Studies on the bioavailability of zinc in humans: intestinal interaction of tin and zinc[1, 2]

Noel W Solomons,[3] MD, J Sergio Marchini,[4] MD, Rosa-Maria Duarte-Favaro,[4] BS, Helio Vannuchi,[1] MD, and Jose E Dutra de Oliveira,[5] MD

ABSTRACT Mineral/mineral interactions at the intestinal level are important in animal nutrition and toxicology, but only limited understanding of their extent or importance in humans has been developed An inhibitory interaction of dietary tin on zinc retention has been recently described from human metabolic studies We have explored the tin/zinc intraction using the change-in-plasma-zinc concentration method with a standard dosage of 12.5 mg of zinc as zinc sulfate in 100 ml of Coca-Cola. Sn/Zn ratios of 2:1, 4:1, and 8:1, constituted by addition of 25, 50, and 100 mg of tin as stannous chloride, had no significant overall effect on zinc uptake. The 100-mg dose of tin produced noxious gastrointestinal symptoms. Addition of iron as ferrous sulfate to form ratios of Sn:Fe/Zn of 1:1:1 and 2:2:1 with the standard zinc solution and the appropriate doses of tin produced a reduction of zinc absorption not dissimilar from that seen previously with zinc and iron alone, and addition of picolinic acid did not influence the uptake of zinc from the solution with the 2:2:1 Sn/Fe/Zn ratio. *Am J Clin Nutr* 1983;37:566–571.

KEY WORDS Zinc, iron, tin, mineral/mineral interaction, intestinal absorption, picolinic acid

Introduction

An important aspect of mammalian metabolism of trace minerals is the competitive interaction of chemically similar metal ions at the intestinal level (1). There appear to be at least two mechanisms of antagonistic mineral/mineral interactions. One type involves direct competition between two minerals simultaneously present in the intestinal luman; thus, Zn/Cu ratios of 1000:1 reduce the uptake of ^{64}Cu from rat intestine (2). The other type involves induction of an intestinal blockade; thus, chronic feeding to rats of diets containing 30 to 240 μg/g of zinc significantly reduced the uptake of copper from everted gut sacs (3). Both types of mineral/mineral interactions can have important implications for human nutrition.

Recently, interest has been focused on a competitive interaction of tin and zinc. Greger and Johnson (4) showed a significantly greater fecal loss of zinc in rats fed a diet containing 206 μg/g of tin as stannous chloride as compared to rats on an unsupplemented diet. The zinc content of tibia and kidney of the tin supplemented rats was also significantly less than that of controls after a 21-day experimental period. Johnson et al (5) fed human volunteers two diets containing 13.5 mg of zinc, but differing in their tin content in a cross-over design for periods of 20 days each. The tin-supplemented diet contained 50 mg of tin, whereas the control diet had <1 mg. Subjects excreted significantly more zinc in stools and significantly less zinc in urine on the tin-enriched diet, and net zinc retention was decreased by tin-feeding as well.

Previous studies in our laboratory of one of our authors (NWS) have shown that the change in plasma zinc concentration after an

[1] From the Division of Nutrology, Department of Internal Medicine, São Paulo University Medical School at Ribeirão Preto, São Paulo, Brazil, and the Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139.

[2] Address reprint requests to: Noel W Solomons, MD, Massachusetts Institute of Technology, Rm 20B-213, 18 Vassar Street, Cambridge, MA 02139.

[3] Associate Professor of Clinical Nutrition, Department of Nutrition and Food Science, Massachusetts Institute of Technology. Recipient of Clinical Investigator Award 1 K08 AM 00715 from the National Institutes of Health. [4] Department of Nutrology, Ribeirão Preto. [5] Professor of Medicine and Head, Department of Nutrology, Ribeirão Preto.

Received August, 31, 1982.
Accepted for publication October 19, 1982.

oral dose of 25 mg of zinc is reduced by simultaneous administration of 50 or 75 mg of ferrous iron (6, 7). Manipulation of conditions known to affect the absorption of iron allowed a mechanistic inquiry into the cellular localization of iron/zinc interaction (7). The demonstration by Greger's group of a tin/zinc interaction (4, 5) potentially offered another model for exploring the intestinal interaction of zinc with a chemically similar mineral in humans. In the present study, we have used the change in plasma zinc concentration after an oral dose of 12.5 mg of zinc to examine the tin/zinc interaction in healthy adult subjects.

