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Studies on the Bioavailability of Zinc in Humans: Mechanism of the Intestinal Interaction of Nonheme Iron and Zinc[1,2]

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ABSTRACT The mechanisms of the previously described competitive zinc:iron interaction were explored in healthy human volunteers, using the increment in plasma zinc concentration after an oral dose of 25 mg of zinc as zinc sulfate as the index of zinc absorption. Ferric iron in a 2:1 Fe/Zn ratio reduced the plasma uptake of zinc, but to a significantly lesser degree than ferrous iron; addition of 1 g of ascorbic acid increased the magnitude of the inhibitory effect of ferric iron to that seen with ferrous iron. An inverse relationship between some indices of iron status in adult women, or of parenteral iron administration in a child, and the magnitude of zinc:iron interaction was observed. Saturation of the intestinal mucosa with consecutive-day doses of therapeutic iron did not influence the uptake of zinc administered alone or in the context of a 2:1 ferrous iron:zinc ratio in solution. The results are most consistent with a combination of an *intraluminal* competition of the two minerals and an *intracellular* competition at a site "distal" to the regulatory step by which iron nutriture modulates the entry of iron into the body, but "proximal" to the site at which the daily administration of therapeutic doses of iron blocked the passage of dietary iron. J. Nutr. *113:* 337–349, 1983.

INDEXING KEY WORDS zinc · iron · intestinal absorption · iron status · mineral–mineral interaction

A decade ago, Hill and Matrone (1) developed the conceptual framework to describe and interpret competitive interactions in biological systems among chemically related minerals from the transition metal series of the Periodic Table of Elements. Radiotracer feeding studies in rats (2, 3) and intestinal perfusion experiments in mice (4, 5) have demonstrated a competitive interaction between zinc and iron. Using the change in plasma zinc concentration as an index of zinc absorption, we have recently demonstrated the existence of a similar zinc:iron competition in human subjects as well (6), demonstrating a substantial and significant reduction in zinc absorption with Fe/Zn ratios of 2:1 and 3:1 comprised with inorganic, *nonheme* iron. Heme iron did not manifest any intestinal interaction with zinc (6). This competition is potentially of considerable nutritional significance, especially in the formulation of vitamin-mineral supplements or of infant formulas (6).

Although the zinc:iron interaction is probably based on chemical similarity between

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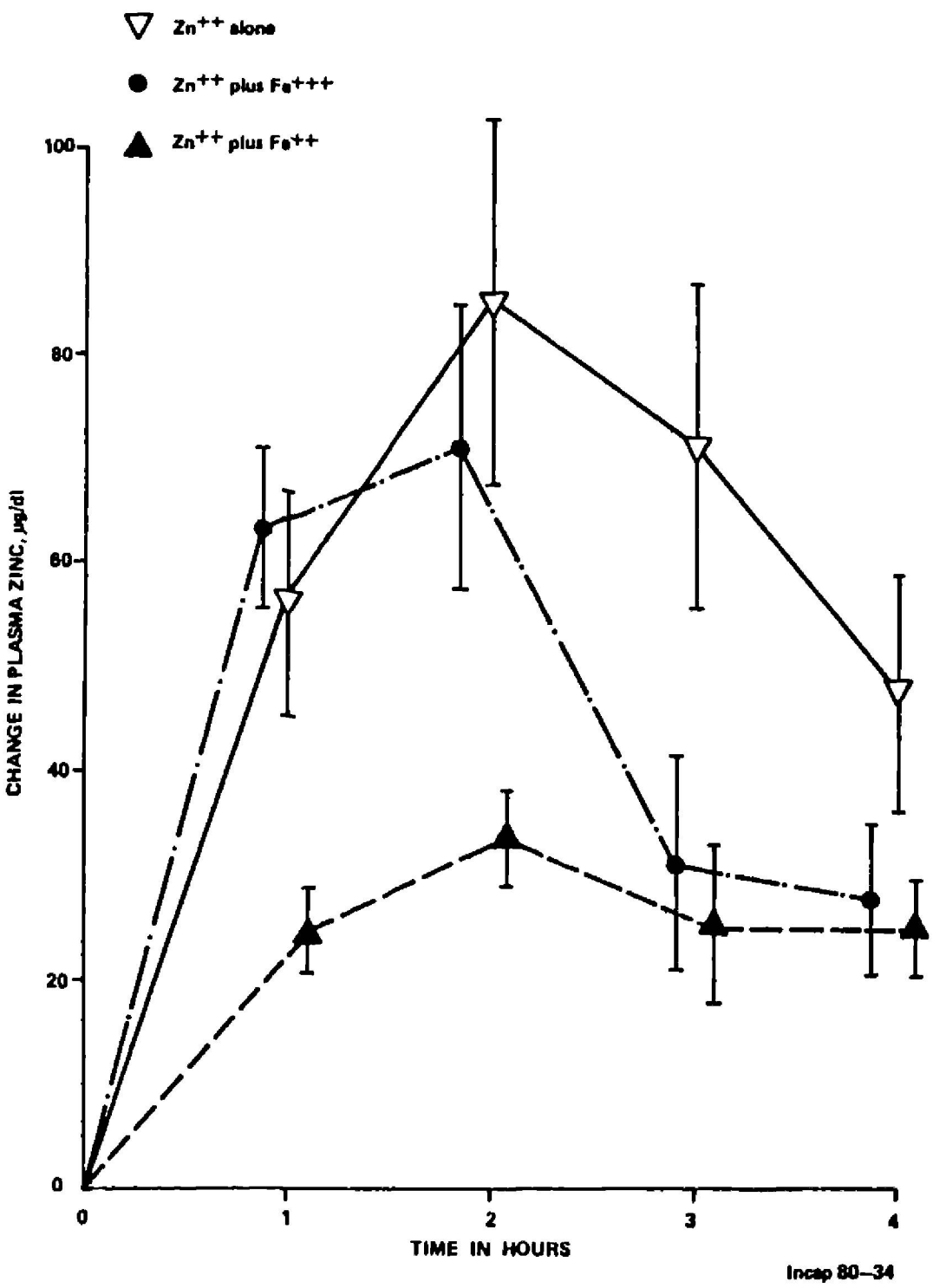

Fig. 1 The change in plasma zinc concentration (mean ± SEM) at 60-minute intervals over 4 hours following an oral dose of 110 mg of $ZnSo_4 \cdot 7H_2O$ (25 mg of elemental zinc) administered alone ($n = 8$), with 248 mg of $FeSO_4 \cdot 7H_2O$ (50 mg of elemental *ferrous* iron) ($n = 7$), or with 241 mg of $FeCl_3 \cdot 6H_2O$ (50 mg of elemental *ferric* iron) ($n = 7$), the latter two treatments constituting 2:1 Fe/Zn ratios. Uppermost and lowest curves as in ref. 6.

the two minerals (1), the nature of the biological interaction in the mammalian intestine is poorly understood. It is not known, for instance, whether the interaction is at the level of the intestinal lumen, the mucosal membrane, an intracellular location, or at the basolateral (serosal) border. Recent advances in our understanding of mammalian iron absorption allow us to predict and localize certain events in the intestinal uptake of iron. In the present study, we have combined contemporary concepts of the mechanisms involved in the regulation of the absorption of *nonheme* iron with the change-in-plasma-zinc-concentration index of zinc absorption to investigate the biology of the zinc:iron interaction in humans.

