Bioavailability of Nickel in Man: Effects of Foods and Chemically-Defined Dietary Constituents on the Absorption of Inorganic Nickel[1-5]

NOEL W. SOLOMONS,*† FERNANDO VITERI,† TERRENCE R. SHULER‡ AND FORREST H. NIELSEN‡

*Massachusetts Institute of Technology, Cambridge, MA 02139; the †Division of Human Nutrition and Biology, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala, Apartado 11-88; and ‡U. S. Department of Agriculture, Science and Education Administration, Grand Forks Human Nutrition Research Center, Grand Forks, ND, 58202.

ABSTRACT By serial determination of the change in plasma nickel concentration following a standard dose of 22.4 mg of nickel sulfate hexahydrate condtaining 5 mg of elemental nickel, the bioavailability of nickel was estimated in human subjects. Plasma nickel concentration was stable in the fasting state and after an unlabeled test meal, but after the standard dose of nickel in water was elevated 48.8, 73.0, 80.0, and 53.3 μg/l, respectively, at hours 1, 2, 3, and 4. Plasma nickel did not rise above fasting levels when 5 mg of nickel was added to two standard meals: a typical Guatemalan meal and a North American breakfast. When 5 mg of nickel was added to five beverages–whole cowmilk, coffee, tea, orange juice, and Coca Cola®–the rise in plasma nickel was significantly suppressed with all but Coca Cola®. Response to nickel also was suppressed in the presence of 1 g of ascorbic acid. Phytic acid in a 2:1 molar ratio with nickel, however, did not affect the rise in plasma nickel. The chelate of iron and ethylenediaminetetraacetate, NaFeEDTA, an iron-fortifying agent suggested for application in Central America, slightly but significantly depressed plasma nickel rise at 2 hours, whereas disodium EDTA depressed plasma nickel levels significantly below the fasting nickel curve at 3 and 4 hours postdose. These studies suggest that the differential responses of inorganic nickel to distinct foods, beverages, and chemically-defined dietary constitutents could be important to human nutrition. J. Nutr. *112*: 39–50, 1982.

INDEXING KEY WORDS nickel · ascorbic acid · phytic acid · EDTA

Nickel has been recognized recently as essential for certain vertebrate species including rats (1–3), chicks (4), swine (5, 6), goats (6), and sheep (7). Biologically-important interactions between nickel and other trace minerals have also been discovered (8–11). In tissue of healthy humans, nickel has been consistently found in concentrations ranging from 0.04 to 2.8 μg/g on a dry basis (12, 13), and Schroeder and Nason (14) estimated that the human body contains 10 mg of nickel, of which 18% is in the skin. It is likely that nickel is metabolically active, if not nutritionally essential, in humans as well.

The nickel contents of common foods from the United States (15, 16), Great Britain (17,

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[4] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

[5] Abbreviations used: Nickel sulfate = $NiSO_4 \cdot 6H_2O$; ethylenediamine-tetraacetic acid = EDTA; sodium iron ethylenediaminetetraacetic acid = NaFeEDTA; disodium ethylenediaminetetraacetic acid = $Na_2EDTA \cdot 2H_2O$.

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18), Sweden (19), and Holland (20) are available. Dry beans, cocoa products, baking soda, and some nuts contain high levels of nickel (>2.0 μg/g); wheat and wheat products, shellfish, processed meats and many vegetables contain intermediate levels (0.2–2.0 μg/g); and whole and dried milk, fresh fruits, meat, eggs and Coca Cola® contain low levels of nickel (<0.2 μg/g). Thomas et al. (17) suggested that contact between food and machinery or cans can contribute to dietary nickel, as processed and canned vegetables contain more than fresh. In some areas of Europe, where nickel is used as a catalyst in hydrogenation, margarine can be a substantial dietary source of nickel (21).

In Sweden, the estimated daily nickel intake ranged from 200 to 4460 μg and averaged 750 μg (19). Early estimates of daily nickel consumption in the U. S. ranged from 300 to 600 μg (15). The average nickel content of nine institutional diets in North Dakota was 168 ± 11 μg, or 75/1000 kcal (22). Based on extrapolation from animal data, the hypothetical human requirement for nickel would be 16 to 25 μg/1000 kcal or about 75 μg of elemental nickel per day (23). Most balanced diets probably exceed that amount of nickel. The issue of nickel in the human diet, however, has further ramifications.

Low-nickel diets have been advocated in the management of nickel-sensitivity dermatitis (21, 24) which apparently is exacerbated by orally-ingested nickel (25). The possibility was advanced that a strict nickel-elimination diet might have implications for human health (23).

Despite broad, general knowledge about customary dietary intakes, little is known about the chemical form of nickel in foods or the factors that affect its biological availability. Concern about the biological availability of trace elements in food is justified by extensive experience with dietary iron (26–28) and dietary zinc (29–32). Vohra et al. (33) showed that nickel was among a host of elements that formed stable complexes with phytic acid in vitro; possibly such complexes would explain interference of unrefined cereal foods with the absorption of nickel (34). In the only study of its kind, Horak and Sunderman (35) have estimated from nickel balance experiments that about 10% of the nickel in a normal diet is absorbed.

Techniques to pursue a detailed exploration of nickel bioavailability are limited. In laboratory animals, Onkelinx et al. (36, 37) used a radioisotope, ^{63}Ni, as a tracer; the radioactive half-life (92 years) of this isotope, however, precluded its use in man. A stable isotope of nickel, ^{64}Ni, could theoretically be used as a tracer because its natural abundance is 1.16% and it can be measured by thermal neutron activation analysis. Technological aspects of this approach, however, await further development. Spruits and Bongaarts (38) reported that plasma nickel concentration rose by about 54 μg/l within two hours of the ingestion of 5 mg of elemental nickel as 22.4 mg of nickel sulfate in a single healthy individual. As the change in plasma zinc concentration has recently been exploited as an index of zinc absorption (34, 35, 39–44), we thought that an analagous approach could be applied to study the effects of meals, beverages, and chemically-defined dietary constituents on the absorption of inorganic nickel.

MATERIALS AND METHODS

Subjects. The subjects were adult volunteers, male and female, in apparent good health and without known or suspected gastrointestinal disease. They agreed to participate after the nature and purpose of the study were carefully explained. No individual with a history of reactions related to nickel sensitivity was included in the study. Experiments were performed in accordance with the Declaration of Helsinki and the protocol were approved by the Committee on the Use of Humans as Experimental Subjects of the Massachusetts Institute of Technology.

Plasma nickel determinations. Blood samples (6 ml) were drawn before and hourly for 4 hours after administration of the dose of nickel. Venous blood was sampled with plastic syringes and stainless-steel needles, and collected in plastic tubes (Falcon® tubes, Division of Becton, Dickinson & Co., Oxnard, CA) containing 50 μl of 20% potassium oxalate. The whole blood was centrifuged and

250 ml of coffee prepared with 3 g of coffee powder (Café Incasa-instantaneo, Industrias de Café, S. A., Guatemala City, Guatemala), lightened with non-dairy creamer (Coffee-mate®, Carnation Co., Los Angeles, CA), and sweetened with 10 g of sucrose.

Data analysis. To compare the mean rise in plasma nickel concentration at a given interval following the dose of nickel, we used the Student *t* test. Relative standard deviation was calculated as the mean divided by the standard deviation times 100.

