Bioavailability of and Interactions Among Trace Elements

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With the advent of present-day tools and techniques of analysis, it is possible and practical to measure accurately the essential trace element content of human foods. Although this is a major step forward in assessing nutrient needs and status, an important unknown remains. To meet nutritional requirements it is important to know not only the quantity consumed but the proportion of that consumed which is absorbed and utilized. A major thrust of modern nutrition research is concerned with the absorption and utilization of nutrients in foods. The term bioavailability has been applied to this concept. Although bioavailability applies to and is an important consideration for all nutrients, it is particularly pertinent for the trace elements because so many factors affect their absorption and assimilation.

Bioavailability of a specific trace element is affected by intrinsic factors such as the physiological status of the animal or person that consumes it. There are numerous intrinsic factors that significantly affect bioavailability (1), but this chapter is concerned with the extrinsic or dietary factors of importance in the bioavailability of those elements that are of prime concern in human nutrition.

Extrinsic factors that affect bioavailability may be divided into major and minor dietary components. Furthermore, each of these components may be classed as an essential or nonessential nutrient. The minor components tend to interact more specifically with the essential nutrients. The purpose of this review is to describe interactions which determine bioavailability of iron, zinc, copper, and selenium. The reader is also referred to other reviews and symposia (1-6) related to the subject.

DEFINITION OF TERMS

There are major discrepancies in the literature regarding the definition of bioavailability, and this has led to confusion. Some investigators have equated bioavailability with absorption. Even the latter term lacks meaning unless it is qualified as to true or apparent absorption.

If bioavailability refers simply to true absorption, there is no justification for it. With that meaning the term bioavailability duplicates a more precisely defined one. For the purpose of this review the following definitions are used or implied.
Bioavailability is the proportion of a nutrient in food which is absorbed and utilized. By utilization is meant the process of transport, cellular assimilation, and conversion to a biologically active form(s). The utilization component of bioavailability is no doubt more important for some elements than others; for example, selenium consumed as selenite or selenate must be reduced and incorporated into the selenocysteine residue of glutathione peroxidase or other seleno-proteins in order to be fully utilized as a biocatalyst. Although utilization is difficult to measure, it is an important component of the bioavailability concept.

True absorption is the proportion of a nutrient in food which moves from the intestinal lumen and through the mucosal cell into the body. To measure true absorption, the fecal excretion of the nutrient must be corrected for endogenous loss which occurs through intestinal secretions and mucosal cell sloughing. The true absorption of isotopic label can be measured directly.

Apparent absorption is the difference between the nutrient content of the food and of the feces without reference to the immediate origin of the nutrient in the feces. Although apparent absorption makes no correction for endogenous loss by way of the intestines, it gives a useful overview of nutrient balance. It reflects the effect of dietary components on the combination of true absorption and endogenous fecal excretion.

TECHNIQUES FOR MEASURING ABSORPTION AND BIOAVAILABILITY OF TRACE ELEMENTS

Absorption

Few apparent absorption studies have been performed with animals, but this is the most common technique used in human experimentation. It is not possible to determine true absorption without the use of an isotope to aid in evaluating endogenous fecal excretion. Approaches used to measure true absorption are discussed below. Major problems in the use of isotopes to measure absorption relate to the method of adding the label, i.e., whether it is added extrinsically or intrinsically, and the isotopic nature of the label. Table 1 lists the radioactive and stable isotopes of the elements under discussion here.

Extrinsic Versus Intrinsic Isotope Labels

Extrinsic labeling of an element in food for measurement of its absorption is both simpler and less expensive than intrinsic labeling. The latter process involves introduction of the isotope biosynthetically into the food under physiological conditions during growth and development of the food organism. It can then be reasonably assumed that the isotope behaves in a manner entirely analogous to the nutrient element intrinsic to the food. In the case of the extrinsic label, one must demonstrate that the isotope added to the food comes into equilibrium with all pools of the element in the food or at least behaves nutritionally in an entirely analogous manner. Proof of equilibrium is best achieved by double labeling, intrinsically with one isotope and extrinsically with another.
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$^a$Italicics indicate isotopes that have been used experimentally.
$^b$Parentheses indicate natural abundance (%) of each stable isotope. Only those stable isotopes of low abundance (the lower the better but usually <10%) provide good sensitivity. The cost of production increases markedly as natural abundance decreases.

$^c$Radioactive isotopes; physical half-lives are in parentheses.

There appears to be an almost universal nonheme pool of iron which can be uniformly labeled by an extrinsic radioiron salt. The label is absorbed to the same extent and rate as the intrinsic nonheme iron pool (4). It is less well established that extrinsically added zinc salts are absorbed to the same extent and rate as zinc intrinsic to the food. Evans and Johnson (7) found that extrinsic 65Zn added to corn meal mush, cornbread, or rat liver was absorbed similarly to that introduced biosynthetically into similar foods. In one trial with rats involving egg and soy flour, Meyer et al. (8) found no difference in the absorption of intrinsic and extrinsic 65Zn, but in another trial involving soy flour the absorption of the extrinsic label was significantly higher than that of the intrinsic 65Zn. In a study involving human subjects who consumed as a source of protein chicken, soy protein isolate, or a combination of these proteins labeled both intrinsically and extrinsically, absorption of the extrinsic label ranged from 80 to 90% of the intrinsic label (9).

