of the intestinal mucosa can readily supply the low-molecular-weight putative ligands that may be required participants in the normal assimilation of Cu (13, 15, 19, 27).

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