Methods

Subjects

The protocol had been approved by the Committee on the Use of Humans as Experimental Subjects of the Massachusetts Institute of Technology. All subjects gave written consent after the nature and purpose of the procedures had been explained. A total of 24 healthy adults without evidence or suspicion of gastrointestinal disease, 15 men and nine women, participated in a total of 40 zinc absorption tests. Sixteen individuals participated on only one occasion while eight subjects were studied on two or more occasions, always in a different experimental treatment. An interval of at least 1 wk, often several months, between studies was observed with repetitions of absorption testing in a given subject.

Absorption tests

The procedure was a modification of the method previously described (8). A sample of 4 ml of venous blood was drawn at approximately 8:00 h after an overnight fast. The test solution of 12.5 mg of zinc as zinc sulfate (JT Baker Chemical Co, Phillipsburg, NJ) in 100 ml of Coca-Cola was administered orally, and blood samples were collected at hourly intervals at 1, 2, 3, and 4 h postdose. Subjects were ambulatory and were allowed to consume water, but no solid foods or other beverages were permitted during the duration of the morning.

Blood was extracted using zinc-free plastic syringes and stainless steel needles, and transferred to plastic Falcon tubes (Becton-Dickinson, Oxnard, CA) containing 0.05 ml of 20% potassium oxalate solution as the anticoagulant. The blood was centrifuged, the red cells discarded, and the plasma stored under refrigeration in Falcon tubes until analysis. Plasma zinc determinations were made by atomic absorption spectrophotometry on a Perkin-Elmer 290B spectrophotometer (Perkin-Elmer, Norwalk, CT), and the plasma zinc concentrations were expressed in μg/dl.

Doses of minerals in oral solutions

The 12.5 mg of zinc in solution was consumed alone, or with addition of various ratios of other minerals as inorganic salts, including tin (stannous chloride, Mallinkrodt Inc, St Louis, MO), and iron (ferrous sulfate, Matheson, Coleman & Bell, Norwood, OH) to produce graded Sn/Zn and Sn/Fe/Zn ratios. In a further experiment, 94 mg of picolinic acid (Sigma Chemical Co, St Louis, MO) was added to the test solution. Six absorption tests were conducted with each dosage except for the one containing an 8:1 Sn/Zn ratio in which only four individuals participated.

Data analysis

The descriptive statistics included calculations of means (± SEM) for the change in plasma zinc for each of the hourly sampling intervals and the area under the curve of individual changes in plasma zinc concentration determined by triangulation. Comparisons were made by the "Student's" *t* test. Although some individuals participated in more than one treatment, each absorption test result was considered as independent in the statistical comparisons between treatments. The power considerations are based on the pooled variance in the test with zinc alone using a two-tailed assumption.

Results

The mean change in plasma zinc concentration (± SEM) in six subjects after ingesting 12.5 mg of zinc alone in 100 ml of Coca-Cola was 45 ± 10, 55 ± 4, 41 ± 4, and 24 ± 3 μg/dl, respectively, at 1, 2, 3, and 4 h postdose. **Figure 1** shows the curves of the change in plasma zinc levels after the oral dose of 12.5 mg of zinc alone, or mixed with 25, 50, or 100 mg of stannous tin to comprise Sn/Zn ratios of 0, 2:1, 4:1, and 8:1, respectively. The mean rise in zinc concentration differed significantly from the pattern of zinc alone only for the 3-h postdose interval when the zinc was administered along with 100 mg of tin ($p < 0.05$). In terms of the average area under the discontinuous curves for the change in plasma zinc levels, expressed in arbitrary units of μg/dl·h, the respecive treatments produced the following values: 154 ± 18; 117 ± 21, 123 ± 9, and 136 ± 12. There are no statistically significant differences between or among these areas under the plasma concentration curves. The experiment involving the 100-mg dose of tin was terminated prematurely when the first four subjects experienced nausea, cramps, and loose stools after ingestion of the dose.