RESULTS

Plasma nickel concentration during fasting and after nickel in aqueous solution. The responses of plasma nickel after ingestion of 5 mg of elemental nickel, as 22.4 mg of $NiSO_4 \cdot 6H_2O$ in water, was monitored over 4 hours in fasting subjects (fig. 1), and the means changes with respect to the zero time nickel level were calculated (fig. 2). The stability of plasma nickel concentration in the fasting state was analyzed during 4 hours of continuous fasting after an overnight fast in another group of individuals. For the 1-hour interval, a cluster of samples was contaminated and the data were invalid. Otherwise, plasma nickel concentrations were stable (fig. 2). In a single subject, studied over a 24-hour period, we found that the excursion of plasma nickel and the kinetics of concentration change were nearly identical (fig. 3) to those in the previous report by Spruits and Bongaarts (38).

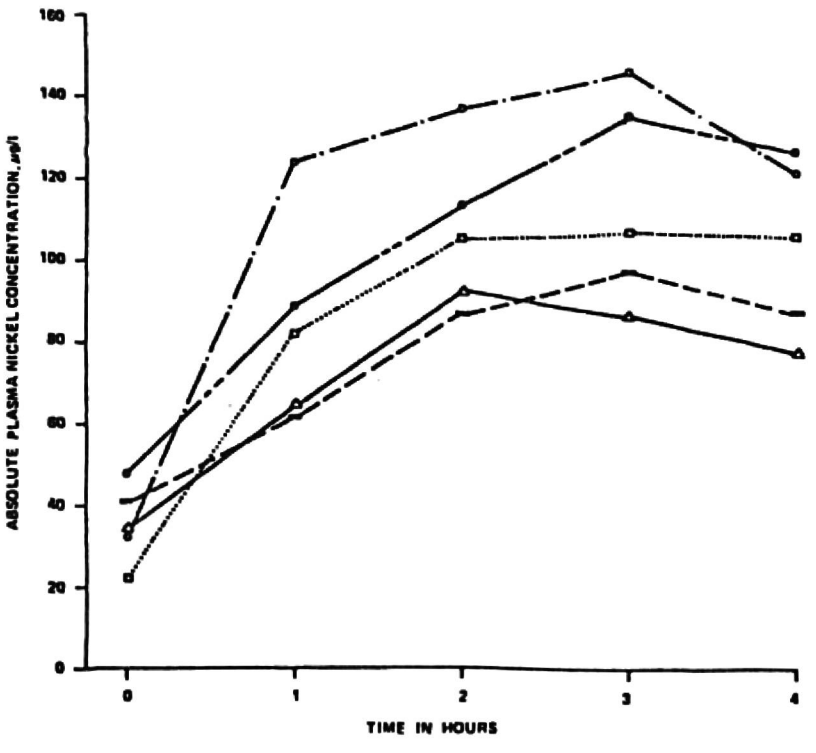

Fig. 1 Individual curves of plasma nickel concentration over 4 hours following a dose of 22.4 mg of $NiSO_4 \cdot 6H_2O$ (5 mg of elemental nickel) in 100 ml of water.

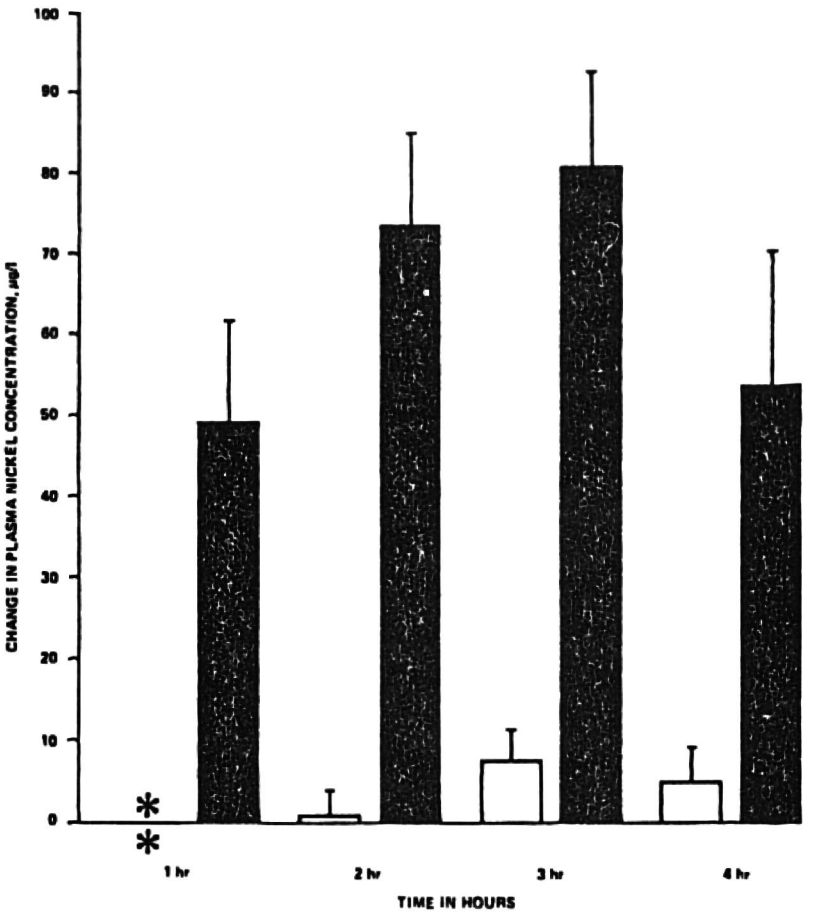

Fig. 2 Change in plasma nickel concentration (mean ± SE) following overnight and continued fasting (open bars) and following ingestion of 22.4 mg of $NiSO_4 \cdot 6H_2O$ (5 mg of elemental nickel) in 100 ml of water (open bars). The number of uncontaminated specimens (‡) at 1 hour was insufficient for inclusion of a mean. Each bar represents five subjects.

Effects of meals on nickel absorption. We measured the effects of two test meals on the absorption of nickel. One meal represented the typical rural diet consumed in Guatemala and the other was a typical North American breakfast. The 22.4 mg of $NiSO_4 \cdot 6H_2O$ were added to the beans and the scrambled eggs in the respective meals. Curves of change in plasma nickel concentration were flat after both meals (fig. 4). The change in plasma nickel at any interval after either meal was not significantly different from the change in fasting subjects. At 1 hour after consumption of the nickel-labeled Guatemalan breakfast, however, insufficient samples were available for inclusion of a mean value.

