Infancy is a stage in the life cycle characterized by several unique features. With the possible exception of the rate in utero, growth during infancy far exceeds that experienced throughout life. This is fully appreciated when one considers that an infant doubles his birth weight within 4 to 6 months and triples it by approximately the first year. Coupled with high nutrient requirements for this exceedingly rapid growth rate is developmental immaturity in metabolic and regulatory functions of infants which limit their tolerance to inappropriate provision of nutrients. At the same time, infants receive for some time after birth a single species-specific food, either human milk or a product formulated to resemble human milk. It is only during infancy that a single food is deemed appropriate as a complete nutritional source, and therefore the composition of this food assumes paramount importance.

Human milk is regarded as the optimal source of nutrients for the young infant, provided the maternal diet is nutritionally adequate and a sufficient quantity is consumed (1). The quantity of nutrients provided by human milk under these conditions as related to physiological parameters of nutrient utilization serves as the standard for infant nutrition and often as the basis for establishing nutrient requirements.

The definition of requirements for trace elements of infants has been hampered by the sensitivity of available analytical techniques. The concentrations of trace elements in human milk are usually 1/10 the levels encountered in most biological materials. Additionally, there are real limitations to the quantity of specimens obtainable from pediatric subjects for research purposes. Primarily for these reasons, knowledge of trace element needs of infants is fragmentary at best. Available information often consists of little more than the concentration of the element in human milk.

In this review, representative data on trace elements in human milk and formulas are presented and discussed in reference to current nutritional guidelines. To the extent possible, bioavailability of trace elements and their interactions with other nutrients in various milks used in infant feeding is also addressed.
IRON

Of all the trace elements, iron has received the most attention in infant nutrition. Iron deficiency as a problem in pediatrics was well documented during the early 1930s (2), and now some 50 years hence it remains the major nutritional disorder. It is apparent that the consequences of an iron deficit encompass more than the effects of an oxygen debt to tissues when anemia develops in its final stages of deficiency (3). An inadequate tissue supply of iron with or without clinical manifestations has an impact on cellular immunity, intestinal function, growth, work performance, behavior, catecholamine metabolism, and thermogenesis (4,5).

The pathogenesis of iron deficiency in infants is complex and involves numerous factors, only one of which is the total concentration of iron in the diet. A number of well-executed clinical investigations have identified several of these factors which include relative iron bioavailability from milks used in infant feeding, introduction of solid foods, individual rate of growth, and amount of fetal iron stores (6).

Among all species studied, milk is notoriously low in iron, and the suckled young is at risk of deficiency (1). The concentration of iron in human milk is highest immediately after parturition (7), as is the case with most minerals, but rapidly declines to the level characteristic of mature human milk (Table 1). It is very difficult to obtain a representative value for the concentration of iron in human milk. Among subjects, variance is great (approximately 50% of the total) and measured amount is influenced by the sampling procedure (9). A large portion of human milk iron is associated with the lipid fraction (10); thus a lipid-rich sample

<table>
<thead>
<tr>
<th>Trace element</th>
<th>Mature human milk (mg or μg/liter)</th>
<th>Infant formulas (mg or μg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (mg)*</td>
<td>0.1-1.6</td>
<td>1.1-17.0</td>
</tr>
<tr>
<td>Zinc (mg)*</td>
<td>0.14-4.0</td>
<td>3.7-12</td>
</tr>
<tr>
<td>Iodine (μg)*</td>
<td>30-70</td>
<td>30-150</td>
</tr>
<tr>
<td>Copper (μg)*</td>
<td>90-630</td>
<td>500-2,000</td>
</tr>
<tr>
<td>Manganese (μg)*</td>
<td>1.9-27.5</td>
<td>70-530</td>
</tr>
<tr>
<td>Fluoride (μg)</td>
<td>5-50</td>
<td>30-100</td>
</tr>
<tr>
<td>Molybdenum (μg)</td>
<td>0.1-1.7</td>
<td>30-70</td>
</tr>
<tr>
<td>Chromium (μg)</td>
<td>40-80</td>
<td>10-20</td>
</tr>
<tr>
<td>Selenium (μg)</td>
<td>8-50</td>
<td>5-10</td>
</tr>
</tbody>
</table>

*The Committee on Nutrition of the American Academy of Pediatrics (8) recommended minimum levels of these trace elements to be contained in infant formula preparations. These recommendations have been adopted by the Congress of the United States into the Infant Formula Bill (Public Law 96-359, September 16, 1980). Minimum levels are expressed per 100 kcal: 0.15 mg for iron, 0.5 mg for zinc, 5 μg for iodine, 60 μg for copper, and 5 μg for manganese. One hundred kilocalories are furnished by 147 ml of formula marketed in the United States for term infants and by either 124 or 109 ml of those marketed for preterm infants.
obtained at the end of a nursing period would yield inflated results (11). For a particular woman, time of sampling (morning versus evening), parity, and age may also affect milk iron concentration. There is no evidence that maternal iron status bears a relationship to milk content. This is well illustrated by the studies of Murray and associates (12) in Nigeria where the spectrum of maternal iron status ranged from frank deficiency to overload. In this population, mean milk iron concentration was remarkably similar among women with depleted, sufficient, or loaded iron stores at 2 weeks and 6 months of lactation.

