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IRON, COPPER, ZINC, AND MANGANESE IN MILK

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CONTENTS

INTRODUCTION 150
METHODOLOGY IN ANALYSIS OF MILK 150
Sample Collection 150
Methods of Estimating Milk Output 151
Analytical Techniques 151
DEVELOPMENTAL CHANGES IN TRACE ELEMENT CONCENTRATIONS DURING LACTATION 152
Patterns of Trace Element Concentrations During Lactation 152
Iron 152
Copper 154
Zinc 154
Manganese 155
DIETARY INFLUENCE ON MILK COMPOSITION—EFFECTS OF MATERNAL TRACE ELEMENT DEFICIENCY OR EXCESS 156
Iron 156
Copper 157
Zinc 157
Manganese 158
Comments 158
TRACE ELEMENT BINDING FACTORS IN MILK 158
Iron 159
Zinc 160
Copper 161
Manganese 162
Comments 162
BIOAVAILABILITY OF TRACE ELEMENTS IN MILK 163
Iron 163
Zinc 164
Copper and Manganese 165
INFANT FORMULAS 166
CONCLUDING REMARKS 167

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INTRODUCTION

The importance of trace elements such as iron, copper, zinc, and manganese in the development of the neonate has been demonstrated in several species (56, 140). Deficiencies of these essential nutrients may lead to high neonatal mortality, abnormal development, or poor growth. As the only food of the neonatal mammal is milk, its capacity to meet the trace element needs of the neonate is crucial.

This review summarizes information concerning the concentrations of iron, copper, zinc, and manganese in milk of several species. The variations in concentration of these trace elements during the course of lactation, as well as the influence of maternal diet are discussed. In addition, current information on the molecular localization (binding) and bioavailability of these elements in milk is described, and implications for infant formulas are discussed. Other trace elements are not included in this review because information regarding their occurrence and properties in milk is limited. The reader is directed to reviews on human milk (59), milk composition (74), infant nutrition (35, 56), and trace elements (114, 140) for topics not considered here.

METHODOLOGY IN ANALYSIS OF MILK

Sample Collection

There are pronounced differences in the composition of milk from different species and the composition of milk even from a single species may be changing constantly. For example, considerable change occurs during a single feeding (37, 111), between morning and evening milk (112), and perhaps more commonly recognized, during the different weeks of lactation (82). Changes in the diet of the mother may influence milk composition (38), and even short-term administration of vitamins or minerals during lactation may affect the concentration of the nutrients in the milk (142). Thus, any one sample does not necessarily represent the nutrient quality of the mother's milk; multiple samples are required to furnish a reliable estimate of most constituents. It should be pointed out, however, that multiple sampling from some species may lead to alterations in milk composition (67).

At any particular nursing there is a striking tendency for fat content to be lower at the beginning of nursing than at the end (37, 58, 88, 111). Furthermore, values for human milk fat increase during the day. Iron concentration, likewise, increases significantly during one feeding (39, 111) and additionally increases during the day (112).

The observed variations in milk composition within a day underline the importance of collecting milk samples at a specific time when making comparative studies. The variation in composition within a feeding stresses the need of carefully selecting and describing the method of sample collection. Although it would be beneficial to evacuate the breast fully and to analyze an aliquot of this sample, from an ethical as well as from a practical viewpoint, this procedure is rarely appropriate, especially in early lactation. An alternative method is to obtain a small sample before nursing and a sample of equal volume after nursing—a procedure that gives a very good estimate of the average composition of the milk during one feeding (82).

Methods of Estimating Milk Output

Two principal methods have been used to estimate the output of human milk. The first requires weighing the infant before and after each meal, and adding all the net weight gains during a 24-hr period to give the daily output of milk (or intake by the infant) (81, 82). Another method is to measure the volume of milk expressed from both breasts, either by hand or with a breast pump. The major disadvantage of the latter method is that one can usually obtain only single meal output rather than total daily output. It is also questionable how valid these expression methods are as a measure of milk volume that an infant is able to suck (106).