In five other subjects, plasma nickel concentrations were monitored over the first 4 postprandial hours after consumption of a typical Guatemalan meal *without* added

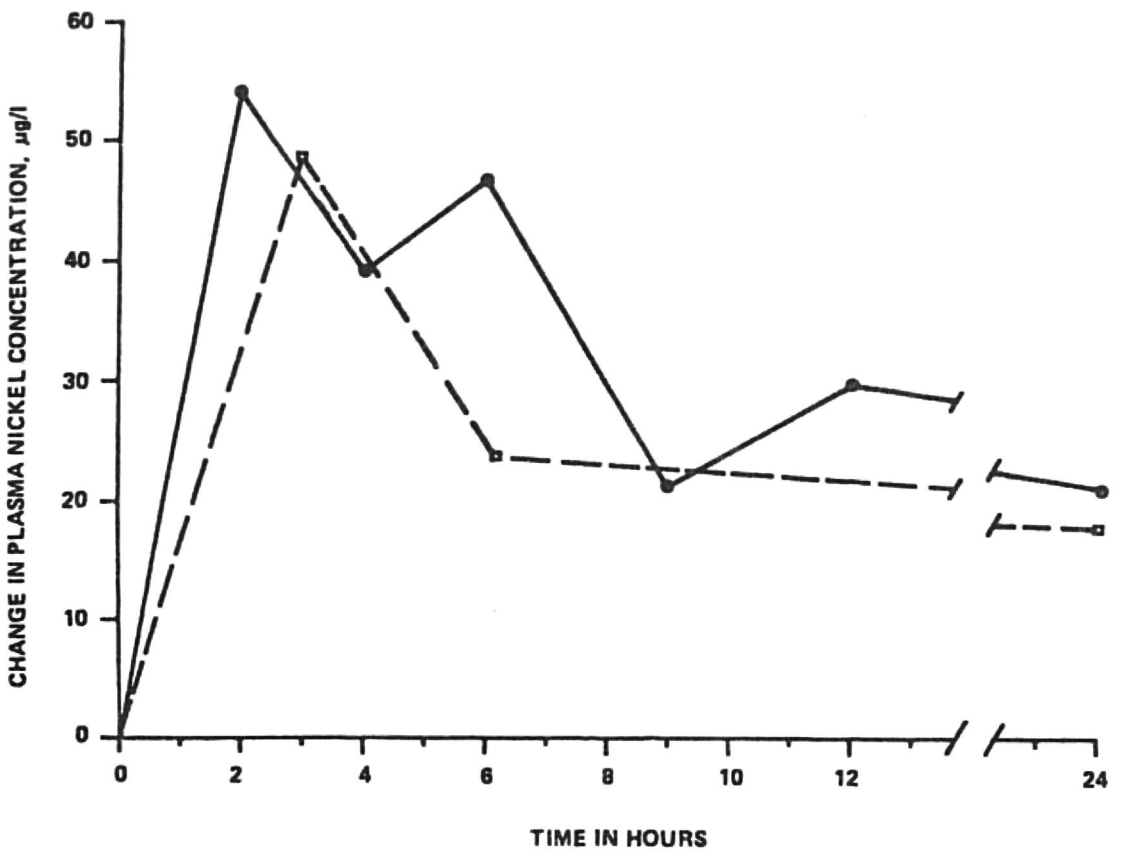

Fig. 3 Plasma nickel concentration at intervals over 24 hours in a single subject following a dose of 22.4 mg of $NiSO_4 \cdot 6H_2O$ (5 mg of elemental nickel) in water. The dotted line represents the subject studied by Spruits and Bongaarts (38). The solid line represents a subject studied in the present study.

nickel. The changes were again similar to those during simple fasting (fig. 4) and no difference was detected between the plasma nickel response to the Guatemalan meal, with or without added nickel.

Effect of common beverages on nickel

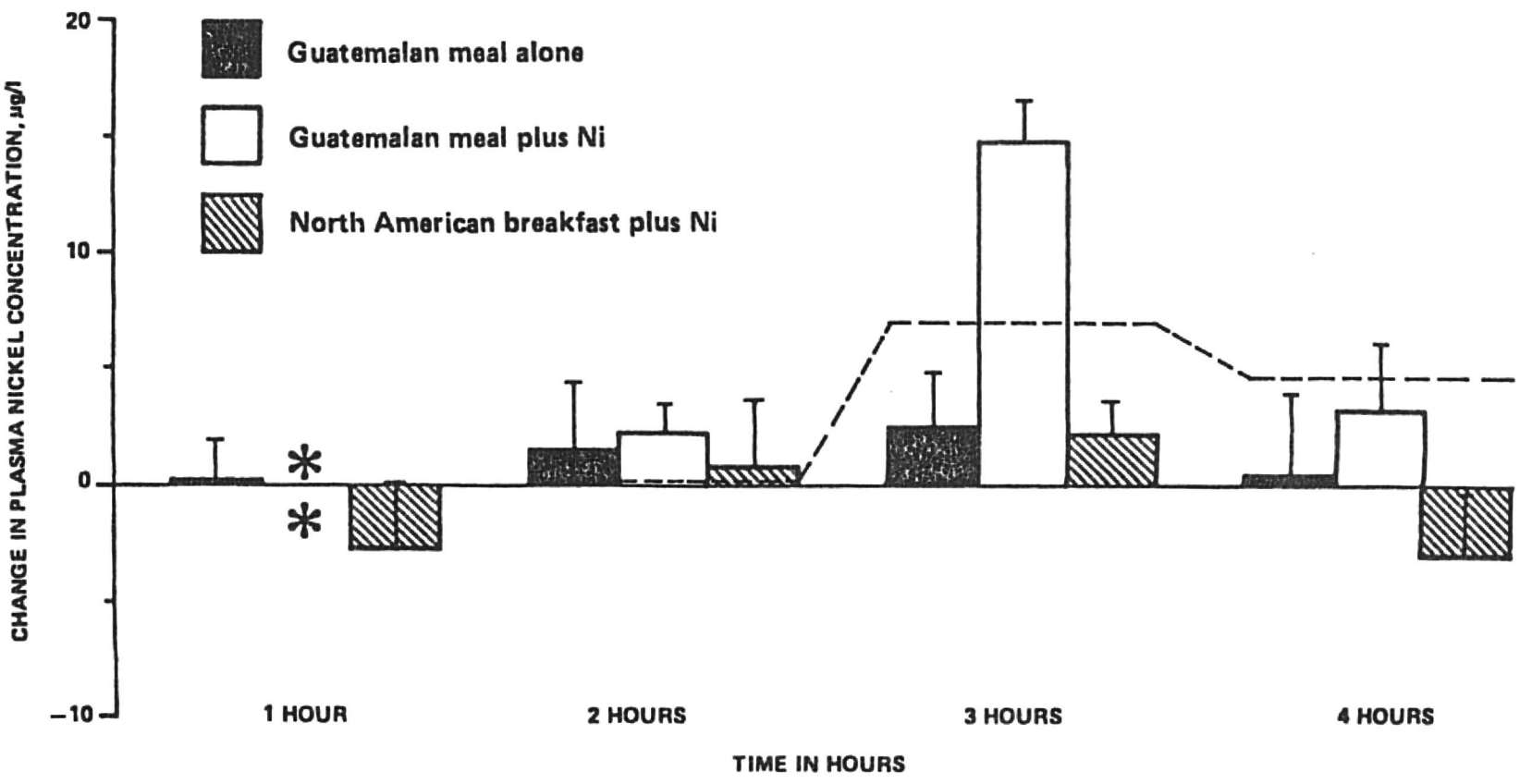

Fig. 4 Change in plasma nickel concentration (means ± SE) at 4 hourly intervals following consumption of a standard Guatemalan meal, with or without 5 mg of nickel, and North American breakfast with 5 mg of nickel. The number of uncontaminated specimens (‡) at 1 hour was insufficient for inclusion of a mean. Dotted line represents the mean of the change in fasting plasma nickel levels. Each bar represents the mean of five subjects.

absorption. When the nickel sulfate was mixed with each of five beverages, plasma nickel levels rose significantly above fasting concentrations at hours 2, 3 and 4 post-ingestion (fig. 5). with respect to the rise in plasma nickel after nickel sulfate in water, tea reduced the elevation at 3 hours ($P < 0.05$), orange juice and coffee at 2 hours ($P < 0.05$) and 3 hours ($P < 0.05$), and milk at 2 hours ($P < 0.025$) and 3 hours ($P < 0.05$). With Coca Cola®, on the other hand, the rise in plasma nickel was not significantly lower at any time interval that the increment produced by the aqueous solution of nickel alone.