Even less is known about the relative absorption of extrinsic and intrinsic Se. This relationship is more important for Se than in the case of Fe and Zn because the source of extrinsic Se used is frequently selenite. Although the intrinsic form is unknown, it is likely organic in nature. Chicken tissues have been labeled intrinsically (10,11) by administration of Na2SeO3 containing an excess of one of the stable isotopes. It appears feasible to label chicken meat sufficiently to permit human absorption studies with intrinsically labeled Se. Data comparing directly the absorption of intrinsic food Se and extrinsically added Se have not been published.

Radioisotopes as Labels

Radioactive isotopes have been widely used to study absorption and metabolism of trace elements in animals but much less so in man. Radioiron is commonly used in human studies (4). A dose of 1.5 to 5 μCi of 55Fe and/or 59Fe is administered
commonly as an extrinsic (Fe III) label of the nonheme iron pool (12,13). In such studies absorption and utilization are evaluated by the incorporation of radioiron into circulating red blood cells. Another technique involves the use of a whole-body counter. By this technique doses of $^{59}$Fe and $^{65}$Zn as low as 0.1 μCi can be used successfully to determine absorption as measured by retention of total radioactivity (14).

Both $^{65}$Zn and $^{69m}$Zn have been used to determine zinc absorption in man (15,16). Zinc-$^{69m}$ has a short half-life and is not useful for long-term studies. It cannot be used for a 2-week period, as is required by the whole-body-counting technique. Zinc-$^{65}$ is more useful for this purpose, but it subjects the person to longer periods of irradiation. The dose of $^{65}$Zn used has varied from 0.5 μCi per meal (17) to 10 μCi (18). Zinc-$^{69m}$ has permitted investigation of the kinetics of zinc movement among organs (15). Although there is no evidence of detrimental effects of the ionizing radiation which results from the experimental doses used, there is justifiable reluctance to use radioactive isotopes with certain segments of the population, e.g., infants and pregnant women.

**Stable Isotopes as Labels**

Because of the desire to avoid experimental exposure to ionizing radiation, there is increasing interest in the use of stable isotopes to study trace element metabolism in man. Stable isotopes can be used safely as labels in all segments of the population and have the added advantage that there is no decay and hence no time restraint in the experimental design or the analysis. There are some disadvantages. Stable isotopes are extremely expensive and available in limited supply. Analysis requires costly instrumentation and in some cases meticulous and time-consuming analytical procedures. There are three commonly used techniques for analysis: neutron activation analysis (19), thermal ionization spectrophotometry (20), and gas chromatography/mass spectrometry (21). Although these methods differ in precision, extent of sample preparation needed, and analytical facilities available to researchers, it is beyond the scope of this review to examine the pros and cons of the various analytical techniques. More pertinent is consideration of the practical problems inherent in the use of stable isotopes for the study of trace element metabolism. In addition to the disadvantages of cost and analytical difficulty, there is an even more basic problem in the use of a stable isotope as an extrinsic label. The amount of isotope which must be added for accurate analysis is frequently too large to be considered a tracer; it adds significantly to the total quantity of the element and thus may perturb the system. Nevertheless, stable isotopes have been employed in human studies.

Janghorbani et al. (22) used $^{58}$Fe to investigate iron absorption in five men who consumed liquid-formula diets based on soy protein isolate and skim milk. The diet supplied 15 mg of iron per day, and during each of four 2-day collection periods each subject received in addition approximately 0.9 mg of $^{58}$Fe per day as FeCl₃. Excretion of $^{58}$Fe was determined in each stool by neutron activation.
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analysis. Collection of at least five stools was required to recover the administered isotope in all subjects. The mean absorption varied from 15 to 49% among the four dietary periods. These values are uncorrected for endogenous re-excretion of absorbed $^{58}\text{Fe}$, which is probably negligible, and hence closely approximate true absorption. Turnlund et al. (23) used $^{58}\text{Fe}$ and thermal ionization spectrometry to measure iron absorption in elderly men who consumed a formula diet containing a total of 10 mg of iron per day. A solution containing 9.76 mg Fe of which 73.3% was $^{58}\text{Fe}$ was given with the meals. Collection of five 3-day fecal pools was required to completely recover the unabsorbed iron. The $^{58}\text{Fe}$ absorbed averaged 7.9%. This value approximates true rather than apparent absorption of dietary iron; however, there would be little endogenous fecal excretion of $^{58}\text{Fe}$ during this period.

Johnson (24) administered $^{54}\text{Fe}$ and measured its excretion in stools collected over a 6-day period. The adult male subjects consumed conventional foods that supplied 9 to 11 mg of iron per day. The isotope (4 mg) was given orally in glucose solution or in orange juice with breakfast. No attempt was made to equilibrate the isotopes with the other food iron. After ashing and chelate formation, the samples were analyzed by mass spectrometry using electron impact ionization. Absorption of $^{54}\text{Fe}$ varied from 25 to 45%. The apparent absorption of total iron measured directly in these subjects averaged 43%.