A third method, which uses radioisotopes, for example, injection of tritiated water (118, 122) is possible in experimental animals. If the specific activity of the milk constituent in question is known, then the activity of this isotope can be determined and total milk output can be estimated.

Analytical Techniques

As the concentration of trace elements in milk is low, there is always a risk of contamination from the environment in dust or equipment, vials, or reagents. Therefore, all materials used either in collection or in storage of the milk samples must be cleaned with nitric acid, thoroughly washed with trace element-free water or alcohol, and stored under dust-free conditions. As an example, contamination from metal receptacles can more than double the iron content of cow's milk (34).

Once collected, milk can be analyzed by a variety of techniques. Earlier, colorimetric methods such as the orthophenanthroline method for iron (127), the diethyldithiocarbamate method for copper (25), and the dithizone method for zinc (110) were employed. During the last 2 decades, however, more sensitive methods that demand smaller sample volumes have been developed. These include atomic absorption spectrophotometry, X-ray fluorescence spectroscopy, and neutron activation analysis. By far the most common procedure is atomic absorption spectrophotometry. This method requires that the organic matter of the sample be completely destroyed prior to analysis. This can be achieved by either ashing the milk with a strong mineral acid (wet ashing), or subjecting the sample to high temperature (dry ashing). The former method is superior with regard to precision (14a,b). It should be noted that the type of acid used can affect the results. Nitric acid appears to be a superior acid for this purpose and is commonly the agent of choice.

DEVELOPMENTAL CHANGES IN TRACE ELEMENT CONCENTRATIONS DURING LACTATION

This section concerns the variations and normal patterns of iron, copper, zinc, and manganese concentrations in milk from man and several other species. In the following section we discuss the influence of maternal diet on the concentrations and patterns of these elements in milk.

Patterns of Trace Element Concentrations During Lactation

IRON

Human A decrease in iron content with lactation time has been reported by several investigators (73, 80a, 135, 142). Levels of 0.6–1.0 μg/ml are common in colostrum (the colostrum period for the human is defined here as the first 10 days of lactation), whereas mature milk (postcolostrum) is found to have concentrations ranging from 0.2–0.4 μg/ml (6, 7, 13, 22, 34, 69, 73, 80a, 102, 105, 112, 135, 142). This decrease of approximately 60% in iron concentration from colostrum to mature milk occurs predominantly during the first 5 weeks of lactation. Thereafter, the iron level does not seem to decrease even in prolonged lactation (135). The decline in iron concentration in human milk is of lesser magnitude than reported for other species (see below).

The values commonly found for the concentration of iron in mature human milk are similar to (101) or somewhat lower than (142) those found in plasma. A mechanism for the uptake of plasma iron by the mammary gland and its secretion into the milk has not yet been established.

Domestic and experimental animals Iron in milk from most dairy animals ranges between 0.2 and 0.3 μg/ml (31, 140). Although several investigators have reported the lack of a developmental pattern of milk iron in dairy animals, a recent report by De Maria (21) showed that in cow, sheep, and buffalo milk, iron concentration decreased by 35–50% during the first 3 days of lactation. Thus, the change in milk iron in most dairy animals may be rapid; however, as in human milk, the change may be observed only when care is taken to collect milk during the first days or even hours of lactation. Goat and horse milk have iron concentrations of approximately

1–2 μg/ml in colostrum, and around 0.3–0.9 μg/ml in mature milk (65a). In sow milk iron concentration has been reported as approximately 3 μg/ml (113, 143).

Iron concentration in rat colostrum was approximately 9–11 μg/ml (64), declining during the first week of lactation to approximately 4–5 μg/ml (61, 77). Rabbit milk contains 2–4 μg/ml (138) and the Australian marsupial *Sentyx brachyeus* has a similar iron concentration (78). For both these species a considerable decline occurred during lactation.