It is now well documented that exclusive breast-feeding provides sufficient iron for at least the first 4 to 6 months of life (13,14). This is due to the superior bioavailability of human milk iron which may well be the highest from any food. Retention of iron from human milk is reported to vary between 20 and 100% (13,15). The mechanism of iron absorption from human milk is not completely understood. Under similar conditions, iron from human milk is absorbed five times as efficiently as a similar amount from cow’s milk (15). Lactoferrin, an iron-binding protein of bacteriostatic importance which is present in high concentration in human milk, has been proposed to account for the high bioavailability. However, heat treatment which destroys lactoferrin does not alter the iron absorption rate (13). Inosine and its metabolites have been shown to enhance iron absorption in rats and human milk content of inosine as its nucleotide increases two-fold from 2 weeks to 3 months of lactation, the time when infant stores of iron are at or near depletion (16). Inosine is not detectable in cow’s milk.

The wide range in the iron content of formulas presented in Table 1 reflects the fact that such preparations are available with or without supplemental iron. The level of iron added to supplemented formulas in the United States is approximately 12 mg/liter, and the iron absorption rate from such products is estimated at 7% (17). In soy-based infant formulas, the protein source contributes 5 mg of iron per liter and therefore the total amount present is 17 mg/liter (18).

The Committee on Nutrition of the American Academy of Pediatrics recommended an iron intake of 1 mg/kg/day (19), whereas the Food and Nutrition Board of the National Research Council recommends 10 mg/day (Table 2). It is obvious that these levels of intake cannot be achieved with exclusive breast-feeding, whereas they are easily met with the use of iron-supplemented formulas. Although there is general agreement that formula-fed infants should receive supplemented iron, there is uncertainty concerning when, how much, and from what source. For infants fed human milk or unsupplemented formula, fortified infant cereals and medicinal preparations are recommended as alternate iron sources to be initiated no later than 4 months of age (19). There is controversy as to whether the time element of this recommendation is appropriate for the breast-fed infant. Using two approaches, it has been demonstrated that when fed with solid foods the iron absorption rate from human milk is drastically reduced (21,22). Moreover, no evidence of iron deficiency was found in a group of 56 breast-fed infants at 6 months (14). Human milk was fed to these infants for at least 6 months, no supplemental iron was
provided, and solids were introduced at 3.5 months. By 9 months, 4% of this group displayed evidence of iron deficiency and by 12 months 7%.

ZINC

Zinc is an essential nutrient in all higher animals including man. It is necessary for growth, sexual maturity, wound healing, and cellular immunity, and is a constituent of many metalloenzymes. Zinc also plays a role in mammalian protein metabolism and nucleic acid synthesis (23).

The importance of zinc in the nutrition of infants was recognized during the 1970s. Serum or plasma zinc concentrations of infants in the United States and Japan were reported to be lower than those of older children and adults, whereas those of Swedish and German infants were similar to values of adults (24). Walravens and Hambidge (25) then demonstrated increased growth rate in 6-month-old male infants fed a zinc-supplemented formula. In 1979 Hambidge et al. (26) observed that the mean plasma concentration of exclusively breast-fed infants at 6 months was not significantly different from that of adults. These studies indicated that previously reported low serum or plasma values for zinc in formula-fed infants were due to inadequate intake. It is important to point out that the concentration of zinc in formulas marketed in the United States when these observations were made was quite similar to that of human milk (9). Thus, as is the case with iron, a greater quantity of zinc is required in a formula preparation to produce the same metabolic response as with human milk feeding owing to differences in bioavailability.

The mean concentration of human milk zinc continuously decreases from approximately 12 mg/liter in colostrum (27) to 1.6, 1.1, and 0.5 mg/liter at 3, 6, and 12 months, respectively (28). The zinc content of mature human milk and its pattern of decline is uninfluenced by maternal dietary intake (29). The concentration of zinc in formulas currently marketed in the United States varies between 3.7 and 12 mg/liter (Table 1). The highest concentration is found in formulas intended for preterm infants, as infants born at 28 to 30 weeks of gestation have total zinc stores estimated at one-third those of full-term infants and are at greatest risk of deficiency (30).

The high bioavailability of human milk zinc is evident from studies employing a rise in plasma zinc concentration as an index of absorption rate. Consumption of 25 mg of zinc with human milk resulted in a significantly higher plasma response than with cow's milk, cow's milk-based formula, or soy-based formula (31). Zinc in human milk is associated with proteins and low-molecular-weight constituents (32). The high bioavailability of human milk zinc remains unexplained. Lönnerdal and co-workers proposed that citric acid formed a ligand with zinc in human milk and that this was the reason for the high zinc absorption rate. Evidence from a variety of experimental approaches has not substantiated that citric acid is the responsible agent (33). Evans and Johnson (34) suggested that picolinic acid, a metabolite of tryptophan, was the bound species with zinc and the effective agent
in human milk. Using an animal model, these investigators provided evidence that picolinic acid increased zinc absorption (35). However, when tested in a number of other systems, the positive effect of picolinic acid on zinc absorption could not be demonstrated. Alternately, Cousins and Smith (36) proposed that the type of protein in milk, its relative digestibility, and the amount of zinc that is potentially available to enter a free- or low-molecular-weight chelated pool explains the differences in bioavailability of milk zinc among species.