Effect of phytic acid and ascorbic acid on nickel absorption. Two chemically-defined dietary substances that affect the absorption of a number of trace elements are phytic acid and ascorbic acid. We tested phytate and nickel in a 2:1 molar ratio by adding 112 mg of phytic acid and 5 mg of nickel to 100 ml of water and allowing it to stand overnight. Phytic acid did not significantly affect plasma nickel (fig. 6). When 1 g of ascorbic acid was added to the standard dose (fig. 6), the rise in plasma nickel was significantly suppressed ($P < 0.05$) at 2 hours and 3 hours, compared to nickel in water.

Effects of NaFeEDTA and $Na_2EDTA \cdot 2H_2O$ on nickel absorption. Uncertainty remains regarding the differential biological behavior of sodium EDTA, a commonly used food preservative, and sodium *iron* EDTA, a substance proposed for iron fortification of foods for humans. When table sugar was fortified with NaFeEDTA at 1 ppm in field trials in four Guatemalan villages, daily consumption was 40 mg (Viteri, F. E. Unpublished datum). For either form of EDTA, 40

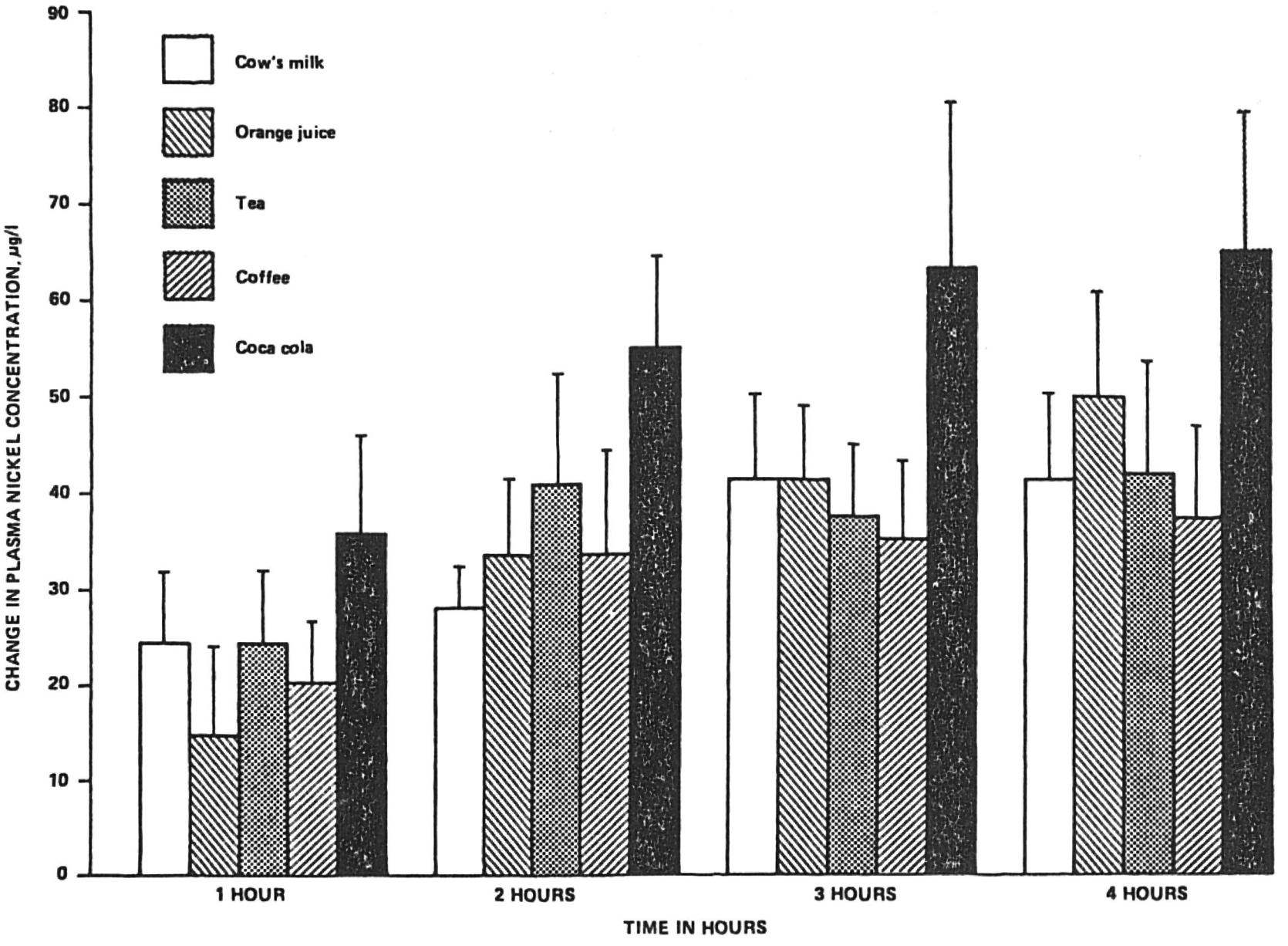

Fig. 5 Change in plasma nickel concentration (means ± SE) at 4 hourly intervals following ingestion of 22.4 mg of $NiSO_4 \cdot 6H_2O$ (5 mg of elemental nickel) in 250 ml of 5 beverages: cow milk; orange juice; tea; coffee; and Coca Cola®. The lower border of the shaded area represents the mean of the changes in plasma nickel level after ingestion of 5 mg of nickel in water. Each bar represents the mean of five subjects.