METHODS

Subjects. The experimental subjects were adult volunteers, both male and female, all in apparent good health, without known or suspected gastrointestinal disease. They agreed to be studied after the nature and purpose of the procedures were explained in full. The studies were conducted concurrently with the studies previously published

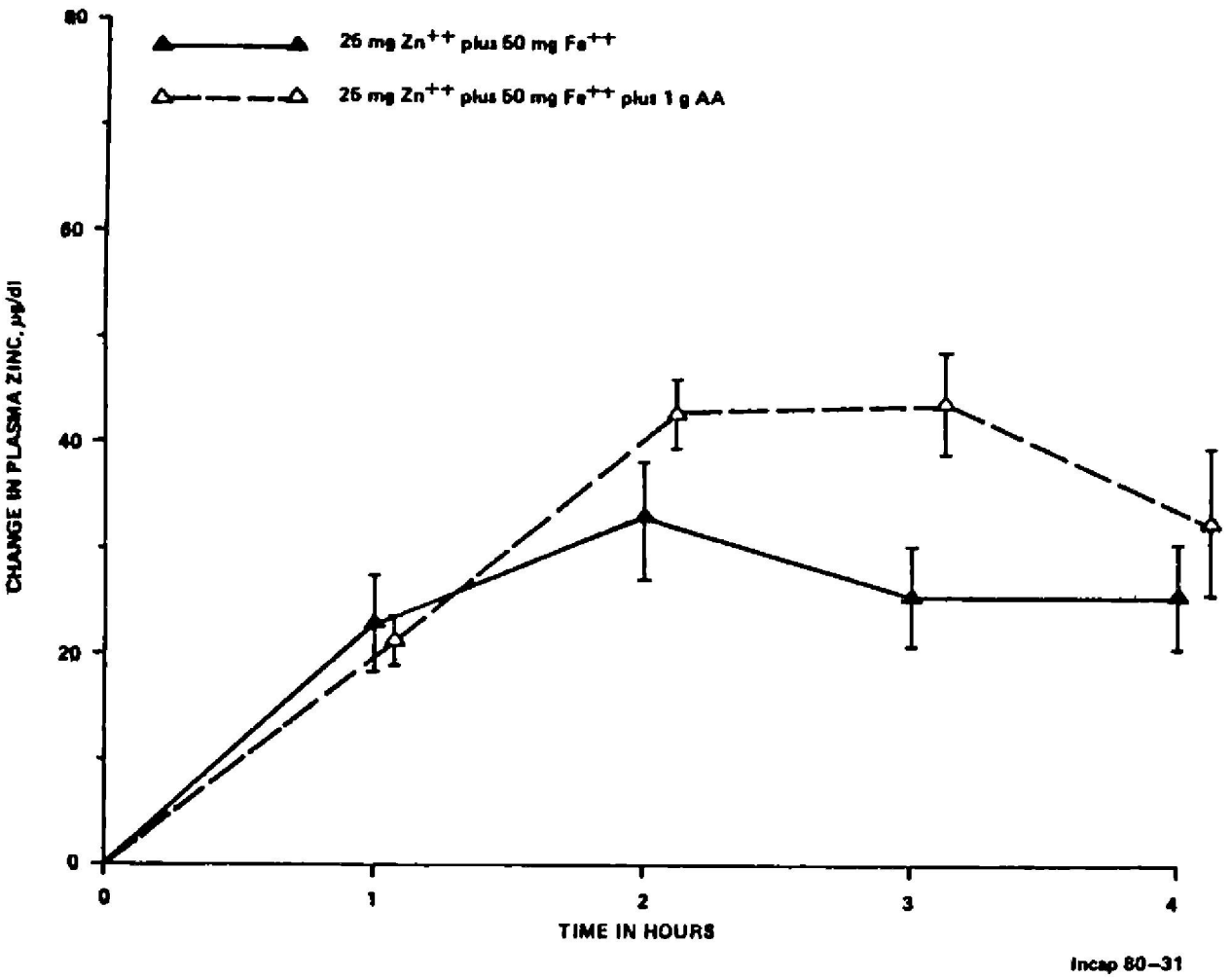

Fig. 2 The change in plasma zinc concentration (mean ± SEM) at 60-minute intervals over 4 hours following an oral dose of 110 mg of $ZnSO_4 \cdot 7H_2O$ (25 mg of elemental zinc) plus 248 mg of $FeSO_4 \cdot 7H_2O$ (50 mg of elemental *ferrous* iron), a 2:1 Fe/Zn ratio, either without (n = 7) or with (n = 7) 1 g of ascorbic acid added. Zinc and ferrous iron curve as in ref. 6.

(6). Data from those experiments serve also as the patterns for absorption of zinc alone and with a 2:1 Fe/Zn ratio. In the course of his rehabilitation from severe protein-energy malnutrition, one 3-year-old child with a slowly resolving anemia was also studied in the Clinical Center of Institute of Nutrition of Central America and Panama (INCAP). The present studies had prior approval of the Human Rights Committee of INCAP and the Committee on the Use of Humans as Experimental Subjects of Massachusetts Institute of Technology.

Administration of absorption tests. Our method for quantifying zinc absorption was identical to that previously reported (7). Tests were begun with a venous blood sample (4 ml) obtained after an overnight fast. Four additional samples were obtained at consecutive, hourly intervals following the administration of the test dose. The subjects remained fasting throughout the test period but were allowed to drink water ad libitum.

In the population studies on the relationship of iron nutriture to zinc:iron interaction, and in the preschool child, a modification of the test was employed; here, only a basal, fasting sample and a 2-hour, postdose sample of blood were obtained. Sufficient blood (10 ml) was collected in the initial sampling for assays to characterize the hematological status of the individuals in that phase of the study.

Because of the strong metallic taste of zinc and iron salts, the minerals were always administered dissolved in 100 ml of Coca-Cola®. For adults, the standard dose of zinc consisted of 25 mg of elemental (stable) zinc as 110 mg of zinc sulfate ($ZnSO_4 \cdot 7H_2O$, mol wt = 287.5, obtained from J. T. Baker Chemical Co., Phillipsburg, NJ). Zinc was administered either alone, or mixed with 50 mg of nonheme iron, to constitute an Fe/Zn ratio of 2:1 on a per weight basis (2.2:1 molar ratio); 50 mg of iron was provided either as 248 mg of ferrous sulfate ($FeSO_4 \cdot 7H_2O$, mol wt = 278.0, obtained from Matheson, Coleman & Bell, Norwood, OH) or 241 mg