There are five stable isotopes of zinc, $^{70}\text{Zn}$ being the least abundant in nature and consequently the most expensive. Experimentally, $^{70}\text{Zn}$ (0.62%), $^{67}\text{Zn}$ (4.1%), and $^{68}\text{Zn}$ (18.6%) have been used to study zinc absorption in man (9,23,24). Endogenous zinc makes up a larger proportion of fecal zinc excretion than in the case of iron. Turnlund et al. (23) administered $^{70}\text{Zn}$ and observed 17.3% absorption, which they referred to as apparent absorption. Under the conditions, the value more closely approximates true absorption of total zinc, as there would be little endogenous $^{70}\text{Zn}$ excreted during the period. There are no rare stable isotopes of copper, but $^{65}\text{Cu}$ (30.9%) has been used to study its absorption in human subjects (24,25). Selenium-74 (0.87%) has been used to determine the absorption of selenite taken orally in aqueous solution (26).

Bioavailability

Although true absorption constitutes a major component of bioavailability, it does not measure utilization or assimilation. Various techniques have been used to obtain an overall measure of bioavailability. Growth rate may be employed as an index when immature animals can be used and the element under study is the only limiting nutrient (1,5). The growth rate of animals fed a food source of the limiting element is compared to that of other animals fed a pure salt of the element. The response may be a single point compared to a standard response curve (27) or the ratio of the slopes of two curves, one being the response to graded levels of the pure element and the other the response to the food source (28,29).

The slope ratio method can be applied to other physiological responses such as hemoglobin production and tissue storage, e.g., bone zinc (28,29) and tissue
selenium (30). An example of the slope ratio method applied to tibia zinc is shown in Fig. 1. Also useful are biochemical parameters such as the response of enzyme activities to graded levels of dietary supplements of pure salts and food sources, e.g., glutathione peroxidase activity in response to dietary selenium levels (31) and liver cytochrome oxidase activity in response to copper (32). Retention of orally administered isotopes over a period of time allows assessment of endogenous loss as well as absorption (14).

Many of the methods used in laboratory animal evaluation of bioavailability are not applicable to human studies. Growth rate response is not possible in adults and cannot be ethically employed with infants. The human tissues available for enzyme assay are limited almost entirely to circulating blood cells; assay of these cells has not been widely used. However, platelet glutathione peroxidase has been employed to evaluate selenium bioavailability in Finnish men (33). This leaves the balance technique as the most widely used tool for determination of trace element bioavailability in man.

Although there are large experimental errors in any chemical balance study, this technique offers one of the most definitive estimates of bioavailability in adult man if performed carefully and with intakes below or near the minimal requirement.

![FIG. 1. Estimation of zinc bioavailability in the rat by use of slope ratios of tibia zinc concentration. These are unpublished data comparing beef and a soy product with ZnCO₃.](image-url)
The balance method has been largely discarded for evaluation of iron absorption (4), but it is still effective for estimating the effect of dietary components on iron (3), zinc (34,35), and copper (34) bioavailability. Few balance data in adult laboratory animals have been published. However, until reliable biochemical indices are found, chemical balance may be the method of choice for evaluating bioavailability in adults of any species.

**EFFECTS OF MAJOR DIETARY COMPONENTS**

**Nutrients**

**Protein and Amino Acids**

That the absorption of iron is enhanced by meat and fish has been demonstrated repeatedly, but the effect is not due to protein per se. Neither egg albumin nor the amino acids found in meat promote iron absorption (4). It is generally accepted that the iron in foods of vegetable origin is less bioavailable than that in animal products (36). The increasing use of soy products in human foods has stimulated interest in their iron bioavailability. Hemoglobin regeneration studies in the rat have shown that the iron in soy protein is as, if not more, available than that in beef, values ranging from 60% (37) to 100% (38) of the iron in FeSO₄. One rat study showed the iron in soy protein to be 70 to 90% as available as that in casein-based diets (39).

Measurements of extrinsic radioiron absorption from single test meals in human subjects have shown dramatic differences due to protein source (40,41). When soy product protein replaced egg albumin protein, the rather low iron absorption was decreased from 65 to 92%. Similar results were obtained when soy replaced beef protein. Iron absorption was increased when the test meal was baked at 200°C and by the addition of ascorbic acid to the meal. Ascorbic acid increased absorption from 0.6 to 3.2%. Addition of meat also had the usual effect of enhancing nonheme iron absorption (41). Whether the “meat effect” is the only factor involved remains to be resolved. It should be pointed out that soy protein contains the metal chelator phytate, which is discussed later. Although the “meat effect” is not adequately explained by the amino acid composition of animal protein, there is evidence that cysteine enhances iron absorption (4). Martinez-Torres et al. (42) showed that the addition of cysteine to vegetable foods, after cooking or at the time of ingestion, doubled the rate of iron absorption. Whereas the addition of cysteine to the diet of growing rats decreased fecal loss of zinc, it had no effect on iron or copper loss (43).