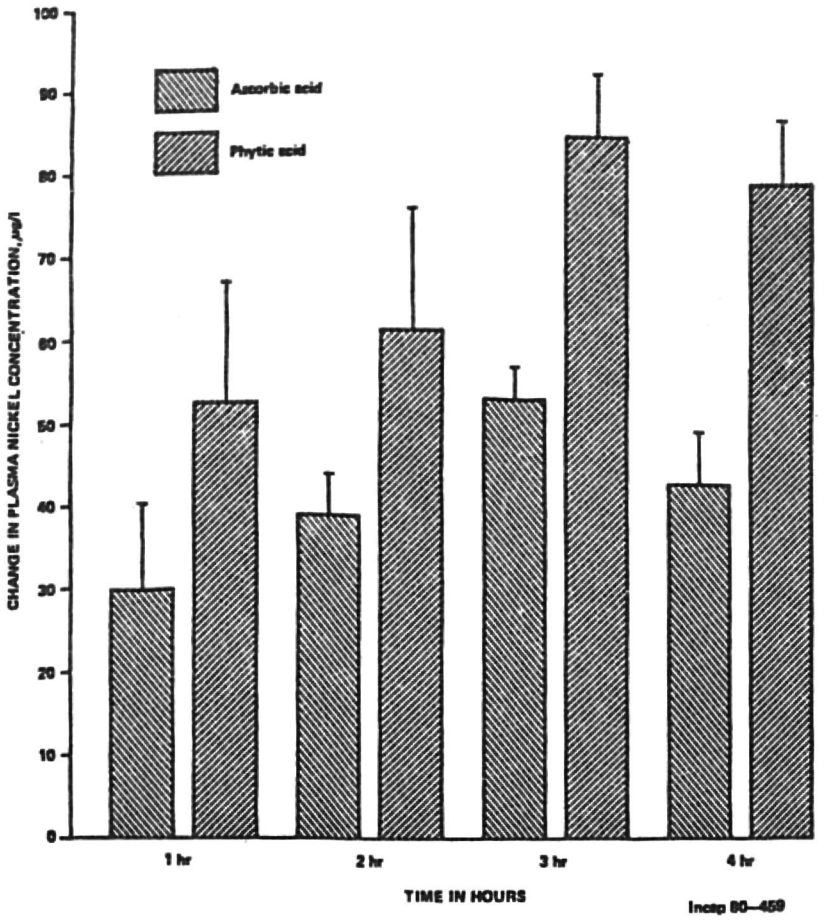

Fig. 6 Change in plasma nickel concentration (means ± SE) at 4 hourly intervals following ingestion of 22.4 mg of $NiSO_4 \cdot 6H_2O$ (5 mg of elemental nickel) with 1 g of ascorbic acid or 112 mg of phytic acid. The lower border of the shaded area represents the mean of the changes in plamsa nickel level after ingestion of 5 mg of nickel in water. Each bar represents the mean of five subjects.

mg represents an EDTA:nickel molar ratio of 1.27:1. We tested each combination in five subjects. The rise in plasma nickel was reduced significantly by NaFeEDTA at the 2-hour interval ($P < 0.05$) and at all hourly intervals by Na_2EDTA. At the third and fourth hours after ingestion of the nickel and Na_2EDTA solution, mean plasma nickel levels were actually significantly lower than the corresponding *fasting* levels ($P < 0.05$).

DISCUSSION

Since the biohazards from radioisotopes of nickel are prohibitive, and the stable isotope tracer technology is not yet established, we exploited the observations of Spruits and Bongaarts (38)–that a 5 mg oral dose of nickel produces a detectable rise in plasma concentration–to develop preliminary observations on factors affecting nickel absorption. This approach is safe and acceptable; none of our subjects exhibited allergic or other adverse reactions or side-effects.

The change-in-plasma-concentration approach was used in early investigations of iron absorption in humans (45–48), but the nutritional requirement of the host for iron was a major determinant variable (49, 50). The same experimental approach has been used more recently in studies of zinc absorption (34, 34, 39–44). In table 1, we compared the variance of the mineral concentrations in plasma between our data for nickel and each of six sets of published data for zinc (24, 29, 42, 43). In terms of relative inter-individual variance, the change-in-plasma-concentration method apparently is not more variable for nickel than for zinc. The analytical determination of plasma nickel, however, is more tedious and complicated than that for other trace elements, because nickel is present at relatively low levels and is much more susceptible to exogenous contamination during handling and processing. In the single

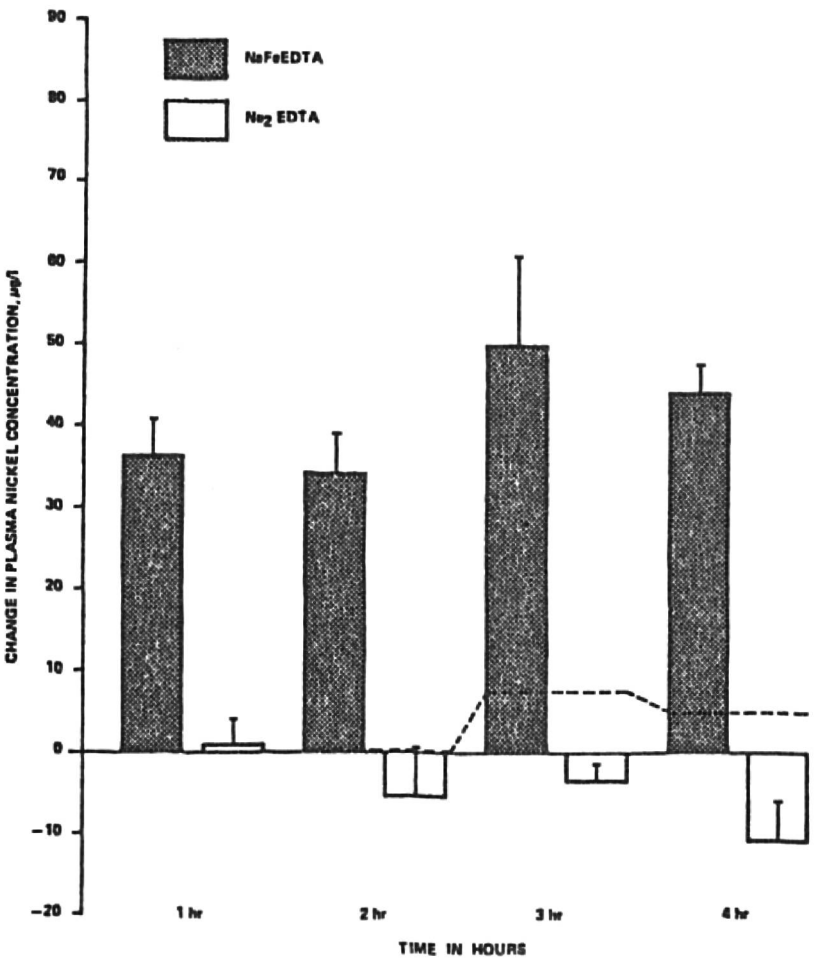

Fig. 7 Change in plasma nickel concentration (means ± SE) at 4 hourly intervals following ingestion of 22.4 mg of $NiSO_4 \cdot 6H_2O$ (5 mg of elemental nickel) with 40 mg of NaFeEDTA or 40 mg of $Na_2EDTA \cdot 2H_2O$ in 100 ml of water. The lower border of the shaded area represents the mean of the changes in plasma nickel level after ingestion of 5 mg of nickel in water. The dotted line represents the mean of the changes in fasting plasma nickel level. Each experiment represents the mean of five subjects.

TABLE 1

Comparative variance in circulating mineral concentrations after aqueous doses

Aqueous mineral dose	Time post-dose			No. of subjects	Reference source
	2 hours	3 hours	4 hours		
5 mg Ni^{++}	107.0 ± 19.4[a] (18.3%)	115.0 ± 25 (21.7%)	104.0 ± 21.4 (20.5%)	5	Present study
25 mg Zn^{++}	151.4 ± 19.4[b] (12.8%)	135.6 ± 4.8 (3.5%)	120.2 ± 13.1 (10.8%)	5	ref. 34
25 mg Zn^{++}	212 ± 42 (19.8%)	—	—	6	ref. 39
25 mg Zn^{++}	—	195 ± 25 (12.8%)	—	8	ref. 42
50 mg Zn^{++}	280 ± 45 (16.1%)	—	221 ± 68 (30.1%)	16	ref. 43
50 mg Zn^{++}	336 ± 20 (6.0%)	—	—	6	ref. 39
50 mg Zn^{++}	—	239 ± 58 (24.3%)	—	8	ref. 42

[a] Means ± SD for nickel concentration in μg/l. [b] Means ± SD for zinc concentration in μg/dl. The number in parentheses are the relative standard deviations.

subject whose plasma nickel was followed for 24 hours in response to a 5 mg dose of nickel, (fig. 3) the curve resembled that recorded by Spruits and Bongaarts (38). Plasma clearance, however, apparently differs between nickel and zinc. Whereas both minerals reached their maximum plasma increments between 2 and 3 hours postingestion, zinc generally returned to baseline values by 6 hours (34) while nickel required more than 24 hours (fig. 3).