As shown in Table 2, the recommendation for dietary zinc from birth to 6 months is 3 mg/day; although this recommended dietary allowance is based on the concentration of zinc in human milk, the values employed (3 to 5 mg/liter) are considerably higher than modern data indicate (Table 1). However, this recommendation is achieved with formula feeding and is consistent with the increased level necessary in such products to maintain adequate zinc status (vide supra).

COPPER

The normal term infant is born with a generous store of copper in the liver (37), making copper deficiency a rare event. As early as 1931, however, Josephs (38) suggested that copper deficiency might be responsible for a resistant anemia in milk-fed infants because cow’s milk is one of the few commonly used foods low in copper. During the last several years, numerous reports of copper deficiency characterized by anemia, neutropenia, and bone demineralization in both preterm and term infants have appeared (39–41).

Neonatal copper deficiency is associated with severe malnutrition, significant malabsorption, and low birth weight (42). Furthermore, fortification of formulas with high amounts of iron may interfere with copper absorption (43). Most recently, copper deficiency was reported in a 6-month-old infant fed cow’s milk and corn flour (44). Actually, the cause of neonatal copper deficiency is poorly understood. Wilson and Lahey (45) were unable to induce hypocupremia in premature infants fed a low copper formula for as long as 10 weeks.

Serum concentration of copper is low at birth (30 μg/100 ml) and rises rapidly to adult levels (70 to 140 μg/100 ml) by 2 to 3 months of age (46). The rise in

<table>
<thead>
<tr>
<th>Trace element</th>
<th>Recommended intake (mg/day)</th>
<th>Trace element</th>
<th>Recommended intake (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>10</td>
<td></td>
<td>Fluoride*</td>
</tr>
<tr>
<td>Zinc</td>
<td>3</td>
<td></td>
<td>Molybdenum*</td>
</tr>
<tr>
<td>Iodine</td>
<td>0.4</td>
<td></td>
<td>Chromium*</td>
</tr>
<tr>
<td>Copper*</td>
<td>0.5–0.7</td>
<td></td>
<td>Selenium*</td>
</tr>
<tr>
<td>Manganese*</td>
<td>0.5–0.7</td>
<td></td>
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</tbody>
</table>

*The available information on estimated requirements is so fragmentary for these trace elements that a “safe and adequate range” of intakes is proposed rather than a recommended dietary allowance.

From ref. 20.
serum copper parallels the synthesis and release of ceruloplasmin from the liver and appears to be uninfluenced by dietary amount in normal infants. The remarkable ability of the fetal liver to accumulate copper is linked to the presence of neonatal hepatic mitochondrocuprein, a protein unique to the fetus which contains 10 times as much copper as any other protein (47). Although not implied by its name, the subcellular location of this protein may be in the lysosome. The concentration of mitochondrocuprein disappears soon after birth, presumably following transfer of copper to ceruloplasmin for bodily redistribution.

The concentration of copper in mature human milk ranges from 0.09 to 0.63 mg/liter, and mean values (0.2 to 0.3 mg/liter) are quite similar for U.S. and European women (9,48). The concentration of copper is highest in colostrum and falls rapidly to that typical of mature milk with no evidence of further decline. There are no data to indicate that levels in milk reflect either maternal status or serum concentration. Cow’s milk is low in copper, and formulas are supplemented to levels indicated in Table 2. The highest amounts are contained in formulas designed for preterm infants, whereas the majority contain between 0.5 and 0.7 mg/liter. The recommended dietary allowance for copper during infancy as well as the minimum level to be contained in a formula preparation are based on the content in human milk. The bioavailability of copper from various milks is unknown. Using chromatographic procedures, Lönnerdal et al. (32) found that copper in human milk is associated with both proteins and low-molecular-weight constituents.

IODINE

The human requirement for iodine is for synthesis of the thyroid hormones which are involved in a variety of metabolic processes. Human milk is reported to contain 30 to 70 μg/liter (49), but modern data are not available. Iodine is unique among the trace elements because the mammary gland avidly accumulates it. The milk/plasma ratio is 10 or greater (50). Consumption of iodized salt by women was found to double the concentration in milk (51). The use of iodine-containing compounds during the milking procedure is largely responsible for the high content of cow’s milk and formulas based on it.

Human milk feeding on one occasion was found to mitigate the symptoms of congenital hypothyroidism (52). The various thyroid hormones have been identified in human milk, but protection from congenital hypothyroidism by breast-feeding is not universal (53).