Subsequently, mixtures of three minerals—tin, zinc, and iron, were studied in two configurations: *1*) with 12.5 mg of zinc, 12.5 mg of ferrous iron, and 12.5 mg of stannous tin; and *2*) with 12.5 mg of zinc, 25 mg of iron, and 25 mg of tin. These mixtures compromised Sn/Fe/Zn ratios of 1:1:1 and 2:2:1, respectively. As shown in **Figure 2**, a marked

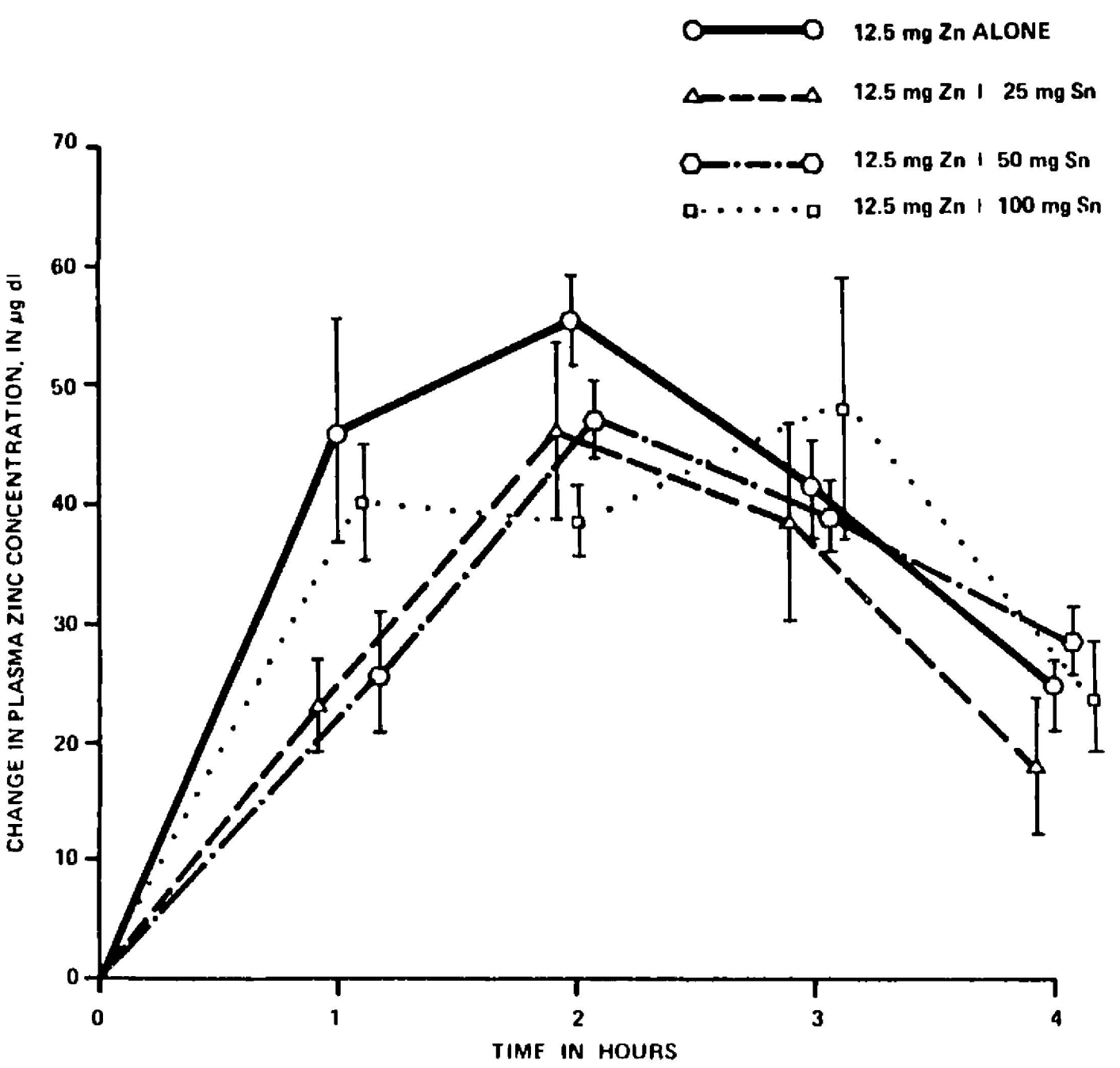

FIG 1. The mean change in plasma zinc concentration over 4 h after the ingestion of 12.5 mg of zinc as 55 mg of zinc sulfate: alone (n = 6), or with 25 mg of tin (n = 6), 50 mg of tin (n = 6), or 100 mg of tin (n = 6) as stannous chloride.

reduction in the excursion of zinc, as compared with that produced by 12.5 mg of zinc alone, was observed, with significant reductions in the rise of plasma zinc at all intervals with the 1:1:1 combination, and at postdose h 1 to 3 with the 2:2:1 mixture. The mean area under the plasma appearance curves, 49 ± 10 and 52 ± 11 µg/dl·h, respectively, were also significantly reduced with respect to the standard curve, 154 ± 18 µg/dl·h ($p < 0.001$). The addition of 94 mg of picolinic acid (PA) to the 2:2:1 Sn/Fe/Zn solution in six absorption studies, produced increments in mean plasma zinc concentrations that were slightly, but significantly, greater than those observed with the corresponding tin-iron-zinc mixture without PA at the 1st ($p < 0.05$) and the 3rd ($p < 0.025$) postdose hours, respectively. The overall areas under the change-in-plasma-zinc-concentration curves, however, 69 ± 5 and 52 ± 11 µg/dl·h, for the treatment with and without PA, respectively, were not significantly different.

Discussion

As recently reviewed (9), the plasma appearance method for assessing zinc absorption in human subjects after ingestion of pharmacological dosages of zinc, ranging from 12.5 to 216 mg, has been used in at least 14 published reports. It has obvious advantages over radioisotopic methods in terms of reduced biohazards, over stable isotope methods in terms of analytical simplicity, and over metabolic balance techniques in terms of tedium, high cost, labor, and convenience. The plasma appearance method has acknowl-