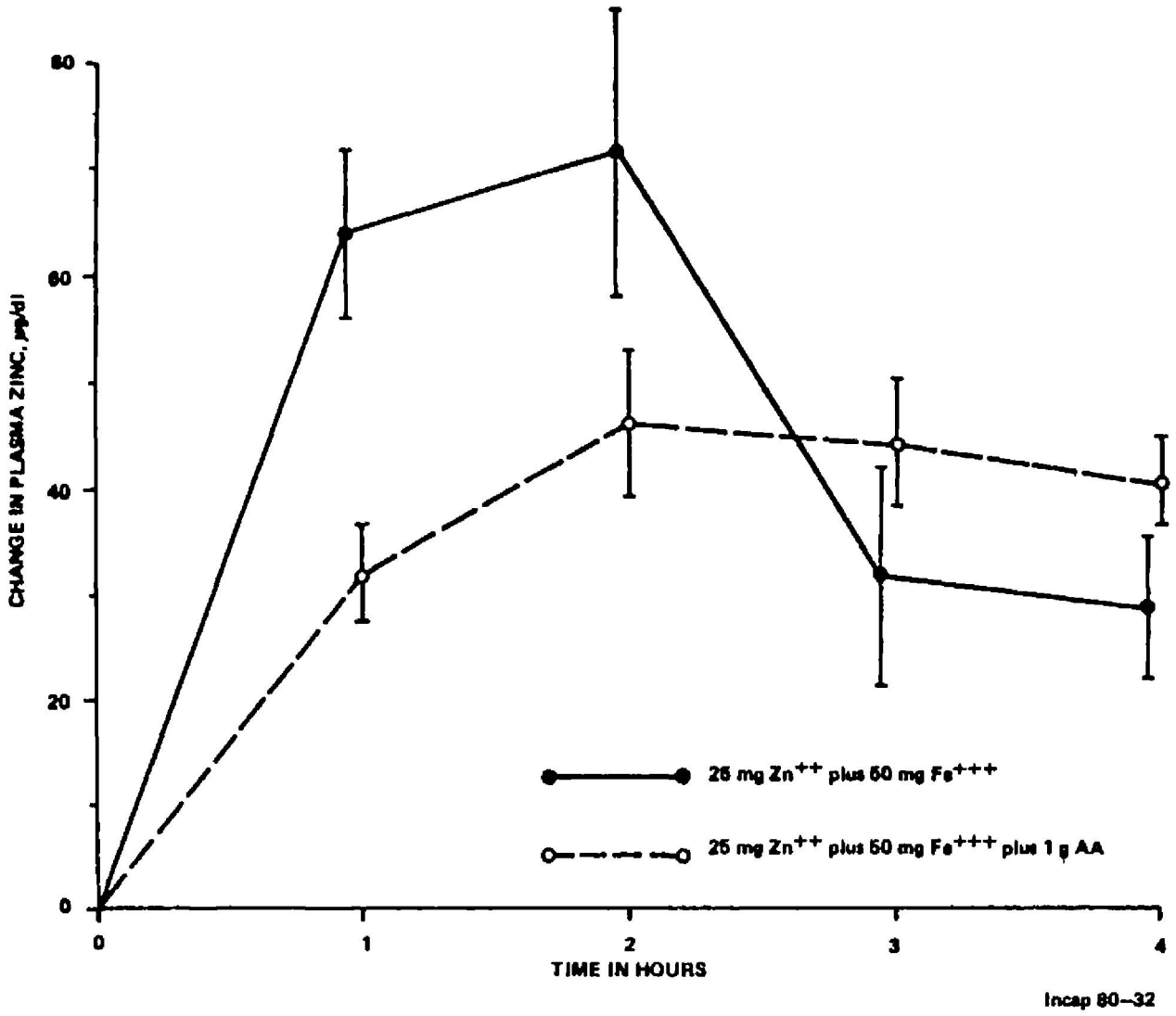

Fig. 3 The change in plasma zinc concentration (mean ± SEM) at 60-minute intervals over 4 hours following an oral dose of 110 mg of $ZnSO_4 \cdot 7H_2O$ (25 mg of elemental zinc) plus 241 mg of $FeCl_3 \cdot 6H_2O$ (50 mg of elemental *ferric* iron), a 2:1 Fe/Zn ratio, either without (n = 6) or with (n = 7) 1 g of ascorbic acid added.

of ferric chloride ($FeCl_3 \cdot 6H_2O$, mol wt = 270.1, obtained from Merck, Munich, West Germany). Also, in some adult experiments, 1 g of ascorbic acid was added to the mineral solution (Redoxon®, Hoffman-La Roche & Co., Basel, Switzerland). The preschool child subject received 100 mg of zinc sulfate (22.4 mg of zinc) either alone in Coca-Cola, or mixed with 222 mg of ferrous sulfate (44.8 mg of iron), to constitute a 2:1 Fe/Zn ratio.

Iron supplementation. In certain experiments, the subjects took therapeutic doses of iron in the form of 325 mg ferrous sulfate tablets (ferrusal, Food Plus, Inc., Moonachie, NJ), once each in the morning and evening, separate from mealtimes. The schedule was either four consecutive days, or the first and third day of a 4-day period (alternate-day schedule).

Determination of plasma trace mineral concentrations. Venous blood was obtained with zinc-free plastic syringes and stainless-steel needles, and collected in plastic tubes (Falcon® tubes, Division of Becton-Dickinson & Co., Oxnard, CA), containing 0.05 ml of 20% potassium oxalate. Whole blood was centrifuged, and the plasma analyzed for zinc concentration by atomic absorption spectrophotometry (Varian Techtron AA5, Varian Techtron Pty., Melbourne, Australia). We used a modification of the method of Sinha and Gabrieli (8), involving a 5:1 dilution of plasma in distilled, deionized water. Iron concentration was determined on the same machine in 5:1 dilutions of plasma treated with trichloroacetic acid.

Hematological determinations. Hematological indices were determined by automated systems. Hemoglobin was measured on a Royco Hemoglobinometer 720-A and hematocrits on a Cell-Crit® 920-A (Royco Co., Menlo Park, CA).

Statistical analysis. The (probability) significance of differences between the mean changes in plasma zinc concentration was computed using the Student's t-test (9). Pear-