The iron concentration of dog milk (~10 μg/ml) is considerably higher than that of human milk and milk from dairy animals, but of about the same magnitude as that of rat milk (85). As with other species, the concentration of iron in dog milk is strongly influenced by the stage of lactation and decreases with time.

The dog, like the rat and some marsupials, is able to secrete a milk in which iron concentration may be some 10 times higher than in serum. This phenomenon suggests that the transfer of iron into, or retention by, the mammary tissue of these species may occur through different mechanisms than in those species in which milk iron content is equal to or lower than its concentration in serum.

Cat milk has iron levels of 5–6 μg/ml in the early part of lactation, later decreasing to about 3 μg/ml (65a). An unusual finding was that colostrum during the first 2 days contained considerably less iron than did milk later in lactation.

Normal levels of iron in milk of the species described above are shown in Table 1.

Table 1 Concentrations of iron and copper in milk from different species

	Iron		Copper	
Species	Colostrum (μg/ml)	Mature milk (μg/ml)	Colostrum (μg/ml)	Mature milk (μg/ml)
Human	0.6–1.0	0.2–0.4	0.3–0.6	0.2–0.3
Cow	0.5–0.7	0.2–0.3	0.2–0.3	0.1–0.2
Buffalo	1.5–1.0	0.2–0.3	0.3–0.4	0.2–0.3
Goat	1–2	0.3–0.4	0.4	0.1–0.2
Sheep	0.4–1.0	0.4–0.6	0.5–1.4	0.2–0.4
Horse	1–2	0.3–0.9	0.6	0.2–0.4
Pig	1–2	1–3	6.0	0.6–1.0
Rabbit	—	2–4	—	—
Rat	9–11	4–5	8–13	1.5–3.0
Dog	10–15	5–10	1.5–2.0	1.5–2.0
Cat	5–6	3	1–2	~1.0

COPPER

Human Copper levels of human milk range from 0.15 to 1.34 μg/ml (13, 22, 47, 73, 80a, 100, 102, 105, 112, 117, 147) and are reported to diminish during lactation (6, 73, 80a, 142, 147). Kleinbaum found that colostrum contained 1.34 μg/ml, with the values decreasing gradually to 0.26 μg/ml 5–6 months after parturition (73). These colostrum values, considerably higher than those reported by other investigators, might be explained by differences in methodology, especially with regard to sample collection (contamination). The concentration of copper in human milk is 3–4 times lower than in serum.

Domestic and experimental animals As in man, the milk copper concentration of most other species is low, with values less than 1 μg/ml (cow, buffalo, goat, sheep, horse, pig, cat) (21, 65a). In several species studied a change in milk copper concentration occurs during lactation, with values decreasing with lactation time (21, 64, 65a).

Although the rat exhibits a decrease with lactation time in milk copper concentration similar to that of most other species, the values may be an order of magnitude higher (64). Thus, values for colostrum may be as high as 12 μg/ml, with mature milk having concentrations around 2 μg/ml. In the dog, however, milk copper concentration is not influenced by the stage of lactation (85). The values for copper concentration (~1.7 μg/ml) are higher than are those in human colostrum (0.3–0.6 μg/ml) but lower than in rat colostrum (~12 μg/ml). The cat has an unusual pattern of milk copper concentration: The values were found to be lowest on the first day of lactation (65a), increasing to approximately 1.5–2.0 μg/ml in early lactation and then falling to 1.0 μg/ml. Normal levels of copper in milk of the species described above are shown in Table 1.

ZINC

Human The zinc content of human milk ranges from 0.65–5.3 μg/ml (6, 9, 13, 22, 80a, 102, 105, 112, 117, 147). Values reported in earlier studies seem to agree with those published recently, indicating good agreement among different analytical techniques used. There is a very pronounced decrease in zinc concentration during lactation, with the concentration dropping from approximately 6 to ~0.6 μg/ml in mature milk. Milk zinc concentrations seem to be similar to those in serum for the major part of lactation (144).