In the study of Snedeker and Greger (43), a high protein diet, 45% lactalbumin, significantly increased the apparent absorption of zinc and its deposition in bone compared to that seen often 15% lactalbumin. A similar effect of high (24.1 g N) versus low (8.1 g N) protein on the apparent absorption of zinc was observed in adult male subjects (44). As in the case of iron, more important than the level of dietary protein is the source of protein. Early animal experiments showed that the
zinc in foods of plant origin, particularly those derived from seeds such as soybean, has low bioavailability (27, 45). Similar observations have been made in human subjects using the balance technique (46). In the latter study there was negative zinc balance when a soy protein diet containing 14 mg of zinc was consumed; the balance was positive when the diet contained 12 mg of zinc of animal origin.

The phytate in seeds is considered to be a major contributor to the low bioavailability of zinc, but it may not be the only negative factor in these foods (27). In addition to the inherent factors, the method of processing soy protein may affect zinc bioavailability (47). Zinc in neutralized soy protein isolate is less available than the acidic product.

Animal studies show that some amino acids affect absorption and utilization of zinc. Amino acids form zinc chelates of varying stability. Cysteine, which forms a stable zinc complex (\(-\log K_d = 18.2\)), improves zinc utilization when added to a soy protein-based diet, and histidine (12.9) decreases the pathology of zinc deficiency (48). Zinc storage in bone is not affected by these amino acids. Addition of arginine to a diet based on casein and egg white decreases tibial zinc (49). It is also clear that synthetic zinc chelators, e.g., ethylenediaminetetraacetate, may be either beneficial or detrimental depending on the stability and solubility of their chelates (50).

Although there is little information concerning the effect of specific proteins on selenium bioavailability, there are differences between foods of plant and animal origin. Cantor et al. (51) found that the selenium in plant products is 60% as effective as selenite Se in the prevention of exudative diathesis in chicks. Selenium of animal product origin is only 25% as effective. Others (52) have found the opposite relationship. Selenium bioavailability studies in the rat show no significant differences between beef kidney and wheat, but the bioavailability in tuna is low (53, 54). Selenomethionine, presumably the major selenium constituent of wheat, is equivalent to selenite (51).

**Carbohydrate**

There are few studies in man that relate trace element absorption to the source of digestible carbohydrate in the diet. There are a few investigations in laboratory animals. Amine and Hegsted (55) found that the absorption of iron by rats is low when starch constitutes 60% of the diet. Absorption is improved substantially when lactose makes up all or even one-third of the carbohydrate component.

Copper absorption and utilization are affected significantly by the source of dietary carbohydrate. Rats fed a low copper diet with sucrose as a source of carbohydrate have lower hepatic and renal copper concentrations than those fed starch with the same dietary level of copper (56). More recent work by this group shows that the detrimental effect of sucrose relates to its fructose moiety (57).

**Vitamins**

Ascorbic acid as a supplement or dietary component affects trace element bioavailability both positively and negatively depending on the element and conditions.
Nonheme iron absorption as measured by an external label added to a single meal is markedly enhanced by ascorbic acid addition. This effect may relate to the reducing property of ascorbic acid as well as its capacity to form soluble iron complexes. A comprehensive review of the related literature has appeared (58). Despite the enhancing effect of ascorbic acid supplementation on iron absorption from a single meal, the long-term iron status of human subjects is not improved by megadoses of ascorbic acid (59).

There is evidence in experimental animals that high levels of dietary ascorbic acid impair copper absorption and utilization. Dietary supplements of ascorbic acid increase the pathology of copper deficiency in the chick (60,61) and, when added with $^{64}$Cu, decrease its absorption from ligated intestinal segments of the rat (62). Increasing the ascorbic acid intake of guinea pigs by a factor of 10 lowers blood and liver copper levels (63).

**Minerals**

Calcium and phosphorus have been implicated in the absorption and utilization of several trace elements, but they are particularly involved with iron and zinc. Calcium, administered in the diet or added to intestinal loops, decreases the absorption of iron in a dose-related manner (64). Phosphates have been reported to decrease iron absorption in experimental animals, but a human study (65,66) indicates that high levels of both calcium and phosphate are required to reduce iron absorption. The calcium and phosphorus content of milk and the phosphoproteins in egg yolk may account for the low bioavailability of iron from these foods (4).

Dietary calcium levels have long been associated with zinc bioavailability, but the detrimental effect of high calcium is dependent on the presence of phytate in the diet (45). Forbes et al. (67) have shown that increasing calcium from 0.4 to 1.2% in the diet of rats lowers zinc bioavailability in soy protein approximately 50% but has little or no effect when egg white supplies all of the protein (Fig. 2). Presumably the difference is due to the phytate in soy protein. Studies with human subjects fed diets based on natural foods relatively low in phytate (68,69) have shown that increasing calcium intake by a factor of 3 or 4 has no effect on zinc balance. Similar observations were made when phosphorus was increased (69).