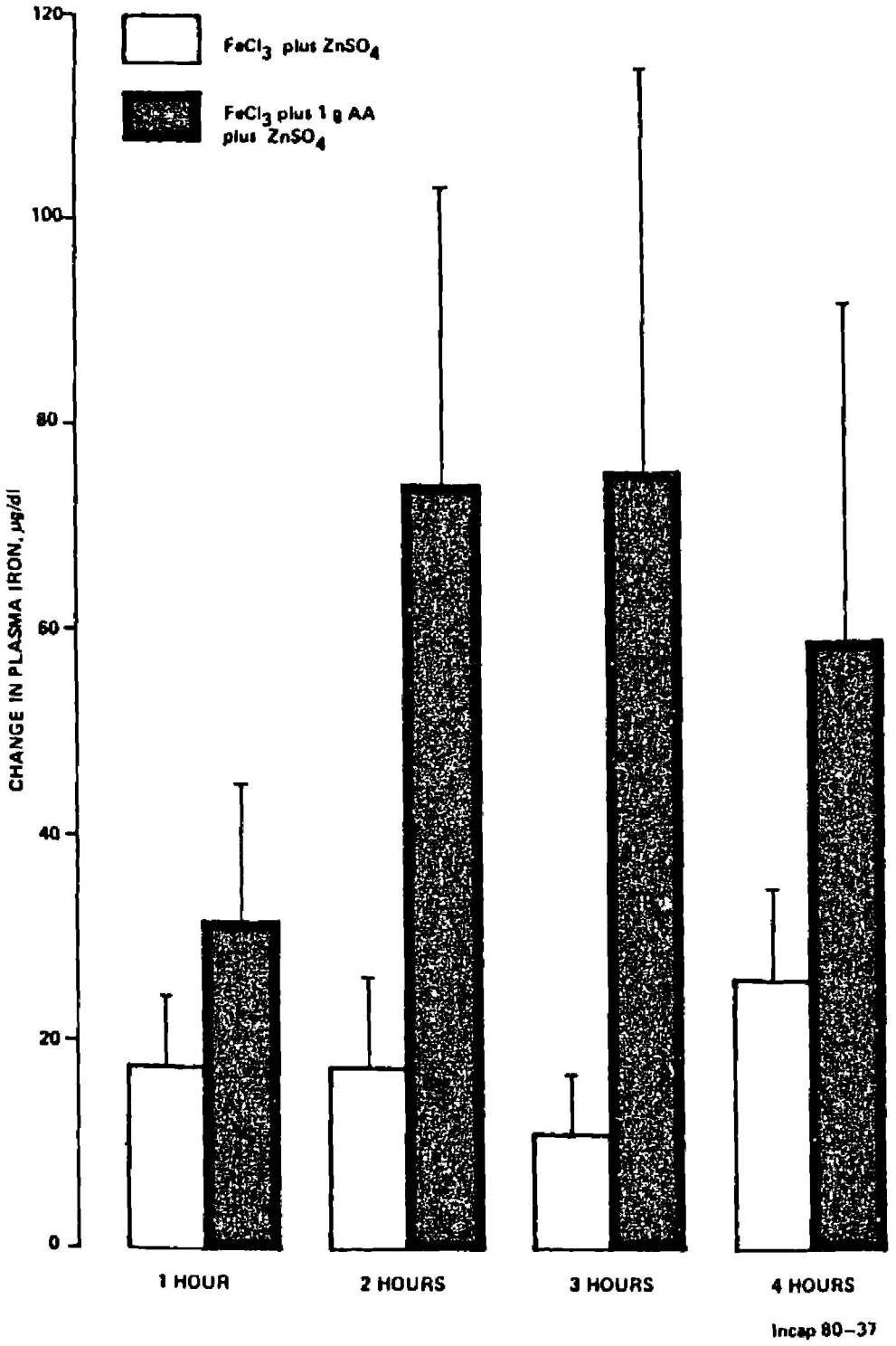

Fig. 4 Change in plasma iron concentration (mean ± SEM) at 60-minute intervals over 4 hours measured concurrently with the experiment illustrated in figure 3. The 50 mg of ferric iron were administered along with zinc either without (open bars) or with (solid bars) 1 g of ascorbic acid.

son's correlation analysis was employed for the regression of hematological data with the 2-hour rise in plasma zinc (9).

RESULTS

Do ferrous and ferric iron produce equivalent inhibition of intestinal zinc uptake?

Our initial demonstration of an intestinal interaction of zinc and iron employed ferrous sulfate as the iron salt (6). We reasoned that if the mineral–mineral competition were related to the chemical similarity between the cations, then the trivalent (ferric) form of iron—a chemically dissimilar and less absorbable species—would produce less inhibition of zinc uptake than the divalent (ferrous) form. Figure 1 represents the pattern of change in plasma zinc concentration when 25 mg of zinc was administered alone, with 50 mg of ferrous iron, and with 50 mg of ferric iron. The 2:1 Fe/Zn ratio, constituted with ferrous sulfate, produced a significant decrement in plasma zinc concentrations at 1 hour ($P < 0.025$), 2 hours ($P < 0.05$) and 3 hours ($P < 0.025$), postdose. On the other hand, a 2:1 Fe/Zn ratio based on addition of

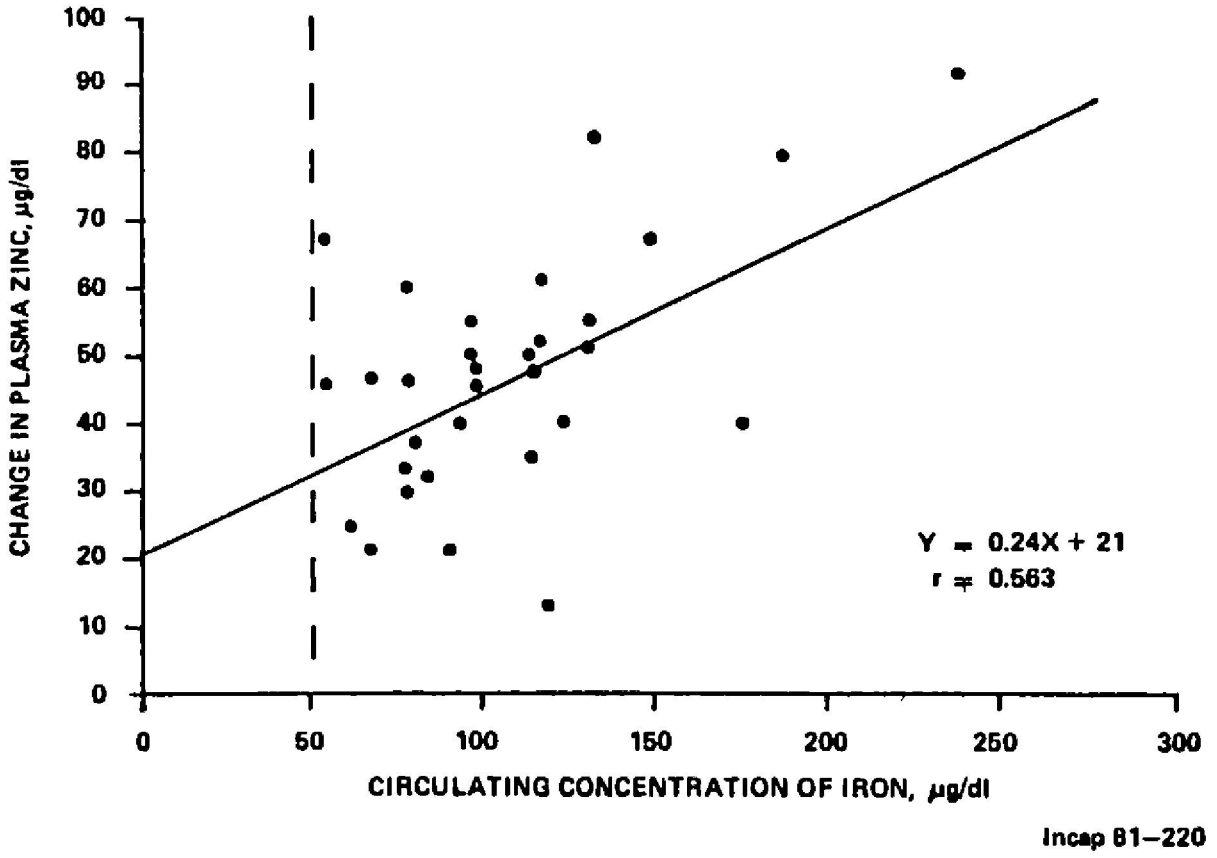

Fig. 5 Linear correlation of the change in plasma zinc concentration at 2 hours after an oral dose of 110 mg of $ZnSO_4 \cdot 7H_2O$ (25 mg of elemental zinc) plus 248 mg of $FeSO_4 \cdot 7H_2O$ (50 mg of elemental ferrous iron) versus the fasting plasma iron concentration in 32 female subjects.

ferric chloride caused a significant reduction in plasma zinc only 3 hours after the dose ($P < 0.05$).