edged disadvantages, however, related to the unphysiological nature of the dose, the dependence of plasma curves on rates of clearance as well as on rates of uptake, and the possibility that only the initial rate of absorption is reflected by the procedure (8). When used under constant conditions of dosage and delivery, however, this method is acceptable for assessing differences among treatments (10). Dosages of 25 and 50 mg of zinc have been used most extensively to date (9), but Oelshlegel and Brewer (11) demonstrated a significant increment in plasma zinc with 12.5 mg doses of zinc and zinc sulfate, zinc carbonate and zinc acetate. We have lowered the dosage to that level to bring it closer to the amount of zinc that would normally be in a meal (~5 mg), and to allow for greater variation in ratios among minerals.

Human tin intakes are quite variable, the most powerful determinant being exposure of food to storage in tin-alloyed containers (12). The mean daily tin intake in the US in 1940 was estimated to be 17 mg/day (13), but daily consumptions ranging from 50 to 200 mg have been observed or projected under circumstances of food storage in tin-lined receptacles (14–16). The tin and zinc content of the experimental diet used by Johnson et al (5), 49.7 and 13.5 mg, respectively, provided a Sn/Zn ratio of about 4:1; their observed fractional reduction in apparent zinc absorption was 16%. Using the plasma appearance method in the present study, we have bracketed this ratio with tin and zinc in 2:1 and 8:1 proportions as well. Throughout our series, no convincing tendency toward reduced uptake of zinc into plasma was observed through this gamut of Sn/Zn ratios.