**Nonnutrient Diet Components**

**Fiber, Lignin, and Tannin**

The effect of dietary fiber on iron absorption is moot. The addition of wheat bran, a fiber-rich product, to bread lowers iron absorption, but it is not clear whether this is due to phytate, fiber, or some other component. Dephynitized wheat bran inhibits iron absorption in human subjects to approximately the same extent as the untreated bran (70). However, the insoluble high-fiber fraction of the dephytinized bran is less inhibitory than a soluble low-fiber fraction. Although the fiber-rich components of corn and wheat bind iron in vitro (71), only lignin and
FIG. 2. Effect of calcium and protein source interaction on zinc bioavailability as measured by growth rate in rats. The egg white protein contained no phytate, whereas the tofu contained approximately 0.5% phytate phosphorus and had a phytate/zinc molar ratio of approximately 30. The single asterisk indicates a significance between 0.4 and 0.7% Ca, and the double asterisk indicates significance between 0.7 and 1.2% Ca. (From Forbes et al., ref. 67. Reproduced with permission.)

mucilage are potent inhibitors of iron absorption, as measured by uptake from dog intestinal loops; cellulose is without effect (72).

The effect of dietary fiber on zinc bioavailability has been studied extensively, but the results make conclusions equivocal. Dietary fiber studies were initiated by Ismail-Beigi and colleagues in Iran (73). They observed that consumption of 10 g of cellulose as filter paper reduced the zinc balance in human subjects. Others have found that cellulose has no effect (74) or that cellulose and hemicellulose reduce zinc balance in omnivores but not in vegetarians (75). The effect of dietary fiber has been reviewed by Davies (76), who was unable to draw firm conclusions relative to the effect of fiber on zinc bioavailability.

The tannins in tea and coffee have a highly detrimental effect on iron absorption (4). These beverages have been studied by Morck et al. (77). They found that a cup of coffee reduces iron absorption from a hamburger meal by 39%, and a cup of tea reduces it by 64%.
**Phytate, Oxalate, and Synthetic Chelators**

Phytate has been the most widely studied dietary component related to trace element bioavailability. Several investigations have shown that phytate decreases iron absorption in man (4). However, the iron of monoferric phytate, the major iron species in wheat bran, is highly absorbed by dogs (78) and rats (79). Phytate occurs in oil seed proteins at levels of 1 to 3%, commonly at 1.5% in isolated soy protein. Although this level of phytate may contribute to the lower iron bioavailability observed in soy protein (4,40,41), as discussed above, this point is not clear and deserves further research. Removal of phytate from soy protein does not improve iron absorption (13).

O'Dell and Savage (80) first demonstrated that phytate decreases zinc bioavailability in experimental animals. This observation has been widely repeated (5), but there are still limited data directly related to man. Clearly, the zinc in seed proteins is less available than that in animal proteins (46), but this correlates only in part with the phytate content of the vegetable products. The effect of phytate on zinc bioavailability is probably best defined by the phytate/zinc molar ratios in the food or diet (81–83). In any case, the detrimental effect of phytate is accentuated by high levels of dietary calcium (67,82–84) and counteracted to a degree by synthetic chelating agents such as ethylenediaminetetraacetate (84,85).

Whereas oxalic acid, either as the sodium salt or as it occurs in spinach, has no detrimental effect on zinc bioavailability in rats (86), human studies show that foods rich in both fiber and oxalic acid, e.g., spinach, decrease the zinc balance (87).

**EFFECTS OF MINOR DIETARY COMPONENTS**

**Interactions of Essential Trace Elements**

Considerable evidence has accrued to show that dietary excess of one essential trace element may have a detrimental effect on one or more of the other essential elements, particularly if the latter is present at a minimal level. Under these conditions deficiency signs of the limiting element are induced, and higher than normal levels are required to meet nutritional needs. Thus an excess of one element may decrease the bioavailability of another. On the other hand, deficiency of one trace element may impair the absorption of another; e.g., copper deficiency impairs iron absorption. The latter effect is referred to as a positive interaction. Only negative interactions—those in which bioavailability is decreased by an excess of another element—are discussed here. An extensive review of mineral interrelationships has been published (88), and Fig. 3 depicts some of the commonly recognized interactions.

**Interactions with Iron**

Intermediate to high levels of manganese interfere with iron metabolism as shown by decreased hemoglobin concentrations and reversal by dietary iron supplemen-
FIG. 3. Trace element interactions of significance in decreasing the bioavailability of iron, copper, zinc, and selenium. This diagram depicts negative interactions which arise among essential trace elements, as well as those between nonessential elements and the essential ones discussed here. Essential elements are indicated in squares and nonessential elements in solid-outline circles. Cobalt is essential only as a component of vitamin B₁₂. Various forms of sulfur (broken circle) interact with Se and, in combination with Mo, decrease Cu bioavailability. Broken lines indicate minor or ill-defined effects.

Interactions with Manganese

Manganese as well as iron is more efficiently absorbed by man and rats that are iron-deficient (89,90).

High levels of zinc also decrease iron bioavailability, but it is not entirely clear whether this is a direct effect or is mediated indirectly through the copper effect on iron metabolism. Increasing the zinc intake of adolescent girls from 11.5 to 14.7 mg per day decreased iron balance from 1.4 to 1.0 mg per day (93). This occurred when the subjects consumed about 30 mg of iron and 1.2 mg of copper per day. This copper level is minimal but not sufficiently low to result in overt deficiency even with the higher zinc level. In support of a direct interaction is the observation that, measured by the load test, zinc absorption is reduced by nonheme iron (94). More data are needed to establish that a direct zinc–iron interaction decreases iron bioavailability.