Does ascorbic acid increase the inhibition of intestinal zinc uptake by iron?

Ascorbic acid increases the bioavailability of nonheme iron (10, 11), presumably by decreasing intraluminal pH, increasing intraluminal solubility, and promoting the reduced (ferrous) oxidation state (11). In a prior series of experiments from our laboratories (12), we failed to demonstrate any independent effect of ascorbic acid on the plasma uptake stable zinc. We reasoned, therefore, that if zinc and iron were competing for a common pathway of intestinal absorption, enhancing the relative bioavailability of the iron in a 2:1 Fe/Zn mixture of iron and zinc salts would increase the inhibition of the intestinal uptake of zinc.

In the first series of ascorbic acid experiments, the 25 mg dose of zinc was administered in the presence of 50 mg of ferrous iron and of 1 g of Redoxon®; the results were compared to studies in which no ascorbic acid had been added. As shown in figure 2, the resultant curves of plasma zinc concentration were identical. However, as the Coca-Cola vehicle is itself acidic, and the solutions were prepared immediately prior to administration, the iron in ferrous sulfate might have been in its maximally absorbable form even without the addition of ascorbic acid. Unfortunately, in this initial set of experiments, the plasma samples were not analyzed for iron concentrations to test this assumption.

In an analogous series of experiments, ferric chloride provided the 50 mg of iron in the test solution. Here, a significant reduction in the increment in plasma zinc levels at 1 hour ($P < 0.01$) was produced by the addition of 1 g of ascorbic acid to the solution (fig. 3). In these ferric iron studies, we did measure plasma iron concentrations and, as expected, the rise in plasma iron was greater in the presence of ascorbic acid (fig. 4).

Does iron nutriture influence the intestinal interaction of iron and zinc?

It is well known that the absorption of iron is inversely related to the total-body iron reserves of an individual (13). Two alternative explanations have been proffered to explain

the mechanism of homeostatic regulation of iron absorption. The "mucosal block" hypothesis proposed that the iron not required for the nutrition of an individual is trapped in the intestinal cell (14); it was subsequently suggested that mucosal ferritin plays the role of intracellular monitor, binding and holding unneeded iron (15, 16). The "transferrin capture" hypothesis proposes that mucosal transferrin, acting at the luminal surface of the enterocyte, captures intraluminal iron in proportion to its cellular abundance (Huebers, H., personal communication). Based on consequences of the intestinal regulation of iron absorption, we reasoned that the iron status of an individual might influence the zinc:iron interaction, if the locus of the intestinal competition were functionally "distal" to the regulatory step for iron. Namely, in individuals with iron depletion and a greater tendency to absorb iron, a greater inhibition of zinc uptake would be seen as compared to individuals with adequate iron stores and consequently less affinity for dietary iron.

In order to enroll a study population with a presumably wide range of iron status, we chose 32 women between the ages of 19 and 50 years, including both premenopausal and postmenopausal subjects. Hemoglobin concentration ranged from 11.3 to 16.2 g/dl, hematocrits from 36 to 47% and fasting plasma iron concentrations from 54 to 248 μg/dl. Each participant underwent a modified zinc absorption test with a 2:1 Fe/Zn ratio solution constituted from 25 mg of zinc and 50 mg of ferrous iron. The correlation coefficients for the regression of hemoglobin or hematocrit levels with the change in plasma zinc concentration at 2 hours were not statistically significant. However, as shown in figure 5, plasma iron (an index of iron nutriture more discriminating and reflective of iron stores) showed a significant correlation with the uptake of plasma zinc from the zinc:iron mixture, $r = 0.563$ ($P < 0.05$).

We also tried to gain a perspective on the role of iron nutriture by studying a preschool child who had been scheduled to receive intramuscular iron therapy. The child was studied on three occasions, using the modified procedure of measuring the rise in plasma zinc only at 2 hours postdose. In the first test he received only 22.4 mg of zinc, and showed an increment in plasma zinc concentration of 230 μg/dl. A week later, the absorption test was repeated with a 2:1 Fe/Zn ratio. The rise in plasma zinc was reduced by 42%. Later that day, the patient received 50 mg of parenteral iron as iron dextran (Imferon®, Lakeside Laboratories, Milwaukee, WI). He showed a mild reticulocytosis over the next 10 days. A third absorption test was repeated, 10 days after the second, once again with a 2:1 Fe/Zn ratio, and the rise in zinc was now 30% below the standard test with zinc alone (fig. 6). The simultaneous changes in plasma iron during the last two

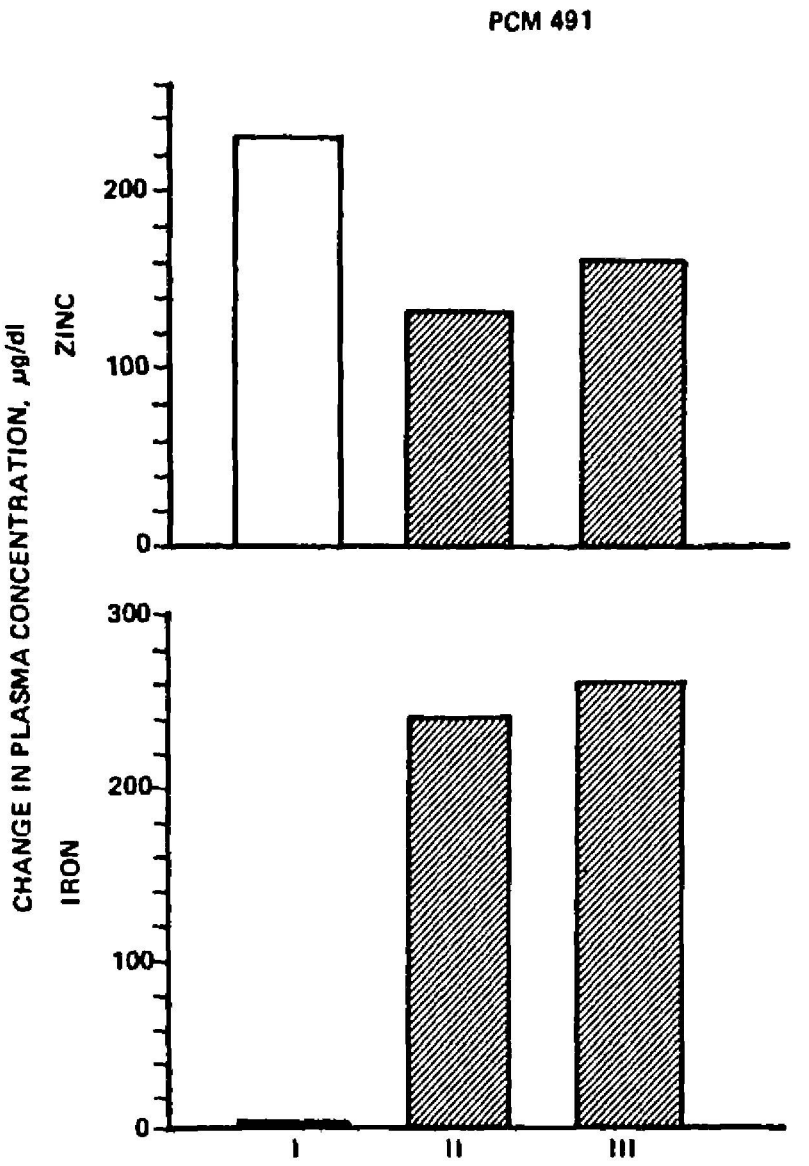

Fig. 6 The change in plasma zinc concentrations (above) and iron concentrations (below) with respect to the respective fasting levels at 2 hours following the dose of 22.4 mg of zinc as 100 mg of zinc sulfate in a preschool child (PCM 491), recovered from protein-energy malnutrition. In tests II and III, the zinc was administered with 44.8 mg of ferrous iron. Between tests II and III, the patient received 50 mg of parenteral iron.