The recommended dietary allowance for iodine during infancy and the minimum level to be present in formulas (Table 2) are based on levels present in milk of women living in nongoitrous areas as milk content falls during maternal deficiency. Soy protein-based formulas are no longer goitrogenic, as they are now supplemented with iodine (49).
FLUORIDE

The essentiality of fluoride for higher animals including man is a matter of dispute. There is irrefutable evidence that fluoride confers maximal resistance to dental caries and may be involved in the maintenance of normal skeletal structure, providing protection from osteoporosis during adulthood. Thus if a reduction in caries rate is considered as a requirement of health, fluoride does assume the role of an essential trace element.

The concentration of fluoride in human milk ranges from approximately 5 to 50 μg/liter, and milk content is similar for women receiving fluoridated water and those receiving water naturally low or high in this element (54). Cow's milk normally contains greater amounts.

The Committee on Nutrition of the American Academy of Pediatrics recently revised its recommendation for fluoride supplementation during infancy (55). This group recommends 0.25 mg/day, the midpoint of the safe and adequate range proposed by the Food and Nutrition Board of the National Research Council (Table 2). Fluoride supplementation of infants is advised only when the water supply contains fluoride at less than 0.3 ppm and human milk provides total nourishment for greater than 6 months. The Committee on Nutrition also recommended that formulas be prepared with low fluoride water to prevent possible fluorosis, as such products may be diluted with water containing high levels of this element.

MANGANESE

In 1931 Orent and McCollum (56) demonstrated the essentiality of manganese for the rat. These investigators noted high neonatal mortality in offspring of manganese-deficient animals. Subsequently, manganese metabolism has been studied in numerous other species (57). Deficiency symptoms in animals in addition to impaired reproductive performance include poor growth, congenital ataxia, and skeletal dyschondroplasia. Manganese is required for the synthesis of prothrombin, a glycolysated protein involved in the blood clotting mechanism, and the synthesis of mucopolysaccharides, structural components of cartilage. Only one case of manganese deficiency has been reported for man and symptoms included hypocholesterolemia, slowed growth of hair and nails, hair depigmentation, and reduced levels of blood clotting proteins (58).

Manganese metabolism is poorly understood during infancy. The human fetal liver does not accumulate manganese as is the case for copper and, to a lesser extent, for iron and zinc. However, there are no data for other tissue contents of manganese, and it is possible that bone represents the storage site for this element. Neonates on days 5 to 8 were reported to be in negative manganese balance when fed human milk, but if and how long excretion of manganese exceeds intake is unknown (59). In this study, a positive correlation (r = 0.8) between iron and manganese in the stools was also noted. Animal studies indicate that high iron intakes interfere with manganese absorption, whereas when iron is low manganese absorption is increased. Likewise, high dietary manganese blocks iron absorption.
These observations in animals suggest that manganese and iron share a common mechanism for absorption (60–62).

The manganese content of human milk was reported during the early 1970s to be 15 µg/liter (63). Recent data from the United States and Finland indicate lower amounts, with the concentration declining from approximately 7 µg/liter at month 1 of lactation to 4 µg/liter in later months (48,64). Actual content may be subject to maternal dietary influences (48). Most interestingly, the serum manganese concentration of breast-fed infants is directly correlated \( r = 0.7 \) with intake from human milk (64).

The quantity of manganese in cow's milk is about 35 µg/liter, whereas U.S. formulas provide between 70 and 530 µg/liter. Differences among human milk, bovine milk, and infant formula in number and type of manganese ligands which may affect bioavailability have been reported (65). High levels of manganese (>1,000 µg/liter) are no longer added to some infant formulas as such supplementation is deemed unnecessary.

There are no firm data upon which to establish a manganese requirement for infants. Manganese is among the least toxic elements, and a wide range of intakes are well tolerated. There is no evidence of deficiency for humans in general and infants in particular. The range of safe and adequate intakes proposed by the Food and Nutrition Board (Table 2) can easily be met with formula feeding, whereas only with the provision of solid foods can breast-fed infants achieve these levels of intake. Cereals rank highest as a dietary source of manganese and are customarily the first infant food. Because animal studies show that manganese absorption is decreased with high dietary levels of calcium, phosphorus, and iron (60–62), it may be necessary to provide more manganese via formulas than present in human milk to achieve similar retention.

**SELENIUM**

Selenium is recognized as a nutritionally essential element for numerous laboratory and production animals. Deficiencies of this trace element are associated with white muscle disease in lambs, calves, and other species, exudative diathesis and pancreatic fibrosis in chicks, hepatosis dietetica in pigs, and liver necrosis in rats. The biological effect of selenium in the metabolic regulation of a wide variety of tissues, e.g., preventing liver necrosis, exudative diathesis, pancreatic degeneration, various myopathies, and various types of cancer in experimental animals, indicates that selenium must have a very basic function in addition to its role as part of the enzyme glutathione peroxidase. Increasing evidence supports the contention that selenium is a dietary essential for humans (66). Dietary supplementation of selenium has been reported to increase growth of children suffering from kwashiorkor, prevent muscle pains in adults receiving long-term parenteral feeding without selenium, and prevent Keshan disease, a cardiomyopathy of unknown etiology which affects children (67,68). This evidence, plus the requirement for selenium for clonal growth of human fibroblasts, supports this trace element as required by humans.
Few studies have focused on selenium nutrition of infants. It is clear, however, that serum, whole blood, and hair concentrations are high at birth and decline to 30 to 50% of newborn values by 5 to 6 months of age (69,70). This is believed due to the low selenium content of milks used in infant feeding, but the clinical significance of these observations is unclear.