The same qualifications previously noted for zinc (34, 44) apply to our data for nickel. These reservations are *a*) the tracer dose is outside of the physiological range; *b*) nickel clearance and enterohepatic circulation or its internal redistribution may affect the curves in unknown ways; and *c*) the change-in-plasma-concentration method probably measures the *rate* of nickel uptake, rather than net absorption. Nonetheless, consistent and differential information was produced in different experimental situations with this method. We consider it reasonable to assume for nickel, as well as for zinc (34), that the area under the discontinuous curve of plasma concentration reflects intestinal absorption of the element.

Background data were not available on nickel bioavailability in man, so we devised treatments based on experience with other divalent trace minerals–iron, zinc, and copper–for which findings regarding intestinal absorption have recently proliferated. The absorption of the aforementioned elements can be depressed by binding- or chelating-substances, competitive inhibitors, or redox reagents; absorption is often enhanced by substances that improve pH, solubility, or oxidation, or by such chelating agents (e.g. amino acids) which are actively absorbed. Those compounds are found, alone or in combination, in the various beverages and meals tested in the present study.

Orange juice contains three potential factors that might affect trace mineral absorption: ascorbic acid, citric acid, and pectins. Tea, and to a lesser extent coffee, contain tannins. Tea is a potent inhibitor of iron absorption (51, 52) and coffee inhibits absorption of iron (31) and zinc (34, 36, 42). Cow milk inhibits the absorption of iron (53) and zinc (39, 54); the constituents of bovine milk that were implicated as inhibitors of trace element absorption are calcium, inorganic phosphates, and proteins. In the present

study, all four of these beverages limited nickel uptake by roughly the same amount. Finally, Coca Cola®, despite its high content of carbonates and phosphates, apparently does not affect the absorption of iron or zinc, and has, in fact, been used as a vehicle for the administration of tracer doses of these minerals in human experiments (28, 55). Coca Cola® was the only one beverage studied that did not inhibit the absorption of nickel.

On the basis of in vitro chemical studies (27), Nielsen (28) postulated that phytic acid might inhibit nickel absorption. The Guatemalan diet is extraordinarily rich in phytate as well as dietary fiber and calcium, all potential inhibitors of the absorption of divalent cations (34). The test meal used in the present study is identical to that used previously (31, 34), except that the 40 g of sweet roll was eliminated. Like iron (31) and zinc (34, 35), nickel was markedly inhibited in its intestinal absorption by the typical, rural, Guatemalan diet.

Two additional observations suggest that dietary components other than phytates are responsible for the reduction in nickel absorption. First, the representative North American breakfast of eggs, bacon, toast, margarine and coffee–relatively poor in fiber, phytates, and calcium, but rich in lipids and nitrates–dramatically reduced the increment in plasma nickel. Second, in marked contrast to the experience of Pecoud et al. (39) with zinc and phytic acid in aqueous solution, in which a 1:6 phytate:mineral ratio produced a 40% reduction in plasma zinc elevation, phytate:nickel molar ratio of 2:1 in aqueous solution here had no effect on nickel uptake. In fact, phytic acid proved to be the only chemically-defined dietary constituent without inhibitory effects. Several investigators (34, 39, 56–58) showed that circulating zinc concentration declined progressively after meals, but we found no evidence for a similar postprandial fall in plasma nickel levels after a standard, unlabeled meal.

Ascorbic acid (AA), a natural dietary reducing- and acidifying-agent, profoundly elevated the absorption of inorganic (nonheme) iron (59, 60), but depressed the absorption of dietary copper (61). AA did not affect the absorption of inorganic zinc or cobalt (44). We found a depression of nickel absorption in the presence of 1 g of AA. The Ni:AA molar ratio in the present study is 1.5×10^{-3}. If we assume that an ordinary breakfast contains between 100 and 200 μg of nickel (15), and that 8 oz. of orange juice contains 100 mg of AA (62), the Ni:AA ratio in our experimental meal would exceed that in a routine breakfast. The nickel:ascorbate interaction should be examined throughout a wide range of Ni:AA molar ratios to determine the nutritional significance of dietary AA on the bioavailability of nickel.

Our interest in NaFeEDTA stems from the proposal for its use as an iron-fortifying agent for the people of Central America (31, 34). The EDTA, itself, reduced the absorption of iron (63). When added to animal feeds, however, EDTA improved the bioavailability of zinc for poultry (64–68) and rats (69). NaFeEDTA is an effective agent for iron fortification (31, 70–72) and, in doses of 15 and 40 mg, did not interact with a 25 mg dose of zinc (34). When we tested the general safety of NaFeEDTA for man by adding 40 mg to the 5 mg dose of nickel, nickel absorption was suppressed only at 2 hours. The $Na_2EDTA \cdot 2H_2O$, however, not only completely attenuated plasma nickel increments, but also caused a net *decrement* in circulating nickel levels at 3 and 4 hours. Although EDTA is commonly used as a preservative in foods, the precise relevance of this finding is not immediately evident. The fact that the competitive action of NaFeEDTA is apparently distinct from that of the iron-free chelate, however, reinforces our notion of the relative safety of NaFeEDTA as an iron-fortifying agent for humans.

Since chemical analyses of human diets consumed in the U. S. have indicated that the total nickel content approximates the theoretical requirement, the major determinant of its nutritional adequacy would be its bioavailability. Additional questions remain regarding other dietary constituents and possible competitive interactions with other trace minerals. The present research, however, permitted a preliminary investigation of dietary factors influencing the absorption of inorganic nickel by man, and set the stage for a more complete elucidation of factors

affecting the bioavailability of nickel in the human diet. Moreover, the present study was conducted with pharmacological doses of inorganic nickel. Experimental evaluation of the absorption of *physiological* doses of nickel must await developments in stable isotope technology. Little is known about the actual chemical forms of nickel in various foods or whether dietary nickel has distinct "organic" forms with enhanced bioavailability analogous to those of iron (73) and chromium (74). If so, the interpretation of the results requires additional qualifications.