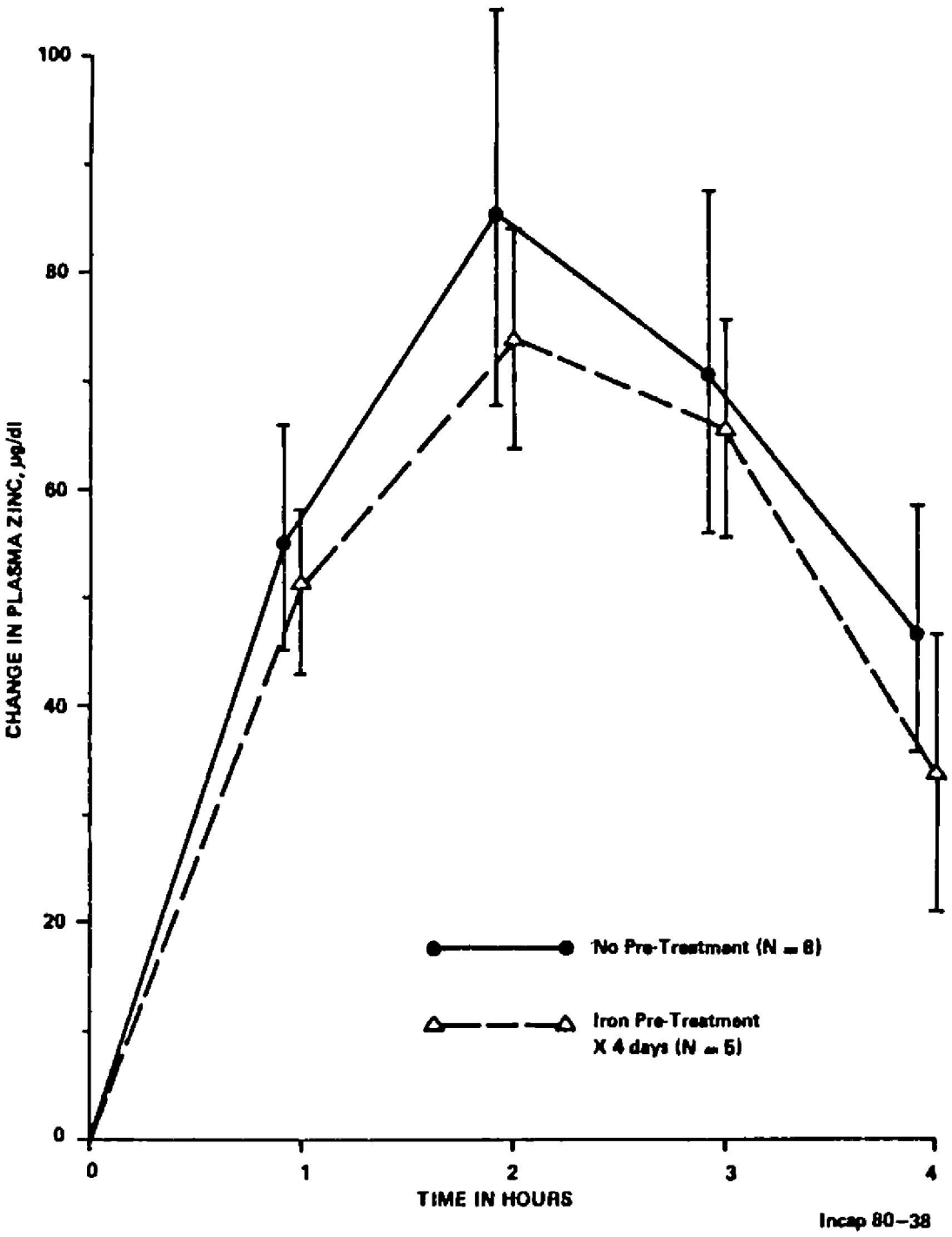

Fig. 7 The change in plasma zinc concentration (mean ± SEM) at 60-minute intervals over 4 hours following an oral dose of 110 mg of $ZnSO_4 \cdot 7H_2O$ (25 mg of elemental zinc) administered after no pretreatment regimen (n = 8) or after four consecutive days of therapeutic iron administration as 325 mg of ferrous sulfate twice a day (n = 5).

absorption studies, on the other hand, differed by less than 7% (fig. 6).

Does consecutive-day administration of iron reduce intestinal zinc uptake?

Experiments at INCAP with preschool children demonstrated that consecutive-day administration of therapeutic doses of iron progressively reduced the rise in plasma iron following each daily morning administration of ferrous sulfate (17, 18). This pattern was not seen, however, with alternate-day dosing with an equivalent daily dosage of iron (17, 18). This has been interpreted as a mucosal saturation or blockade of iron uptake. If zinc shared this absorptive mechanism in common with iron, treatment with consecutive-day doses of therapeutic iron might similarly reduce the plasma zinc. Four-days' consecutive ingestion of 650 mg of ferrous sulfate (130 mg of iron) did not affect plasma zinc uptake of 25 mg of zinc administered alone (without iron) to five subjects as compared to the curve of individuals with no pretreatment (fig. 7).

Does consecutive-day administration of iron reduce the inhibition of intestinal zinc uptake by iron?

Having shown no interaction between oral zinc and prior consecutive-day administration of therapeutic amounts of iron (above),