As evidenced for other trace elements, the selenium level is high in colostrum (40 to 80 µg/liter), but levels fall to those typical of mature mammary secretions (11 to 53 µg/liter) by 2 weeks and remain unchanged until 5 months of lactation (71,72). Bioavailability of selenium from human milk is unknown, as is its molecular form. However, a significant correlation exists between human milk protein and selenium content, suggesting that it is located at least in part in the protein fraction (71). Approximately 25% of the selenium in human milk is estimated to be contained in the enzyme glutathione peroxidase (73). The selenium content of formulas marketed in the United States contains 5 to 9 µg/liter, approximately half the amount present in human milk. Corresponding differences in selenium intakes via formula and human milk are reflected in serum concentrations of 3-month-old infants (71). The infant’s requirement for selenium has not been defined. A range of safe and adequate dietary intakes of selenium has been estimated primarily by extrapolation from the selenium requirement of mammalian animal species. This extrapolation makes the broad assumption that this dietary concentration of selenium will also meet the human requirement (Table 2). These estimates do not seem to reflect the actual selenium intakes of infants in the United States, as recent data have shown that approximately 60% of the human-milk-fed and 95% of the formula-fed infants had selenium intakes below the 10 to 40 µg/day proposed by the National Research Council (71). Although not actually determined, the addition of solids would be expected to increase selenium intake during infancy.

**CHROMIUM**

Chromium is an essential trace element required for normal glucose tolerance. It is biologically active in the trivalent state as an organic complex. One of these complexes has been identified in Brewer’s yeast and was found to contain, in addition to trivalent chromium, a dinicotinic acid–glutathione moiety (74).

The concentration of hair chromium which is used as an index of status is high in full-term and low in preterm infants. Human fetal bone ash content of chromium is directly related to gestational age, indicating that premature infants are at greatest risk for a low chromium state. After chromium administration, children recovering from protein-calorie malnutrition often exhibit improved growth as well as improved glucose tolerance (75).

The falling hair chromium concentration during infancy is viewed as physiological in part but may also be related to the low amounts of this element found in milks. The wide range in values indicated in Table 1 for chromium in human milk may be the result of analytical problems. Until quite recently human milk was reported to contain between 40 and 80 µg of chromium per liter (76). A recent
study from Finland, however, provided a range of 0.19 to 0.69 \( \mu g/\text{liter} \) and a mean of 0.4 \( \mu g/\text{liter} \) (77), values considerably lower than previously reported. These authors were also unable to relate maternal dietary chromium intake with content in human milk. Cow’s milk is reported to contain less chromium than human milk and is present in a nonbiologically active form (75). The bioavailability of chromium from human milk and formulas has not been established.

Human requirements for chromium are difficult to establish because the amount needed depends on the biological form. For example, the quantity required to improve glucose tolerance in adults is 200 \( \mu g/\text{day} \) as chromic chloride but only 10 \( \mu g/\text{day} \) when furnished in Brewer’s yeast. Based on the chromium density (25 \( \mu g/1,000 \text{ kcal} \)) of the American food supply, an average availability of 1 to 2\%, and the recognition that this density meets the requirement of most healthy Americans, the Food and Nutrition Board of the National Research Council has proposed a range of safe and adequate intake of 50 to 200 \( \mu g/\text{day} \) for adults. The recommended range for infants (Table 2) is extrapolated on the basis of expected food intake from the adult range.

**MOLYBDENUM**

As an integral component of xanthine oxidase, aldehyde oxidase, and sulfite oxidase, molybdenum is an essential trace element. Molybdenum and copper interact in a complex manner, and high levels of copper are effective in alleviating molybdenum toxicity in cattle, rats, rabbits, and chicks. There are no reports of molybdenum deficiency in humans, and metabolism during infancy is virtually unexplored. One report (78) indicated that human milk from Indian women contains an average of 0.72 \( \mu g/\text{liter} \), with values ranging from 0.1 to 1.7 \( \mu g/\text{liter} \). Cow’s milk is reported to contain 30 to 70 \( \mu g/\text{liter} \); cow’s milk-based formulas would presumably have intermediate values, but data are unavailable. Balance studies in adults have detected retention on intakes of 2 \( \mu g/\text{kg/day} \) (76), and the safe and adequate range of intakes for infants (Table 2) was derived by extrapolation from these balance studies.