Interactions with Zinc

As mentioned above, iron and zinc interact at the level of the intestinal mucosa, and zinc absorption is impaired by iron (94). In a subsequent study (95), it was found that ferrous iron inhibits zinc absorption more than does ferric iron. Surprisingly, addition of ascorbic acid to the ferric iron dose decreased rather than increased the effect.

There is a strong interaction between zinc and copper. Although the nutritional effect of excess zinc on copper metabolism is much stronger than vice versa, there is evidence that excess copper decreases zinc absorption. Copper inhibits $^{65}$Zn absorption from isolated rat intestinal segments maintained in situ (96). Similar studies show that 1 μM Cu$^{2+}$ decreases $^{65}$Zn absorption in the intestine of zinc-supplemented but not of deficient rats (97). In a factorial study (98) designed to investigate copper–zinc interrelations, 2 ppm Cu$^{2+}$ in drinking water resulted in lower serum and kidney zinc levels than did 1 ppm Cu$^{2+}$ when the water contained 25 ppm zinc. There was no effect at higher zinc levels. Although the data suggest that excess copper deters zinc absorption, the effect is minimal and probably of little practical significance.
Interactions with Copper

The copper–zinc interaction is expressed most dramatically when dietary copper is limiting and zinc is in excess. In fact, it is one of the best defined interactions which results in decreased bioavailability. Not only does zinc interfere with copper absorption from intestinal segments (99), it also induces definite signs of copper deficiency (100–102). That the zinc–copper interaction has physiological significance in human nutrition is shown by certain studies (93,103). Hypocupremia and anemia developed in a sickle cell anemia patient treated with zinc for 2 years, and copper therapy reversed the effect, increasing the hemoglobin and the hematocrit (103).

Interaction of Nonessential with Essential Trace Elements

No attempt is made here to define the essential trace elements for man, but the following discussion is concerned with the effects of those elements which are generally recognized as nonessential. Only their effects on the bioavailability of essential elements is considered.

Interactions with Iron

There is substantial evidence that cadmium interacts with iron to decrease its bioavailability. Hill et al. (104) added Cd to chick diets limiting in iron and copper. Cadmium at levels of 25 to 400 ppm decreased growth rate and hemoglobin levels and elevated the mortality rate. Iron supplementation ameliorated the Cd effect by decreasing mortality and increasing the growth rate. Supplementation of a rat diet with 400 ppm Fe had a marked effect in preventing the anemia induced by 100 ppm Cd (105). Japanese quail fed a diet containing 75 ppm Cd and 100 ppm Fe developed severe anemia and exhibited retarded growth as well as other signs of functional iron deficiency. Ferrous iron was more effective than ferric iron in correcting the anemia, and high levels of ascorbic acid were beneficial.

There is also evidence for a direct cobalt–iron antagonism inasmuch as cobalt is more efficiently absorbed in iron deficiency (92) and cobalt supplementation accentuates iron deficiency anemia (88). There is mutual antagonism between iron and cobalt in their absorption from intestinal segments (88). So far as is known, cobalt is a dietary essential only as a component of vitamin B_{12}.

Interactions with Copper

In addition to its effect on iron metabolism, cadmium impairs Cu absorption and utilization as demonstrated in growing chicks (88) and in the absorption of Cu from the rat intestine (106). Dietary Cd increases the proportion of Cu sequestered in the intestinal mucosa, where it is associated with metallothionein.
Dietary silver accentuates the signs of copper deficiency as indicated by reduced growth rate, increased mortality, anemia, and failure of aortic elastin to form crosslinks (88). All of these signs are eliminated by adequate copper supplementation. Thus dietary Ag at 100 to 200 ppm decreases Cu bioavailability.

Another copper interaction of considerable importance in ruminant animals, although of little significance in man and simple-stomached animals, is the three-way relationship of Cu, Mo, and S. This complex interaction has been reviewed by Mills and Bremner (107). Molybdenum decreases Cu bioavailability, and in ruminants any form of dietary sulfur accentuates the effect. This has been attributed to the formation of insoluble cupric thiomolybdate (CuMoS₄) and related oxythiomolybdates. Not only does tetrathiomolybdate impair copper absorption, it prevents utilization within tissues and induces a conditioned copper deficiency.

**Interactions with Zinc**

Cadmium injection into male rats produces severe testicular damage, and protection is afforded by prior or simultaneous injection of zinc (108). In spite of this suggested interaction of Cd and Zn, there is little evidence for significant dietary interaction (88, 109).

There is an interaction between lead and zinc which occurs at the level of the enzyme δ-aminolevulinate dehydratase (110). Lead toxicity in children results in decreased activity of this enzyme (ALA-D), and its activity has been used to monitor the body burden of Pb. *In vitro* addition of Zn²⁺ to blood from Pb-exposed rats increases its ALA-D activity (110).