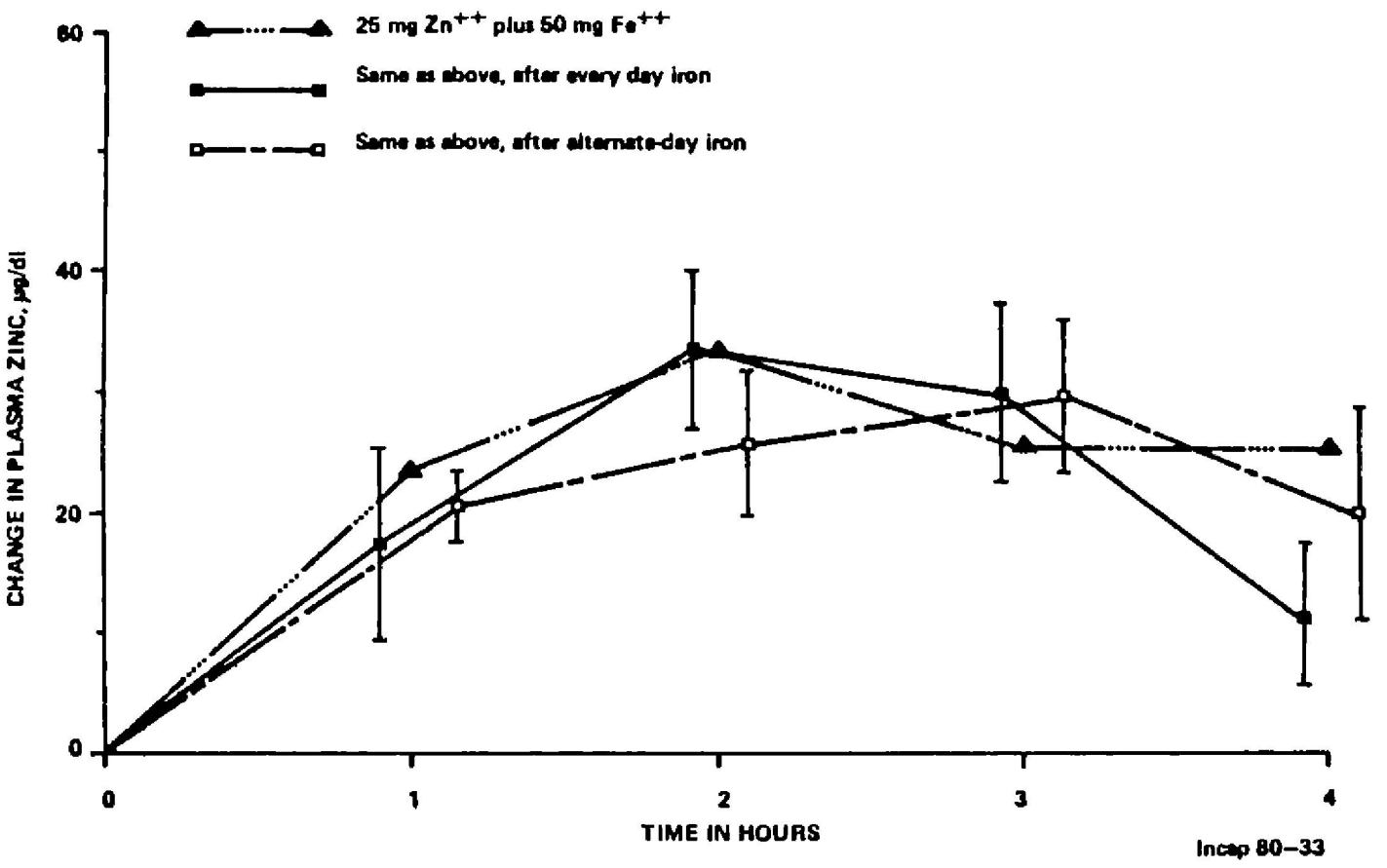

Fig. 8 The change in plasma zinc concentration (mean ± SEM) at 60-minute intervals over 4 hours following an oral dose of 110 mg of $ZnSO_4 \cdot 7H_2O$ (25 mg of elemental zinc) plus 248 mg of $FeSO_4 \cdot 7H_2O$ (50 mg of elemental *ferrous* iron) administered after no pretreatment regimen (n = 7), after four consecutive days of therapeutic iron administration as 325 mg of ferrous sulfate twice a day (n = 5), or after 4 days of alternate-day therapeutic iron administration with the same dosage (n = 5). Reference curve, as in figs. 1 and 2, after ref. 6.

we concluded that the effect of iron administration on iron absorption (17, 18) could be used as a probe of zinc:iron interaction. We reasoned that if, in the scheme of transcellular transport, the competition between zinc and iron were functionally distal to the site of mucosal blockade produced by consecutive-day (but not alternate-day) iron administration, then differential patterns of plasma zinc uptake in the presence of a 2:1 Fe/Zn ratio might be observed with the two forms of pretreatment. To explore this hypothesis, the plasma uptake of zinc from a 2:1 Fe/Zn solution containing 25 mg of zinc and 50 mg of ferrous iron was studied in subjects receiving therapeutic doses of ferrous sulfate either every day or every other day during the 4-day period prior to the absorption tests. As compared to results obtained in subjects with no prior treatment (fig. 8), no differences in the pattern of zinc:iron interaction were observed. In accordance with the prior experience in children (17, 18), adult subjects also tended to have a reduced plasma excursion of iron after consecutive-day treatment, as compared to alternate-day, as shown by the decrement in plasma iron rise ($P < 0.05$) at 1 hour and 3 hours postdose (fig. 9).

DISCUSSION

Despite the elegance and precision afforded by the use of laboratory animal models, the final arbiter in the description of human physiology is experimentation with human subjects. The earlier demonstration of a competitive zinc:iron interaction in the human intestine (6) underscored the relevance and validity of the concept of mineral–mineral competition in human intestinal physiology. Our present studies elucidate the mechanisms underlying this interaction.

The long radioactive and biological half-life of ^{65}Zn (19), the excessively short half-life of ^{69m}Zn (20–22), and the complexity and expense of stable isotope technology (23–26) have limited the experimental options for studying zinc absorption in healthy human subjects. Recently, however, the change in plasma or serum zinc concentration after oral doses of pharmacological amounts of stable zinc (12.5–108 mg) have been used as an in-