Domestic and experimental animals For several species studied, milk zinc concentrations are similar to those of human milk, at a level of approximately 1–5 μg/ml for mature milk (2, 10, 21, 84a, 110, 129). However, in other species, milk zinc concentration is considerably higher. In the pig, goat, rat, cat, and dog, the concentration of zinc in mature milk is approximately 5–10 μg/ml (64, 65a, 84a, 85, 103, 136).

In dairy animals, a precipitous drop in milk zinc concentration with lactation time occurs (21), with values decreasing as much as 50% during the first 72 hr of lactation, after which little change occurs. In other species, such as the rat, concentrations of zinc do not decrease during the first days of lactation (~13 μg/ml), but after the first week, a continuous decrease in milk zinc occurs until the end of lactation (5 μg/ml) (64).

In canine milk, concentration of zinc decreases slightly during lactation, although the percentage drop is small. This species seems to have the highest concentration of zinc reported in mature milk (85).

Zinc in cat milk did not exhibit a developmental pattern; levels of 5–6 μg/ml are found throughout lactation. However, as with iron and copper, zinc levels were lower on the first day of lactation (65a). Normal levels of zinc in milk of the species described above are shown in Table 2.

MANGANESE

Human Information is very limited regarding the manganese concentration of human milk and its variation during lactation, with only a few studies of manganese in human milk having been reported. In contrast to the pattern of decline found for iron, copper, and zinc, a parabolic curve for milk manganese occurs, with values averaging 6 μg/liter in early lactation, 4 μg/liter in mid-lactation (2–6 months), and 6–8 μg/liter in late lactation (142, 146). McLeod & Robinson (94) earlier reported values of approximately 15 μg/liter for mature breast milk. The discrepancy between these reports could be explained either by a difference in analytical techniques or in manganese status of the two populations. The levels of manganese in human milk are about one tenth of those reported for other species (see below); plasma levels are similar to those reported for milk (144).

Domestic and experimental animals A developmental pattern has been observed for manganese in cow's milk, with levels of 100–160 μg/liter in colostrum and 20–50 μg/liter in mature milk (3, 21, 130). The manganese content of sheep, goat, and buffalo milk is approximately the same as in cow's milk (10, 47, 94). In these species, in contrast to man, an increase of manganese in late milk has not been reported.

The manganese concentration in rat milk is of the same magnitude as that of most dairy animals, with values ranging from 100 to 500 μg/liter. Like human milk, a parabolic curve has been reported for manganese concentra-

Table 2 Concentrations of zinc and manganese in milk from different species

	Zinc		Manganese	
Species	Colostrum (μg/ml)	Mature milk (μg/ml)	Colostrum (μg/ml)	Mature milk (μg/ml)
Human	3–6	0.5–1.0	0.006	0.004–0.015
Cow	5–7	3–5	0.10–0.16	0.02–0.05
Buffalo	6–9	4–6	0.10–0.16	0.02–0.05
Goat	13.0	5–6	0.10–0.16	0.02–0.05
Sheep	5–15	1–2	0.10–0.16	0.02–0.05
Horse	2.4	1–2	—	—
Pig	10.7	4.5–6.0	—	—
Rat	12–15	5–12	0.5–0.6	0.1–0.3
Dog	8–13	6–10	0.15	0.15
Cat	5–6	5–6	0.30	0.30

tion in rat milk (64): levels of 500–600 μg/liter in colostrum, about 100 μg/liter in mid-lactation and around 300 μg/liter in late lactation.

In contrast to other species studied, manganese concentration of canine and feline milk was not different by the stage of lactation (65a, 85). The concentration of manganese approximated 150 μg/liter in dog milk and 300 μg/liter in cat milk.

Normal levels of zinc in milk of the species described above are shown in Table 2.