High levels of dietary tin (>500 ppm) are toxic to rats, and there is evidence that Sn interferes with zinc metabolism. Because the human diet may contain as much as 200 mg of Sn per day, Johnson et al. (111) studied the effect of Sn on trace elements (Zn, Cu, Fe, and Mn) metabolism in adult males. Subjects were supplied diets containing either 0.11 or 50 mg of Sn. Those who consumed the high Sn diet excreted more Zn in the feces and were in negative Zn balance. Only zinc excretion was affected by the high Sn level. In a single-dose load test, Solomons et al. (112) found no effect of Sn on Zn absorption when given to human subjects at an 8:1 Sn/Zn ratio. The effect of Sn on Zn bioavailability deserves further study.

**Interactions with Selenium**

When vitamin E is limiting, high levels of silver acetate produce signs of toxicity in rats and chicks. These signs are indistinguishable from those of Se deficiency under the same conditions (113). It is postulated that Ag⁺ forms a complex with Se and limits its availability for glutathione peroxidase biosynthesis. This could occur at the tissue level.

As reviewed by Levander (114), Ganther and co-workers first suggested that the mercury in tuna complexes Se and decreases its bioavailability. Later they recognized poor availability of Se in other fish meals that were low in mercury. Thus there is no evidence for a nutritional Hg–Se interaction.
SUMMARY

Bioavailability of a trace element refers to that fraction of the element in a food which is absorbed and utilized by a person or animal. Several dietary components affect bioavailability, and their major effect is exerted on the true absorption of the element. Some dietary factors also affect tissue utilization, i.e., conversion of the element to its biochemically active form. There are numerous examples of trace element interactions which decrease bioavailability. Although many of these negative interactions occur at the intestinal level, some interactions occur at the tissue level and thus impair utilization.

ACKNOWLEDGMENT

This review and the original work reported from this laboratory were supported in part by the U.S. Public Health Service, NIH grant HL11614.

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DISCUSSION

Dr. Chandra: Dr. O’Dell, have you studied bioavailability using mixed feeds or so-called cafeteria diets in human beings or in animals?

Dr. O’Dell: No, we have assayed primarily diet components rather than the total diet because it is simpler. It is important to determine the bioavailability of trace elements in the total human diet because that is the way we are nourished, but at this point there is need for improvement in methodology.

Dr. Mertz: I have two comments. First, at least in the United States there is still misuse of the word bioavailability. Bioavailability must include utilization. We should make the point very strongly that bioavailability is not just absorption. The second point is this: Dr. O’Dell has shown very well the importance of speciation and of interactions for bioavailability. It occurred to me that with very few exceptions our dietary intake is always greatly in excess of what we really need. In terms of public health measures we could do a lot of good by influencing nutritional status through measures that increase bioavailability. This would probably circumvent some of the pitfalls that we have seen in the past when fortification was implemented without an adequate understanding of bioavailability. Such efforts have not only not solved the problem, as is the case with our iron fortification program in the United States, but have also created problems of undesirable interactions and imbalances. I want to emphasize one practical point: The importance of bioavailability is greatest in your own
field, pediatrics. When we are concerned with exclusive foods, which infant formulas are, it is not enough to imitate the real thing by putting certain amounts of trace elements into those formulas. Dr. Hurley has shown in the past few years the tremendous importance of the chemical form in which a trace element is present in milk, and I would say that speciation effects in infant food are among the greatest challenges in pediatric nutrition.

Dr. Thompson: I disagree with the previous speaker and with Dr. O'Dell over the term bioavailability. I cannot disagree with the definition, but what it defines. For clinical studies it seems to me that to define bioavailability as assimilation or utilization is to define the unmeasurable, because it is not possible, for instance, to tell whether zinc going into a tissue is being utilized or stored. Most dietary zinc passes through the liver into the plasma, and therefore plasma bioavailability is a more meaningful term, as for the bioavailability of bile acids or drugs. Then the amount of a drug X or zinc that has reached the systemic circulation is known. The problem, then, is hepatic extraction, for if this varies there are then problems in calculating the oral bioavailability. Secondly, Dr. O'Dell has highlighted the problem with the complex interaction between the various trace elements and substances in the diet. It has been emphasized that different types of milk affect the bioavailability of zinc, and this is an example of how important are the other constituents in the diet.

Dr. O'Dell: There is no point in emphasizing definitions further except that I perceive that the plasma level of an element or its movement through the plasma is a measure of absorption, not bioavailability. If one is satisfied with the information supplied with measurement of absorption, it is a valid and useful parameter which should not be confused with bioavailability.

Dr. Zoppi: Regarding relationships between absorption of trace elements and fibers in children and infants, we have demonstrated (J Pediatr Gastroenterol Nutr 1982; 1:91–5) that undigestible fibers (approximately 1 g/kg/day) inhibit the absorption of zinc and copper. I believe that this observation is important for the practical pediatrician.

Dr. Haschke: There is evidence from studies in animals that iron has an antagonistic effect on copper absorption. The purpose of our recent study was to find out if copper absorption in normal infants was influenced by the iron content of the formula. Metabolic balance studies were performed in Dr. Fomon's metabolic unit in Iowa City with seven healthy infants fed two cow's milk-based formulas identical in composition except for concentration of iron (formula S 8004: 3 mg/liter, formula S 8003: 12 mg/liter) (Fig. D-1). The net absorption of copper was significantly lower with the formula containing 12 mg/liter (S 8003) than with the formula containing 3 mg/liter (S 8004). These results in normal infants demonstrated that there is antagonism between copper and iron at the intestinal level. The mechanism of this interaction, however, is poorly understood.