The apparent contrast of our results to the findings from metabolic studies (5) might be explained, however, on the basis of the rela-

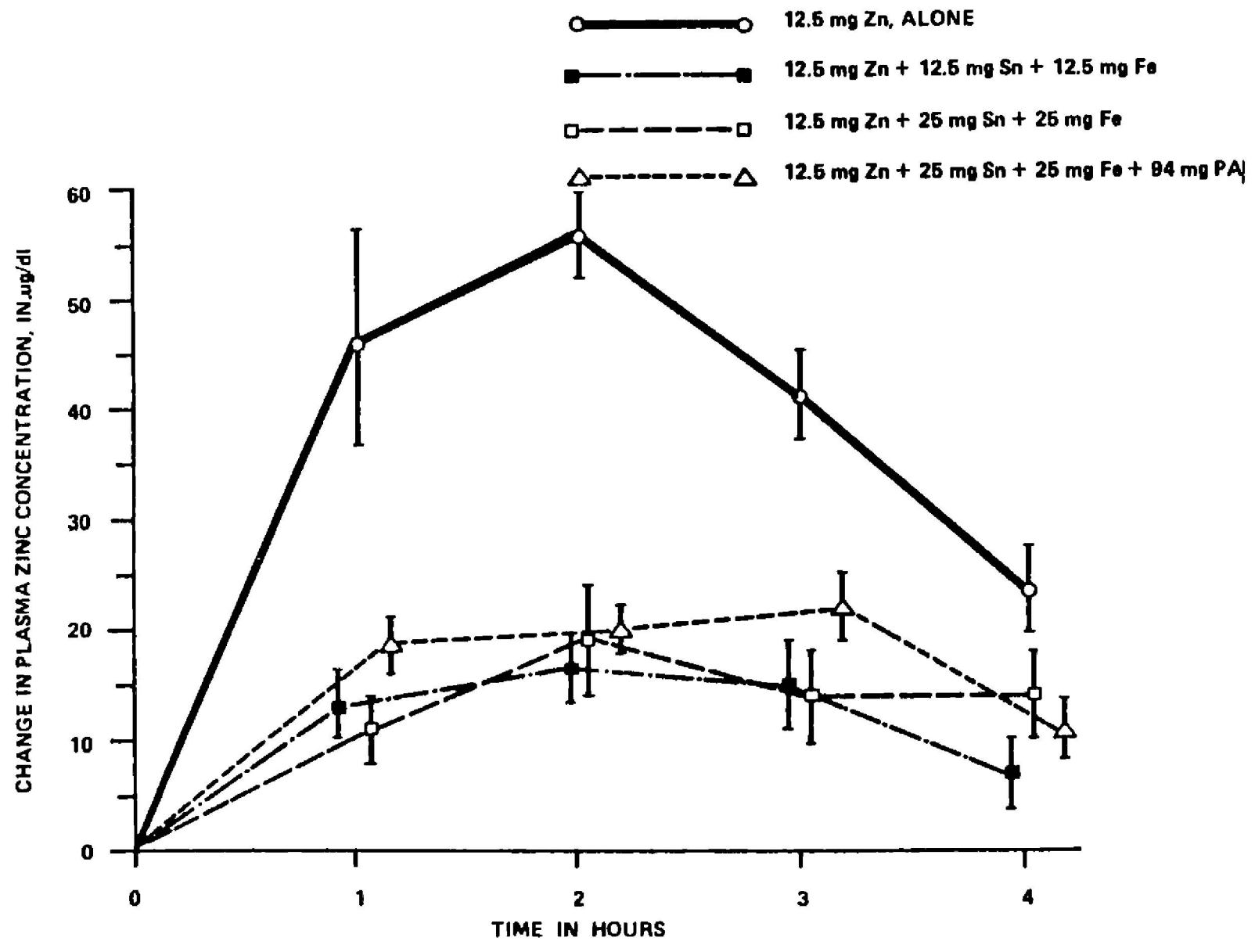

FIG 2. The mean change in plasma zinc concentration over 4 h after the ingestion by six subjects each of 12.5 mg of zinc: alone, with 12.5 mg of tin and 12.5 mg of ferrous iron, and with 25 mg of tin and 25 mg of ferrous iron without or with 94 mg of PA.

tive insensitivity of the plasma appearance technique—with coefficients of variation for the mean rise in plasma zinc of 18 to 53%. By contrast, the metabolic balance method used by Johnson et al (5) had less variance and each participant was used as his own control in a cross-over design. They thus had inherently greater statistical power to determine small differences between dietary treatments. We have estimated, based on the observed variance in the means of plasma zinc concentration change, that with six subjects per treatment group, a reduction of plasma zinc excursion of about 40% would have been required for a predictable 90% certainty of finding significant differences at the 0.05 probability level.