DIETARY INFLUENCE ON MILK COMPOSITION—EFFECTS OF MATERNAL TRACE ELEMENT DEFICIENCY OR EXCESS

In this section, we discuss the influence of the trace element content of the maternal diet on milk composition. In a subsequent section, the effects of potential perturbations on the suckling are discussed.

Iron

In the human, data reveal little correlation between maternal iron intake and the concentration of iron in breast milk. A number of investigators (62, 101, 148) have reported that supplementation of iron to mothers with an adequate iron status has no appreciable effect. Furthermore, Karmarkar & Ramakrishnan (62) and Murray et al (101) found that the iron content of mother's milk was not reduced even when the iron status of the women was poor. Loh & Sinnathury (79) similarly observed that although the iron content of breast milk of Chinese, Malaysian, and Indian women varied with their ethnic origins, it appeared to be independent of maternal levels of hemoglobin, serum iron, and total iron-binding capacity. However, in the rat supplementation with an iron chelate of a diet adequate in iron has been shown to increase milk iron content (29, 44, 66), and conversely, dietary iron deficiency during pregnancy and laction caused lower than normal concentration of iron in milk (44, 133, 134). Ezekiel & Morgan (31) reported that the concentration of milk iron in rats may also be depressed by repeated bleeding. In cows, sows, and goats (2, 26, 113), iron supplementation did not appreciably effect milk iron content.

Copper

In humans, the addition of copper to a diet already adequate in this element has little influence on the copper concentration of the milk (72, 100, 148). To our knowledge, no reports are available regarding the effect of suboptimal concentrations of dietary copper on the copper concentration of human milk.

In both rats and sheep, consumption of copper-deficient diets resulted in milk with significantly lower than normal copper concentration (8, 71). The copper concentration of milk in rats may also be lowered by diets high in zinc (14). This may have important implications when considering the use of zinc supplements by lactating mothers. In some dairy animals, copper supplementation does not appear to increase copper concentration of milk when the diet is adequate in the element (2, 27). However, some reports on cow's milk show that the copper content of milk may be increased by supplementation (102). In contrast, dietary supplementation with a copper chelate did increase rat milk copper content (66). Interestingly, the shape of the developmental curve for milk copper appears to be similar in copper-deficient and copper-supplemented dams (66, 71).

Zinc

Zinc supplementation of a zinc adequate diet did not appreciably affect the concentration of this element in human milk (72, 148). There are no reports of the effect of dietary zinc deficiency on the zinc concentration of human milk. One report has suggested that low levels of zinc in breast milk might have been related to zinc deficiency in two infants (153). However, dietary supplementation to their mothers did not increase the zinc content of their milk. It would be of great interest to analyze the milk of women in geographical regions where zinc deficiency or marginal zinc status occurs in significant numbers, especially in view of the severe effects of zinc deficiency during the perinatal and early postnatal periods.

In dairy animals, and in the sow, the zinc content of milk can be increased by dietary supplementation (2, 97, 136). In the rat, some investigators have found an effect of zinc supplementation on milk zinc content (17), whereas others have not (66). This discrepancy may be due to a difference in the zinc content of the basal diet, as the zinc level of the diet where an effect was observed may have been marginally deficient (64). Since female rats fed a zinc-deficient diet beginning at parturition produced milk low in zinc (103, 104), the observed positive effect of supplemental zinc (14) may have been due to a correction of a marginal deficiency.

Manganese

In humans, as well as in cows, the manganese concentration of milk may be elevated by increasing the dietary manganese intake (2, 148), but it is not known whether or not such manipulation can obliterate its normal developmental pattern. The report by Vuori et al (148) of a significant correlation in humans between dietary intake and milk manganese concentration is particularly interesting. Similar correlations were not found between maternal intake and breast milk concentrations of iron, copper, or zinc. An important question is whether the increase in milk manganese concentration was due to a correction of suboptimal manganese status or if manganese is indeed different from iron, copper, and zinc with respect to the influence of dietary supplementation. The latter seems unlikely, since, in general, concentrations of manganese in animal tissues seem to be under close homeostatic control (141). This question might be clarified by better information on the dietary intake of lactating mothers and its correlation with milk concentration. It can be noted, however, that a difference in manganese concentration of milk from mothers from Finland (146) and New Zealand (94) was reported. This difference is not necessarily due to a difference in manganese status, but as Vaughan et al (142) have indicated that manganese intake affects milk manganese concentration. Investigation is warranted.