**CONCLUSION**

Tremendous strides have been made in our knowledge of trace element nutrition of infants during the last decade. Particularly noteworthy is the recognition of the importance of mineral-mineral interactions and bioavailability from various milk and mixed diets. It is now well recognized that the ability of formulas to furnish trace elements in adequate amounts cannot always be predicted on the basis of compositional analysis alone. Adequacy of such preparations must be demonstrated in studies employing criteria of evaluation in addition to growth. In such studies the human milk-fed infant should serve as the control. Fetal stores can exert a strong influence on trace element needs during infancy, and often the preterm infant has increased requirements. To assess the daily intakes of trace elements by infants, knowledge of their concentration and molecular form in human milk and
metabolism in exclusively breast-fed infants displaying normal growth is urgently needed. Particular emphasis should be paid to those trace minerals known to be essential in animals but unconfirmed in man. Such minerals include nickel, vanadium, and silicon and possibly tin, arsenic, and cadmium.

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28. Kirksey A, Ernst JA, Roepke JL, Tsai TI. Influence of mineral intake and use of oral contracep-

**DISCUSSION**

**Dr. Gibson:** I would like to deal with three topics discussed only briefly by Dr. Picciano. Firstly, the increasing use of soya-based infant milk formulas for feeding full-term and particularly preterm infants is a cause for concern in view of the poor bioavailability of zinc (Momcilovic et al. J Nutr 1976;106:913-7; Sandstrom et al. Am J Dis Child 1983;137:726-9) and possibly iron (Cook et al. Am J Clin Nutr 1981;34:2622-9) in these formulas. This has been attributed to the presence of phytate in the soya protein concentrate. However, the
decreased availability of zinc in these formulas may also be due to the higher levels of calcium (0.6–1.4%) and phosphorus (0.5–1.0%) which occur in the soy-based compared to the cow's milk-based formulas (Ca 0.33–0.45%; P 0.26–0.35%).

Although the levels of fortification of zinc and iron in some soya-based formulas are higher than those of cow's milk-based formulas to compensate for the poor bioavailability of these two trace elements, there is evidence that the level of zinc added is still not sufficient to meet the requirements of full-term infants, and certainly not the higher nutritional needs of very-low-birth-weight preterm infants. A longitudinal study conducted on full-term and preterm Canadian infants demonstrated that all infants fed soya milk showed a marked decline in zinc status during early infancy.

A second point of perhaps more widespread concern relates to the level of zinc fortification used in the cow's milk-based formulas (i.e., 3–5 mg/liter). In a recent study (MacDonald et al. Acta Paediatr Scand 1982;71:785–9) these levels were found to be insufficient to prevent a decline in zinc status, as indicated by a significant fall in hair zinc concentrations in male full-term formula-fed infants during the first 6 months of infancy. In contrast, female full-term infants and male and female breast-fed infants of this study did not exhibit a decline in zinc status (Table D1). Hambidge (Walravens, Hambidge. Am J Clin Nutr 1976;29:1114–21) emphasized that male infants appear to have a higher requirement for zinc during infancy. If the level of zinc in these cow's milk-based formulas is not sufficient to prevent a decline in zinc status of male full-term infants, it is questionable if these formulas meet the higher zinc requirements of preterm infants.

We recently compared the trace element status of a group of very-low-birth-weight preterm infants with a group of full-term infants during the first 12 months of infancy. Sixty-four percent of the preterm and 36% of the full-term infants of this study at 3 months were fed a variety of cow's milk-based formulas. The remainder received soy-based formulas (12% in both the preterm and full-term groups) and mother's own breast milk (10% in the preterm and 47% in the full-term group) at 3 months. None of the preterm infants were fed specially adapted preterm milk formulas at any time.

Multiple regression analysis demonstrated that dietary zinc intake was the only significant variable in the regression equation for length in the preterm infants at 3 months, whereas weight at birth and zinc intake were both significant in the regression equation for weight at 3 months. At 12 months, the regression equation for weight for the total sample of infants was significant, the significant variables being weight at birth and, again, zinc intake. Hence dietary zinc intake played a more important role in explaining the length and weight of these infants at 3 and 12 months, respectively, than did any of the other variables such as intakes

<table>
<thead>
<tr>
<th>TABLE D1. Hair zinc changes from 1 to 6 months</th>
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<tbody>
<tr>
<td>No. of infants showing increasing hair zinc levels</td>
</tr>
<tr>
<td>Bottle-fed infants</td>
</tr>
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<td>male</td>
</tr>
<tr>
<td>female</td>
</tr>
<tr>
<td>Breast-fed infants</td>
</tr>
<tr>
<td>male</td>
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<tr>
<td>female</td>
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</tbody>
</table>
of protein and energy, incidence of early illness, socioeconomic index of father, sex, midparent height, and age of introduction of solid foods.

The findings of the longitudinal study cited above emphasize the necessity for adequate intakes of dietary zinc for the achievement of maximum growth potential in very-low-birth-weight preterm infants and have important implications for the nutritional management of these infants. Obviously, it is essential to determine the optimal levels of zinc supplementation for any milk formulas designed specifically for preterm infants. This requires detailed knowledge of all the factors affecting the bioavailability of zinc. At the present time, such detailed information is not yet available.