LITERATURE CITED

1. Nielsen, F. H., Myron, D. R., Givand, S. H., Zimmerman, T. J. & Ollerich, D. A. (1975) Nickel deficiency in rats. J. Nutr. *105*, 1620–1630.
2. Schnegg, A. & Kirchgessner, M. (1975) Zur Essentialität von Nickel für Tierische Wachstum. Z. Tierphysiol. Tierernähr g u Futtermittelkde *36*, 63–74.
3. Schnegg, A. & Kirchgessner, M. (1975) Veränderungen des Hamoglobingehaltes, der Erythrozytensahl und des Hamatokrits bei Nickelmangel. Nutr. Metab. *19*, 268–278.
4. Nielsen, F. H., Myron, D. R., Givand, S. H. & Ollerich, D. A. (1975) Nickel deficiency and nickel-rhodium interaction in chicks. J. Nutr. *105*, 1607–1619.
5. Anke, M., Grün, M., Dittrich, G., Groppel, B. & Hennig, A. (1974) Low nickel rations for growth and reproduction in pigs. In: Trace Element Metabolism in Animals (Hoekstra, W. G., Suttie, J. W., Ganther, H. E. & Mertz, W., eds.) Vol. 2, pp. 715–718, University Park Press, Baltimore.
6. Anke, M., Hennig, A., Grün, M., Partschefeld, M., Groppel, B., & Ludke, H. (1977) Nickel-ein essentialles Spurenelement. Arch. Tierernährung *27*, 25–38.
7. Spears, J. W., Hatfield, E. E., Forbes, R. M. & Koenig, S. E. (1978) Studies on the role of nickel in the ruminant, J. Nutr. *108*, 313–320.
8. Nielsen, F. H., Zimmerman, T. J., Collings, M. E. & Myron, D. R. (1979) Nickel deprivation in rats: nickel-iron interaction. J. Nutr. *109*, 1623–1632.
9. Schnegg, A. & Kirchgessner, M. (1976) Zur Interaktion von Nickel mit Eisen, Kupfer und Zink. Arch. Tierernährung *26*, 543–549.
10. Spears, J. W., Hatfield, E. E. & Forbes, R. M. (1978) Interrelationship between nickel and zinc in the rat. J. Nutr. *108*, 307–312.
11. Spears, J. W., Hatfield, E. E. & Forbes, R. M. (1977) Nickel-copper interrelationship in the rat. Proc. Soc. Exp. Biol. Med. *156*, 140–143.
12. Sunderman, F. W., Jr., Nomoto, S. & Nechay, M. (1971) Nickel metabolism in myocardial infarction. 2. Measurement of nickel in human tissues. In: Trace Substances in Environmental Health. IV. Hemphill, D. D., ed.) Vol. 4, pp. 352–356, University of Missouri Press, Columbia.
13. Casey, C. E. & Robinson, M. F. (1978) Copper, manganese, zinc, nickel, cadmium, and lead in human foetal tissues. Br. J. Nutr. *39*, 639–646.
14. Schroeder, H. A. & Nason, A. P. (1971) Trace-element analysis in clinical chemistry. Clin. Chem. *17*, 461–474.
15. Schroeder, H. A., Balassa, J. J. & Tipton, I. H. (1962) Abnormal trace metals in man: nickel. J. Chronic Dis. *15*, 51–65.
16. Furr, A. K., MacDaniels, L. H., St. John, L. E. Jr., Gutenmann, W. H., Pakkala, I. S. & Lisk, D. J. (1979) Elemental composition of tree nuts. Bull. Environm. Contam. Toxicol. *21*, 392–396.
17. Thomas, B., Roughan, J. A. & Watters, E. D. (1974) Cobalt, chromium, and nickel content of some vegetable foodstuffs. J. Sci. Food Agr. *25*, 771–776.
18. Evans, W. H., Read, I. & Lucas, B. E. (1978) Evaluation of a method for determination of total cadmium, lead, and nickel in foodstuffs using measurement by flame atomic absorption spectrophotometry. Analyst *103*, 580–594.
19. Dencker, I., Fristedt, B. & Ovrum, P. (1970) Jarn, kopper, krom, nickel, bly och kobolt i föda. Läkartidningen *68*, 4027–4040.
20. Ellen, G., Van den Bosch-Tibbesma, G. & Douma, F. F. (1978) Nickel content of various Dutch foodstuffs. Z. Lebensm. Unters. Fursh. *166*, 145–147.
21. Rudzki, E. & Grzywa, Z. (1977) Exacerbation of nickel dermatitis by margarine. Contact Derm. *3*, 344.
22. Myron, D. R., Zimmerman, T. J., Shuler, T. R., Klevay, L. M., Lee, D. E. & Nielsen, F. H. (1978) Intake of nickel and vanadium by humans. A survey of selected diets. Am. J. Clin. Nutr. *31*, 527–531.
23. Nielsen, F. H. (1980) Evidence for the essentiality of arsenic, nickel, and vanadium and their possible nutritional significance. In: Advances in Nutritional Research (Draper, H. H., ed.) vol. 3, pp. 137–172, Plenum, New York.
24. Kaaber, K., Veien, & Tjell, J. C. (1978) Low nickel diet in the treatment of patients with chronic nickel dermatitis. Brit. J. Dermatol. *98*, 197–201.
25. Christensen, O. B. & Möller, H. (1975) External and internal exposure to the antigen in the hand eczema of nickel allergy. Contact Derm. *1*, 136–141.
26. Horak, S. E. & Sunderman, F. W. (1972) Fecal nickel excretion by healthy adults. Clin. Chem. *19*, 429–430.
27. Vohra, P., Gray, G. A. & Kratzer, F. H. (1965) Phytate-acid-metal complex. Proc. Soc. Exp. Biol. Med. *120*, 447–449.
28. Nielsen, F. H. (1976) Newer trace elements and possible application in man. In: Trace Elements in Human Health and Disease. Essential and Toxic Elements. (Prasad, A. S., ed.) vol. 2, pp. 379–399, Academic Press, New York.
29. Sayer, M. H., Lynch, S. R., Jacobs, P., Charlton, R. W., Bothwell, T. H., Walker, R. B. & Mayet, F. (1973) The effects of ascorbic acid supplementation on the absorption of iron in maize, wheat, and soya. Br. J. Haematol. *24*, 209–218.
30. Cook, J. D. (1977) Absorption of food iron. Federation Proc. *36*, 2028–2032.
31. Viteri, F. E., García-Ibáñez, R. & Torún, B. (1978)

Sodium iron NaFeEDTA as an iron fortification compound in Central America. Absorption studies. Am. J. Clin. Nutr. *31*, 961–971.