Dr. O'Dell: You are suggesting that high levels of iron impair copper absorption. It is well known that copper deficiency impairs iron absorption, but it is less well established that excess iron decreases copper absorption.

Dr. Picciano: I would like to elaborate on the bioavailability of minerals from soy. At the University of Illinois it has been shown that there is great variation among various cultivars of soybean; even using the same cultivar, if you vary the method of processing, e.g., drum-drying versus spray-drying, you get entirely different bioavailabilities for zinc. Thus processing of the soy has a very potent influence on the bioavailability of the mineral, and there are tremendous differences among different varieties of soy. It was not clear to me from your report what was being measured in terms of bioavailability. There are two issues. One is the bioavailability of the zinc from a native product, and the other is bioavailability from the diet to which the specific protein source has been added. You used
a zinc supplement as well as different levels of soy. Were you measuring total zinc absorption when it was provided from soy protein plus a supplement of zinc, or were there graded levels of soy protein?

Dr. O'Dell: In our studies supplementary zinc came from zinc carbonate, beef, or soy. We measured the bioavailability of the zinc intrinsic to the natural products, not the zinc added to them. I am aware that Drs. Erdman and Forbes studied the effect of processing soy protein on zinc bioavailability. They made the pertinent observation that the zinc in neutralized soy protein is less available than the isoelectric product. In other words, the sodium salt decreased bioavailability more than the acid form.

Dr. Van Caillie-Bertrand: I want to focus on the interaction between copper and zinc. How do you differentiate between redistribution in the tissues and bioavailability? The reason I ask you this question is as follows: In 1958 a study in The Netherlands showed that you could treat Wilson's disease patients with zinc sulfate. I have been doing that since 1980 and have followed two children with Wilson's disease treated with pharmacological doses of zinc sulfate. We have seen in both children a normalization of the urinary excretion of copper: Instead of having an elevated value, we found something below 80 μg/24 hr. We have also observed that the serum copper level fell below 10 μg/dl, and that liver function tests returned to normal. The weight and height gains were normal. A few months ago, we did a liver biopsy. We were surprised because in one child the liver copper content decreased as we expected, but in the other child it rose from approximately 350 μg/g dry weight to about 1 mg. We did not expect this at all and thought that we were dealing with redistribution of copper in the tissues. My question is this: In which way is metallothionein, whose synthesis is stimulated by zinc, influenced by copper?

Dr. O'Dell: It is generally believed that zinc impairs copper absorption by inducing metallothionein into the intestinal mucosa. Copper is bound more tightly to metallothionein than zinc. Hence copper displaces the zinc and is held in the mucosa until it sloughs off. The large doses of zinc that you used probably decreased copper absorption and thus
prevented its accumulation by the Wilson's disease patient. There is reason to believe that it specifically decreased liver storage of absorbed copper.

*Dr. Prasad:* Dr. Brewer and I have been involved in a similar study for 3 to 4 years. We administered zinc every 4 hr, and our total daily oral dose was 150 mg. To our great surprise we found that it took several weeks before we saw an effect of zinc on the copper balance. It was not an instantaneous effect, and thus the competition between zinc and copper was not at the luminal level; rather, once we increased the body zinc level, we began to see negative copper balance. Therefore I agree with you that our approach is mainly preventive. However, whether we can actually decrease the body burden of copper by giving oral zinc remains to be studied.

*Dr. Chandra:* One other condition in which also copper accumulates markedly in the body is a unique liver disease seen mainly in the Indian subcontinent, so-called Indian childhood cirrhosis. We have conducted some studies in which we gave 150 to 300 mg of zinc daily and measured urinary copper excretion, plasma copper levels, as well as copper content in liver biopsies. Preliminary analysis suggests that the progression of the disease seems to be arrested. If diagnosed early, one may even expect reversal of the liver damage.

*Dr. Gebre-Mehdin:* Just a comment on the interaction between iron and copper. We have a situation in Ethiopia where the diet provides something like 2 to 300 mg of iron per day. Although the availability of this iron is low, it is still an example of a unique situation in the world. Recently we had the opportunity of looking at the serum and breast milk concentrations of a few trace elements, and to our great surprise we found that breast milk from Ethiopian mothers contained about the lowest amount of copper that we have ever come across. We believe that this has something to do with the iron situation. In Sweden we looked at children with recurrent infections and found excessively high serum levels of copper in those children who were low in iron. We thought this could be due to the effect of infections on ceruloplasmin synthesis. However, when we investigated children who were on elimination diets for allergy of various kinds, we again found high levels of serum copper mainly in children with low iron.

*Dr. O’Dell:* This is an interesting observation that agrees with the earlier comments of Dr. Haschke. In this connection, I should mention the work of Dr. Noel Solomons which showed that ferrous iron decreases zinc absorption.