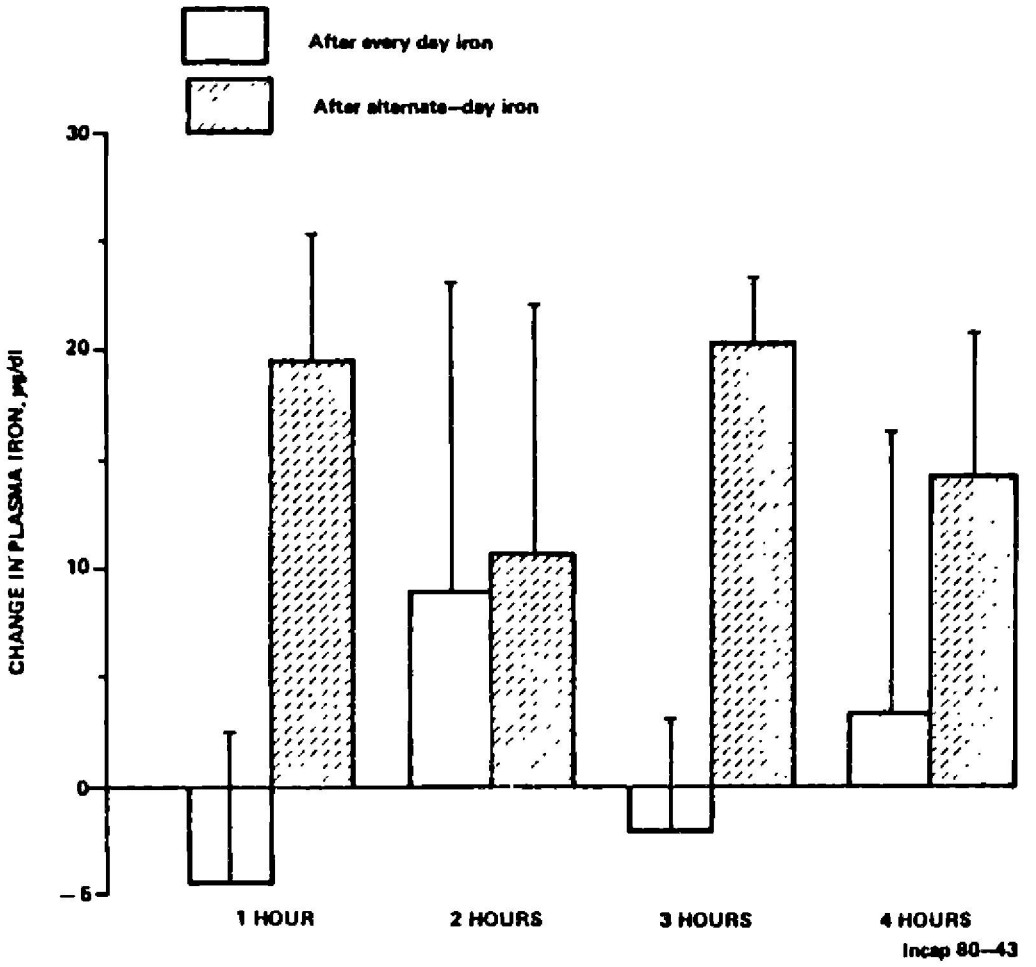

Fig. 9 Change in plasma *iron* concentration (mean ± SEM) at 60-minute intervals over 4 hours measured concurrently with the experiment illustrated in fig. 8. The 50 mg of ferrous iron were administered with zinc after consecutive-day (open bars) or alternate-day (stippled bars) pretreatment with therapeutic iron.

dex of zinc absorption (6, 7, 12, 27–36). There are several acknowledged limitations to the interpretation of experimental results obtained with this method. They involve: the size of the zinc load in relation to normal dietary levels; the possible interindividual differential clearances of absorbed zinc from the circulation; and whether the curves of plasma uptake reflect only the *rate* of zinc absorption or *net* absorption as well (7). However, the method is simple, well tolerated and without risk to subjects; it offers a valuable tool for exploring intestinal events in human zinc absorption (7, 33).

The present study amply confirms the operation of a competitive interaction between inorganic zinc and nonheme iron in the human intestine. We were indebted to the recent, rapid progress in the conceptual formulation and experimental description of the mechanisms of iron absorption in humans. We used these newer findings regarding iron absorption to address hypotheses about the alterations in zinc:iron interaction which might be predicted from recognized changes in the behavior of iron.

Faithful to the predictions of Hill and Matrone (1), ferric iron (a chemically less similar ion) produced less inhibition of zinc absorption than did ferrous iron. However, it is also recognized that ferric iron is less biologically available than ferrous iron. Whether it is the divalent ionic nature, per se, or the greater access of ferrous iron to various sites in the absorptive pathway that determine the greater interaction with zinc cannot be conclusively resolved.

In the experiments with perfused murine intestinal segments (5), both the uptake of ^{65}Zn into the mucosa and the transfer of ^{65}Zn from mucosa to carcass were reduced by the presence of iron in the perfusate. This would suggest both an intraluminal and an intracellular influence of iron. The action of ascorbic acid is intraluminal, affecting a greater absorbability of iron. The addition of ascorbic acid to the solution constituted from ferrous iron and zinc produced no change in zinc:iron interaction. However, Grebe et al. (37) reported that when ^{59}Fe in the ferrous form was used as a tracer in human iron absorption experiments, ascorbic acid had no

additional enhancing effect. It was with the ferric chloride solution that our addition of ascorbic acid had a measurable effect on the zinc:iron interaction, reducing the plasma uptake of zinc. This is consistent with an intraluminal zinc:iron interaction. It could result from a competition for access to surface binding sites, or from a change in the partition of iron and zinc among intraluminal binding ligands. Evans et al. (38) postulated that a low-molecular-weight binding ligand was instrumental in the intestinal absorption of dietary zinc. Recently, Evans and Johnson (39) have suggested that the mechanism of action of picolinic acid in enhancing zinc absorption might be its favoring of zinc (relative to iron) in regard to intraluminal binding affinities.

In our studies, however, differential effects on zinc:iron interaction were not limited to intraluminal manipulations. A suggestive effect of iron status, as assessed by fasting plasma iron concentration, on intestinal interaction of iron and zinc was observed in a group of 32 women. It is unfortunate that serum ferritin, an even more definitive index of iron stores (40) was not included in this design. Although limited to a single subject, the serial observations on zinc uptake in the presence of iron, before and after a parenteral iron injection calculated to increase total-body iron by 20%, are revealing. They suggest that increasing iron status, rapidly and directly by the intramuscular route, reduces the magnitude of the iron:zinc interaction. This was apparent in this child despite the lack of influence of iron therapy on the rise in plasma iron after a standard dose of ferrous sulfate. Both sets of findings are consistent with the concept that when the body requires more iron, its enhanced passage into or through the mucosa reciprocally reduces the entry of zinc. It does not matter whether the mucosal block or the transferrin capture mechanism is evoked to explain the homeostatic regulation of iron absorption. With either mechanistic supposition, it would appear that the reduction of iron requirements enhances the passage of zinc, and the augmentation of iron requirements inhibits the uptake of zinc.

Our previous demonstration of a blockade of the uptake of therapeutic doses of iron by their consecutive-day administration suggested several probes of the zinc:iron interaction. If the site of the mucosal saturation effect, itself, were shared in common between iron and zinc, then treatment with consecutive-day doses of ferrous sulfate pills would have been expected to reduce the uptake of zinc into the plasma as well. This was not seen, and suggests that some levels of the absorptive regulation of iron are *not* shared by zinc. Moreover, the locus of interaction of iron and zinc appears to be functionally proximal to the site of the mucosal blockade, since the consecutive-day administration of iron had no effect on the magnitude of zinc uptake in the presence of an inhibiting dose of ferrous iron.

Although gross nutritional effects of various dietary components can be monitored in human subjects, access to the cellular and subcellular mechanisms subtending these effects is often difficult to obtain through human experimentation; we cannot reproduce the isolated perfused intestinal segment studies, isotopic partition analyses and cellular fractionation procedures that have been used so elegantly in animal models. However, judicious exploitation of the recognized behavior of dietary iron in the human intestine and in vivo manipulations have allowed us to probe the interaction of iron and zinc and to speculate on the mechanistic nature of this phenomenon in the human alimentary tract. It would appear that the combined action, at an intraluminal site and at an intracellular locus, suggested by the murine studies of Hamilton et al. (5), is also true for the competitive inhibition of zinc absorption by iron in the human intestine. However, this is not necessarily the case, as the effect of increasing the absorbability of iron with ascorbic acid may only have served to increase its relative accumulation at some interior cellular location of primary interaction. This latter site would appear to be situated along the pathway of iron absorption *distal* to the regulatory control step exerted by the iron nutriture of the host. The mucosal blockade produced by steady doses of therapeutic quantities of iron did not prevent the inhibition of zinc absorption by the standard

competitive dose of ferrous sulfate in solution.

We have attempted to keep the relative proportions of the two minerals within the ranges achieved in adult diets, vitamin-mineral supplements and infant formulas (6). Given the pharmacological nature of the test solutions used, however the extrapolation of our findings to physiological, dietary levels of these two minerals is premature. The confirmation of the operation of the zinc:iron interaction with physiological amounts of zinc and iron will have to await the application of stable isotope technology to this interesting issue.

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