If, however, our findings of no difference in zinc absorption through the range of Sn/Zn ratios be accepted as explaining the true physiology, an intriguing possibility, considered by Johnson et al (5), that the action of tin in increasing fecal losses of zinc and reducing whole-body retention might not be due to an inhibition of the uptake of dietary zinc, but rather due to enhancement of excretion of endogenous zinc, emerges. It is known, for instance, in both rats (17) and cattle (18), that the distal segments of the intestinal tract play an important role in zinc conservation, and the importance of an enteropancreatic circulation of zinc for maintaining normal human zinc balance has recently been cited (19). If tin, indeed, has no "first pass" effect on the absorption of an oral dose of zinc, it is possible that it might act on the biological availability of endogenous zinc excreted into the intestinal lumen.

Whatever be the resolution of the discordance between our findings and those of Johnson et al (5), it is clear that the combination of tin and zinc in progressively higher Sn/Zn ratios does not offer the anticipated tool for exploring an additional human model of mineral/mineral interactions involving zinc, at least using the plasma uptake approach; and we are constrained in the use of higher Sn/Zn ratios by the adverse symptoms experienced by subjects when the 8:1 ratio was applied. Benoy et al (20) reported the occurrence of similar gastrointestinal manifestations when volunteers ingested a solution of tin in fruit juices of 140 mg/dl in amounts corresponding to 5 to 7 mg/kg. Although our absolute dose was less than half of the former on a per kg body weight basis, the concentration of tin in the test solution was roughly equivalent to that of Benoy et al (20), suggesting that the dilution of the tin, rather than its total delivery, may be a determinant factor in human gastrointestinal sensitivity to stannous salts.

The addition of iron to the Sn/Zn solution produced a significant reduction in zinc uptake into plasma. The effect with a 1:1 Fe/Zn ratio in the presence of the additiional one part of tin was qualitatively more pronounced than we had observed with this ratio of iron and zinc alone (6). The 2:1 Fe/Zn ratio with two parts of tin produced a percentage reduction in the area under the curve comparable to that in our previous report. Interestingly, the addition of 94 mg of PA to the 2:2:1 Sn/Fe/Sn mixture (the Zn/PA molar ratio being 4:1) did not have a profound effect of releasing the zinc from the absorptive inhibition by ferrous iron. We have previously observed a complete reversal of the intestinal iron: zinc competition as the only demonstrable effect of PA on zinc absorption with the plasma zinc appearance method in human subjects (21). Evans and Johnson (22) found PA capable of reducing the iron/zinc interaction from iron-rich diets in rats. No mechanistic assessment of the factors in the four-way combination of zinc, tin, iron, and PA and the resultant plasma zinc uptake can be offered until more detailed studies of the phenomenon have been conducted.

In conclusion, with a range of Sn/Zn ratios, we have been unable to demonstrate clear inhibition of the plasma appearance of zinc in normal volunteers ingesting 12.5 mg of zinc in an aqueous solution, but the addition of iron to the tin/zinc mixture produced the same or slightly more than the expected reduction in plasma zinc concentration previously observed with zinc and iron alone. Certainly no antagonism and no unequivocal evidence of synergism of inhibitory effect on zinc absorption can be derived from our experiments with iron and tin combined. In the presence of tin, the previously observed reversal of iron inhibition of zinc absorption by PA was not seen. The recent demonstration in rats (4) and humans (5) of a competitive

interaction between dietary zinc and tin represents a potentially important nutritional observation. The interaction of the two minerals in experiments based on the change in plasma zinc concentration did not approach the magnitude seen previously with the combination of iron and zinc using this same experimental approach. It is possible that the mechanisms of interaction of iron on zinc and tin on zinc in the human intestine are fundamentally different. More detailed investigation with a combination of experimental techniques will be necessary to further characterize and contrast the physiology of mineral/mineral interactions involving zinc and chemically similar divalent cations in the diet.