Comments

The levels of trace elements in milk from domestic and experimental animals can be influenced by either dietary deficiency or excess. The functional implications of these changes in milk trace element concentrations are discussed in a following section. Although there is little evidence to suggest that the trace element concentration of human milk is affected by dietary intake, neither the levels of supplementation used nor the degree of deficiency observed were as high as those reported in experimental animals. More research on the effect of dietary intake on milk trace element concentration is necessary before concluding that human milk differs from that of other species in being resistant to dietary influence.

TRACE ELEMENT BINDING FACTORS IN MILK

In previous sections the trace element concentration of milk was shown to have distinct developmental patterns which suggests metabolic regulation.

In all cases, concentrations of trace elements of a given species during a major part of lactation fall within a fairly narrow range (even if these concentrations may be modified to some degree by dietary deficiency or excess), again suggesting metabolic regulation. In this section we examine the distribution (binding) of these elements among the various components of the milk. In the following section we discuss possible effects of such binding on bioavailability of the elements.

Iron

Three individual proteins in milk have been reported to bind iron in various degrees: lactoferrin, transferrin, and casein (11, 29, 39, 77, 78, 90, 91). Lactoferrin and transferrin have similar molecular weights (~86,000) and structures; however, immunologically and functionally they differ. Transferrin was first detected in serum whereas lactoferrin was first detected in milk (90). Recently, transferrin has been detected in milk and lactoferrin has also been found in serum (90). However, the concentrations of these proteins are usually considerably higher in the fluids in which they were originally detected than in the others. Both lactoferrin and transferrin bind two molecules of iron per molecule of protein, but lactoferrin has a much higher binding affinity than transferrin and does not release iron until the pH is lower than 2; transferrin releases its iron at or below pH 4. Casein, the major protein of most milks, is an aggregate of several types of casein (α, β, γ, and κ) as well as of calcium, phosphate, and citrate (76), that together form micelles of considerable size.

In human milk, concentrations of lactoferrin are high (39, 82, 91), usually around 1–3 mg/ml. Lactoferrin is thus one of the major human milk proteins. Transferrin concentration is very low in human milk (39, 91), with values around 0.01 mg/ml. Earlier, lactoferrin was reported to be saturated with iron to 10–30% (11, 90), but recently it has been shown that the iron saturation of human milk in vivo is very low (~1–4%); in fact, lactoferrin can account for only a minor part (~10–30%) of the total iron in human milk (39). The major iron-binding compounds in human milk were localized in the milk fat fraction and a low-molecular-weight fraction (<15,000) (39). Recently, iron in human milk fat was reported to be bound to xanthine oxidase, an enzyme that is part of the fat globule membrane (40).

The localization of a major part of iron in human milk in the fat fraction is in accordance with reports that substantial amounts of iron occur in bovine milk fat (70, 120, 131). Concentrations of lactoferrin and transferrin in cow's milk are low, whereas the casein content is high (51, 91). Hegenauer et al have shown that casein is the major iron-binding protein in cow's milk and that the phosphate groups of α-casein are responsible for this binding (54). Casein concentration is high in rat milk and this protein has also been reported to be the major iron-binding protein in the milk of this species (78).