Nevertheless, as Dr. Picciano noted, an attempt has been made by the infant formula manufacturers to produce specially adapted preterm milk formulas. These milks have a higher content of certain trace elements, e.g., Zn 12 mg/liter, Cu 2 mg/liter, Fe 3 mg/liter, as well as a higher sodium and protein content. Furthermore, their calcium/phosphate ratio is higher, e.g., 1.8 versus 1.4, and this may have a detrimental effect on zinc absorption (Sandstrom, Cederblad. Am J Clin Nutr 1980;33:1778–83). Energy and nitrogen balances and growth parameters have been compared in babies fed preterm milk formulas and breast milk (Brooke et al. Arch Dis Child 1982;57:898–904). However, to date, the trace element status of preterm infants fed these highly adapted milk formulas has not been adequately studied.

We recently compared the serum zinc and copper concentrations of enterally fed preterm infants, most of whom were given especially adapted preterm milk formula, with a group receiving parenteral nutrition supplemented with zinc and copper. The latter were infused

![FIG. D-1. Serum zinc levels (mean ± SEM).](image-url)
at levels of 350 \( \mu g \) Zn and 20 \( \mu g \) Cu/kg/day, concentrations recommended for preterm infants by the Expert Panel of Nutrition, Advisory group of the American Medical Association (J Parenter Enter Nutr 1979;3:263). The preterm milk formula contained 12 mg Zn and 2 mg Cu/liter (Fig. D-1). Mean serum zinc concentrations in the preterm group fed the specially modified milk formulas declined significantly during the first 14 days, whereas in the parental group mean serum zinc concentrations remained relatively constant from week to week (Friel et al. J Pediatr, in press). Furthermore, the latter were significantly higher than the corresponding concentrations for the enteral group at days 14 and 21. The decline in mean serum zinc levels in the enteral group cannot be attributed solely to an effect of birthweight and gestational age, as a comparable decline has been documented in full-term formula-fed infants (Walravens, Hambidge. Am J Clin Nutr 1976;29:1114-21). Instead, the fall is attributed to inadequate levels of zinc in the specially modified milk formulas, exacerbated in preterm infants by a combination of poor absorption of enteral zinc and poor resorption of endogenous zinc by the immature gastrointestinal tract. It is not clear from these results if the higher mean serum zinc concentrations in the parenteral group are indeed indicative of improved tissue zinc stores.

The third topic I would like to briefly discuss deals with selenium intakes of infants during the first year of life. Dr. Picciano noted that there were no data on selenium intakes of infants, once mixed feeding was established. We recently determined the selenium intakes of a group of full-term and pre-term infants at 3, 6, and 12 months of age. Dietary intake data were collected via 3-day diet records. Test weigh results were used to assess the mean daily volume of breast milk consumed by the breast-fed infants. Mean daily intakes of selenium were calculated using company product information, the literature, and laboratory analysis.

Pre-term infants had a significantly lower mean selenium intake at 3 months compared to the full-term infants. This difference was in part due to the greater number of breast-fed infants in the full-term group, breast milk containing more selenium than formula milk. However, even when mixed feeding was firmly established (i.e., 6 months), mean selenium intakes (expressed in micrograms per day) for both groups were below the U.S. Food and Nutrition Board adequate and safe range for full-term infants. Only at 12 months did mean selenium intakes for both groups fall within the U.S. safe and adequate range. At this time, milk was no longer the major food source of selenium. Instead, meats provided the greatest source of selenium (41% and 34% respectively) at 12 months. It is possible that the U.S. safe and adequate range for selenium is too high for 3- and 6-month-old full-term infants.

Dr. Lombeck: Three comments: First, on zinc: only some values are published about the zinc content of formulas. Recently Higashi et al. from Japan showed that the zinc content of serum and hair decreased similarly in breast- and bottle-fed infants, although the zinc content of the formula was very low. In addition, the urinary zinc excretion was reduced in bottle-fed infants in comparison to breast-fed ones.

Second, on manganese: Literature data—I just checked them for a supplementation study—show still an incredible broad range of physiological manganese concentrations in body fluids, from less than 1 \( \mu g \) liter to hundreds of micrograms per liter. The figures of Dr. Picciano concerning the manganese content of milk fit well with the data published by Vuori (Finland). Problems arise with respect to the serum values. In adults and children, the so-called normal values amount to 0.5 ng Mn/ml serum—that means 10% of what was mentioned here. Probably higher values can be caused by contamination.

Third, on selenium: The data of Drs. Picciano and Gibson concerning the selenium intake of bottle-fed infants or young children fit well with my data. In exclusively bottle-fed infants
the selenium intake is below 10 μg/day. The data on breast-fed infants are somewhat lower in the United States than in Europe. In Western Germany the selenium intake rises to 33 μg/day (range 8–79 μg Se/day) in the second half-year of life. This rise depends on the cereal content of the food. I measured the Se intake with different foodstuffs. In older infants and young children 40 to 50% of the daily Se intake derives from cereals, 17% from milk, and the rest from fruits, vegetables, and commercially available meals. The typical children's menus consisting of vegetable plus meat have an average Se content of 23 ng/g, which is comparable to that of cow's milk. Some data were puzzling in my recent study, e.g., the selenium values of bananas. We investigated several samples because young German children are fed bananas rather frequently. The selenium content of the bananas differed widely: Some samples measured around 10 ng Se/g, another group around 33 ng Se/g, and the last group more than 100 ng Se/g. I suspect the bananas were imported from different countries. Regional differences are well documented for cereals. Because of these regional differences we have to measure the true selenium intake of children in different countries and cannot calculate it from published food tables.