The authors thank the members of the nursing staff of the Clinical Research Unit of the University Hospital at the University of São Paulo Medical Campus at Ribeirão Preto for their cooperation and technical assistance. We are grateful to the statistical advice of Mr Rafael Flores of the Institute of Nutrition of Central America and Panama in Guatemala City.

References

1. Hill CH, Matrone G. Chemical parameters in the study of in vivo and in vitro interactions of transition elements. Fed Proc 1970;29:1474–81.
2. Van Campen DR, Scaife PU. Zinc interference with copper absorption in rats. J Nutr 1967;91:473–6.
3. Fischer PWF, Giroux A, L'Abbe MRI. The effect of dietary zinc on intestinal copper absorption. Am J Clin Nutr 1981;34:1970 5.
4. Greger JL, Johnson MA. Effect of dietary tin on zinc, copper and iron utilization by rats. Food Cosmet Toxicol 1981;19:163 6.
5. Johnson MA, Baier MJ, Greger JL. Effect of dietary tin on zinc, copper, iron, manganese and magnesium metabolism of adult males. Am J Clin Nutr 1981;35:1332–8.
6. Solomons NW, Jacob RA Studies on the bioavailability of zinc in humans. Effect of heme and nonheme iron on absorption of inorganic zinc. Am J Clin Nutr 1981;344:475–81.
7. Solomons NW, Pineda O, Viteri FE, Sandstead HH. Studies on the bioavailability of zinc in humans. Mechanisms of intestinal interaction of nonheme iron and zinc. J Nutr (in press)
8. Solomons NW, Jacob R, Pineda O, Viteri FE. Studies on the bioavailability of zinc in man: effects of the Guatemalan rural diet and of the iron-fortifying agent, NaFeEDTA. J Nutr 1979;109:1519–28.
9. Solomons NW. The biological availability of zinc in humans. Am J Clin Nutr 1982;35.1048 75.
10. Casey CE, Walravens PA, Hambidge KM. Zinc absorption and plasma response. Am J Clin Nutr 1981;34:1443–4.
11. Oelshlegel FJ, Brewer GJ. Absorption of pharmacological doses of zinc. In: Prasad AS, Brewer GJ, eds. Zinc metabolism: current aspects in health and disease. New York, NY: Alan R Liss, 1977:299–311.
12. Monier-Williams GW. Trace elements in food. New York, NY: J Wiley & Sons, 1949:138.
13. Kehoe RA, Cholak J, Story RV. Spectrochemical study of normal ranges of concentration of certain trace metals in biological materials. J Nutr 1940;19:579 92.
14. Calloway DH, McMullen JJ. Fecal excretion of iron and tin by men fed stored canned foods. Am J Clin Nutr 1966;18:1–6.
15. Woolfe ML, Manu-Tawiah W. Tin content of canned evaporated milk manufactured in West Africa. Ecol Food Nutr 1977;6:133–5.
16. Greger JL, Baier M. Tin and iron content of canned and bottled foods. J Food Sci 1981;46:1751–4, 65.
17. Antonson DL, Barak AJ, Vanderhoff JA. Determination of the site of zinc absorption in rat small intestine. J Nutr 1979;109:142–7.
18. Miller WJ. Absorption, tissue distribution, endogenous excretion and homeostatic control of zinc in ruminants. Am J Clin Nutr 1969;22:1323–31.
19. Anon. On the enteropancreatic circulation of endogenous zinc. Nutr Rev 1981;39:162–4.
20. Benoy CJ, Hooper PA, Schneider R. The toxicity of tin in canned fruit juices and solid foods. Food Cosmet Toxicol 1971;9:645–56.
21. Solomons NW, Pineda O, Viteri FE, Sandstead HH. The effect of picolinic acid on absorption of pharmacological doses of zinc by the human intestine. XII International Congress on Nutrition, San Diego, CA, abstracts, 1981:167.
22. Evans GW, Johnson EC. Effect of iron, vitamin B-6 and picolinic acid on zinc absorption in the rat. J Nutr 1981;111:68–75.