32. Reinhold, J. G., Faraji, B., Abadi, P. & Ismail-Beigi, F. (1976) Decreased absorption of calcium, magnesium, zinc and phosphorus by humans due to increased fiber and phosphorus consumption as wheat bread. J. Nutr. *106*, 493–503.
33. Ismail-Beigi, F., Reinhold, J. G., Faraji, B. & Abadi, P. (1977) The effect of cellulose added to diets of low and high fiber content upon the metabolism of calcium, magnesium, zinc and phosphorus by man. J. Nutr. *107*, 510–518.
34. Solomons, N. W., Jacob, R. A., Pineda, O. & Viteri, F. E. (1979) Studies on the bioavailability of zinc in man. Effects of the Guatemalan rural diet and of the iron-fortifying agent, NaFeEDTA. J. Nutr. *109*, 1519–1528.
35. Solomons, N. W., Jacob, R. A., Pineda, O. & Viteri, F. E. (1979) Studies on the bioavailability of zinc in man. 2. Absorption of zinc from organic and inorganic sources. J. Lab. Clin. Med. *94*, 335–343.
36. Onkelinx, C., Becker, J. & Sunderman, F. W. Jr. (1973) Compartmental analysis of the metabolism of ^{63}Ni(II) in rats and rabbits. Res. Commun. Chem. Pathol. Pharmacol. *6*, 663–675.
37. Onkelinx, C. (1977) Whole-body kinetics of metal salts in rats. In: Clinical Chemistry and Chemical Toxicology of Metals (Brown, S. S., ed.) pp. 37–40, Elsevier, North Holland, Amsterdam.
38. Spruits, D. & Bongaarts, P. J. M. (1977) Nickel content of plasma, urine, and hair in contact dermatitis. Dermatologica *154*, 291–300.
39. Pecoud, A., Donzel, P. & Schelling, J. L. (1975) Effects of foodstuffs on the absorption of zinc sulfate. Clin. Pharmacol. Ther. *17*, 469–474.
40. Schelling, J. L., Muller-Hess, S. & Thonney, F. (1973) The effect of food on zinc absorption. Lancet 2, 968–969.
41. Andersson, K. E., Bratt, L., Dencker, H. & Lanner, E. (1976) Some aspects of intestinal absorption of zinc in man. Eur. J. Clin. Pharmacol. *9*, 423–428.
42. Oelshlegel, F. J. & Brewer, G. J. (1977) Absorption of pharmacological doses of zinc. In: Zinc Metabolism: Current Aspects in Health and Disease (Brewer, G. J. & Prasad, A. S., eds.), pp. 299–311, Alan R. Liss, Inc., New York.
43. Sullivan, J. F., Jetton, M. & Burch, R. E. (1978) A zinc tolerance test. J. Lab. Clin. Med. *93*, 485–492.
44. Solomons, N. W., Jacob, R. A., Pineda, O. & Viteri, F. E. (1979) Studies on the bioavailability of zinc in man. 3. Effects of ascorbic acid on zinc absorption. Am. J. Clin. Nutr. 32, 2495–2499.
45. Crawley, J. (1952) Iron absorption test in anaemia: the use of intravenous iron preparations. Edinburgh Med. J. *59*, 478–497.
46. Henley, E. D., Christenson, W. N., Grace, W. J. & Wolff, H. (1956) Absorption of iron from the gastrointestinal tract. Am. J. Clin. Nutr. *4*, 609–618.
47. Joseph, H. (1958) Absorption of iron as a problem in human physiology. Blood *13*, 1–54.
48. Hallberg, L. & Sovell, L. (1960) Absorption of a single dose of iron in man. Acta Med. Scand. (Suppl. 358) *168*, 19–42.
49. Gross, S. J., Stuart, M. J., Swender, P. T. & Oski, F. A. (1976) Malabsorption of iron in children with iron deficiency. J. Pediat. *88*, 795–799.
50. Massa, E., MacLean, W. C. Jr., de Romaña, G. L., de Martinez, Y. & Graham, G. G. (1978) Oral iron absorption in infantile protein-energy malnutrition. J. Pediat. *93*, 1045–1049.
51. Disler, P. B., Lynch, S. R., Charlton, R. W., Torrance, J. D., Bothwell, T. W., Walker, R. B. & Mayet, F. (1975) The effect of tea on iron absorption. Gut *16*, 193–200.
52. de Alarcon, P. A., Donavan, M. E., Forbes, G. B., Landaw, S. A. & Stockman, J. A. III. (1979) Iron absorption in the thalassemia syndrome and its inhibition by tea. N. Engl. J. Med. *300*, 5–8.
53. Schulz, J. & Smith, N. J. (1958) A quantitative study of the absorption of food iron in infants and children. Am. J. Dis. Child. *45*, 109–119.
55. Solomons, N. W. & Jacob, R. A. (1981) Studies on the bioavailability of zinc in man. Effect of heme and nonheme iron on the absorption of inorganic zinc. Am. J. Clin. Nutr. *34*, 475–481.
56. McBean, L. & Halsted, J. A. (1969) Fasting versus postprandial plasma zinc level. J. Clin. Pathol. 22, 623.
57. Hetland, O. & Brubakk, E. (1973) Diurnal variation in serum zinc concentration. Scand. J. Clin. Lab. Invest. 32, 225–226.
58. Walker, B. E., Dawson, J. B., Keller, J. & Losowsky, S. (1973) Plasma and urinary zinc in patients with malabsorption syndrome and hepatic cirrhosis. Gut *14*, 943–948.
59. Brise, H. & Hallberg, L. (1962) Effect of ascorbic acid on iron absorption. Acta Med. Scand. (Suppl 376) *171*, 51–58.
60. Monsen, E. R., Hallberg, L., Layrisse, M., Hegsted, D. M., Cook, J. D., Mertz, W. & Finch, C. A. (1978) Estimation of available dietary iron. Am. J. Clin. Nutr. *31*, 134–141.
61. Van Campen, D. & Gross, E. (1968) Influence of ascorbic acid on the absorption of copper by rats. J. Nutr. *95*, 617–622.

61. Tablas de Composición de Alimentos por Uso en América Latina (1961) p. 16, Institute of Nutrition of Central America and Panama, Guatemala City.

63. Cook, J. D. & Monsen, E. R. (1976) Food iron absorption in man. 2. The effect of EDTA on absorption of dietary nonheme iron. Am. J. Clin. Nutr. *29*, 614–620.
64. Morrison, A. B. & Sarrett, H. P. (1958) Studies on zinc deficiency in the chick. J. Nutr. *65*, 267–280.
65. Savage, J. E., Yohe, J. M., Pickett, E. E. & O'Dell, B. L. (1964) Zinc metabolism in the growing chick: tissue concentration and effect of phytate on absorption. Poultry Sci. *43*, 420–426.
66. O'Dell, B. L., Yohe, J. M. & Savage, J. E. (1964) Zinc availability in the chick as affected by phytate, calcium and ethylenediamine-tertraacetate. Poultry Sci. *43*, 415–419.
67. Kratzer, F. H., Allred, J. B., Davis, P. N., Marshall,

B. J. & Vohra, P. (1959) The effect of autoclaving soybean protein and the addition of EDTA acid on the biological availability of dietary zinc for turkey poults. J. Nutr. *68*, 313–322.
68. Davis, R. M., Norris, L. C. & Kratzer, F. H. (1962) Interference of soybean protein with the utilization of trace minerals. J. Nutr. *77*, 217–223.
69. Forbes, R. M. & Yohe, J. M. (1960) Zinc requirements and balance studies with the rat. J. Nutr. *70*, 53–57.
70. Garby, L. & Areekul, S. (1974) Iron supplementation in Thai fish-sauce. Ann. Trop. Med. Parasitol. *68*, 467–476.
71. Layrisse, M. & Martinez-Torres, C. (1977) Fe(III)-EDTA complex as iron fortification. Am. J. Clin. Nutr. *30*, 1166–1174.
72. Martinez-Torres, C., Romanao, E. L., Renzi, M. & Layrisse, M. (1979) Fe(III)-EDTA complex as iron fortification. Further studies. Am. J. Clin. Nutr. *32*, 809–816.
73. Hallberg, L. & Björn-Rasmussen, E. (1972) Determination of iron absorption from whole diet—A new two-pool model using two radioiron isotopes given as haem and nonhaem iron. Scand. J. Haematol. *9*, 193–197.
74. Mertz, W., Toepfer, E. W., Roginski, E. E. & Polansky, M. M. (1974) Present knowledge of the role of chromium. Federation Proc. *33*, 2275–2280.