Dr. Chandra: What is the effect of supplementation during lactation on the trace mineral content of serum and milk of lactating women? High zinc intake may reduce levels of copper, manganese, and even calcium.

Dr. Picciano: The human data are less convincing. The animal model is exceedingly reproducible. When you put a lactating animal on a deficient diet, milk levels drop. The best data I have seen relative to this issue are for iron. The Murrays studied lactating subjects with iron overload, iron deficiency, and iron adequacy. Over the whole spectrum of possible iron status, they found no differences in milk iron content. I believe Dr. Hambidge showed there was a slight increase in milk zinc at one point for zinc-supplemented lactating women. I suspect that selenium is influenced by maternal intake because we see a geographical pattern of distribution even in the United States. In general, available information suggests that milk mineral levels drop in the face of maternal dietary inadequacy, but once the ceiling is reached with maternal dietary sufficiency milk levels do not appear to spike over that level as has been noted with vitamins in human milk.

Dr. Haschke: It is interesting that most formulas have higher manganese concentrations than breast milk. A study by Collipp et al. expressed major concern on the high manganese intake of some formula-fed infants because elevated hair manganese was found in disabled children.

Dr. Picciano: The very high manganese content of some milk-based proprietary infant formulas was based on data obtained from balance studies in adolescents and extrapolation to the infant as to what suspected manganese requirements would have been. The available information now is that it was probably excessive, but there were absolutely no data to indicate that it was harmful, as manganese is one of the least toxic elements. With regard to the report you referred to concerning excessive manganese intake and neurological disorders: I know it exists, but I have not read it. One concern about excessive manganese stems from the reciprocal relationship that has been observed in balance studies between manganese in food and iron excretion and the possible sharing of the same site for absorption by manganese and iron. Half of the bottle-fed infants we studied were receiving an iron-fortified formula and the other half a noniron-fortified formula, and we could detect no differences between the subgroups.

Dr. Zlotkin: I recently had the opportunity to take postmortem samples from a group of infants who died either of sudden death or from traffic accidents between the ages of 0 and 6 years, including some infants who were born prematurely. Fifty-eight liver samples were
analyzed by neutron activation analysis for selenium. During the first year of life, there is a marked decline in the selenium concentration of the liver. After the first year and up to around 6 years of life, there is a gradual increase in the selenium concentration and then a leveling off after 6 years. With respect to the total selenium concentration, we saw a plateau between birth and the first year and then a gradual increase in the total selenium of the liver. We also noted a significantly lower total selenium content in the liver of preterm infants. Obviously the liver size is much smaller. My interpretation of the results would be two-fold. First, this decline in selenium concentration could mean either inadequate intake or, much more likely, a normal physiological response. Second and perhaps more important, there is a more marked difference in liver selenium concentration at the beginning of the life for the preterm than the full-term infant. If we are going to talk about “at risk” groups, it seems that preterm infants are born at greater risk for deficiency, including selenium, than the full-term infant.

Dr. Picciano: My position would be that until you can prove that the observed decline in selenium concentration is not physiological, you have to assume that there is an influence of diet. You are taking the opposite position that, unless you can prove a dietary effect, it is physiological.

Dr. Hurley: In reference to selenium, it resembles what happens to liver copper, in rats anyway. The fact that it is lower in the preterm than in the full-term infant is also consistent with the idea that selenium is stored for use during the early neonatal period, but this is purely hypothetical. I wanted to make a comment (but I was glad Dr. Lombeck said it first) that manganese is a neglected element we know little about and which deserves more attention. You showed some serum values and questioned whether that was the good index of manganese status. We have been interested in manganese for a long time and have been trying to develop a way of determining manganese status. We found in rats given manganese-deficient diets that the whole blood manganese concentration correlated very well with the concentration of liver manganese. Manganese is very different from zinc in that is does not decline rapidly in the plasma, and in fact dietary manganese deficiency takes a long time to produce any effects. In the manganese-deficient rats, when there was a difference in the liver manganese concentration it also showed up in the whole blood manganese concentration.

Dr. Picciano: Did you measure it in plasma also?

Dr. Hurley: No. We did not because it is too low to be easily measured even with the graphite furnace. Even whole blood manganese determinations are very difficult.

Dr. Lombeck: About liver selenium content: I think the data fit with those from C. Casey from New Zealand, who published high prenatal liver selenium values; this might reflect prenatal selenium storage. About manganese: it is easier to measure whole blood manganese because it is at least 20 times higher than serum manganese (0.5 ng Mn/ml). The data I refer to are from some published and unpublished investigations based on neutron activation analysis.