To prevent the risk of iron deficiency anemia, iron is frequently given to infants, either as a supplement to breast milk or in a formula based on cow's milk (36). Not much is known regarding the binding of excess iron to breast milk. In cow's milk, however, extensive studies have regarded the localization of added iron. Hegenauer et al (52, 53) have demonstrated that iron will bind to different components of cow's milk depending on the nature of the iron supplement used. Density microcentrifugation experiments (54) have shown that ferrous sulfate, a common iron supplement, donates less iron to the casein micelle than many other iron complexes tested. Unhomogenized milk supplemented with ferrous salts results in milk with a high iron content in the milk fat fraction. The affinity of Fe(II) for the milk fat fraction is responsible for the intense lipid peroxidation and objectionable "off-flavor" associated with iron-supplemented milk (52, 53). Homogenization of milk, by causing adsorption of casein onto the fat globule membrane, is responsible for the increased affinity of the milk fat for added iron.

The Fe(III)-nitrilotriacetate complex donates iron rapidly and specifically to the casein fraction of cow's milk, which has a binding capacity for iron approximately 100 times greater than the iron concentration added to formula for pediatric use. Casein-bound iron is stable throughout the fractionation of milk by isoelectric precipitation, ultracentrifugation, and anion-exchange chromatography in denaturing media. Alpha-casein is the principal sequestrant of supplemental iron added to cow's milk. Hegenauer et al have drawn particular attention to the facile exhange of iron between casein and the ferric chelates of nitrilotriacetate and lactobionate, which appear to be promising milk and food supplements on the basis of relative inertness in promoting lipid oxidation (52, 53) and on nutritional evaluations (12, 66).

Zinc

In contrast to iron, studies on the localization of zinc in milk began quite recently. In part, these studies were stimulated by the observation that the disease acrodermatitis enteropathica, a genetic recessive disorder of zinc metabolism, could be treated with human milk (99), whereas cow's milk was not efficacious. The symptoms usually appear after weaning from the breast to cow's milk, and they can be overcome either by human milk or by zinc supplements (99). Since the zinc concentration of these two milks is similar, this observation led to the hypothesis by Eckhert et al that the localization of zinc in human milk is different from that in cow's milk, making it of increased bioavailability to humans (24).

Supporting this hypothesis is the observation that in human milk a significant fraction of zinc is associated with a low-molecular-weight zinc binding ligand (ZBL), whereas in cow's milk zinc is bound almost exclusively to high-molecular-weight compound(s) (24). The ZBL from human milk has now been isolated and identified as citrate (87). Others have claimed that the low-molecular-weight zinc complex in human milk is zinc picolinate (28). In our opinion, however, this identification is inappropriate because of methodological problems (80, 86). Furthermore, the identification of citrate as the low-molecular-weight ZBL in human milk has recently been confirmed by Martin et al (89).

The concentration of citrate was 0.99 ± 0.12 mM in human milk ultrafiltrates and 1.72 ± 0.11 mM in corresponding samples from bovine milk. Thus, although the citrate concentration was somewhat lower in human milk than in bovine milk, the amount of zinc complexed to citrate in human milk was about 4 times higher. It is reasonable to suppose that the high-molecular-weight zinc binding compound(s) of cow's milk is casein, which is present in cow's milk at a concentration about 10 times that of human milk (51). This protein forms hard curds in the stomach of the newborn infant and a considerable portion therefore may pass the gastrointestinal tract virtually undigested (35). It is possible that zinc bound to casein may be inaccessible to the young infant.

In human milk, on the other hand, we have recently demonstrated that casein binds only a small fraction of the total zinc. The major zinc-binding protein in human milk is serum albumin (82a). We have also found that a significant portion of the zinc in human milk is bound to the fat fraction. Alkaline phosphatase, bound to the fat globule membrane, appears to be the major zinc-binding protein of milk fat (G.-B. Fransson, B. Lönnerdal, manuscript in preparation).

Copper

The localization of copper in human milk has received little attention. Assuming that human milk may contain low-molecular-weight complexes of copper, as well as of zinc, Williams et al (151) suggested that human milk may also be therapeutic in the treatment of Menkes' kinky hair disease, a sex-linked genetic disorder in humans manifested by abnormal intestinal copper absorption and many characteristics similar to those of copper-deficient animals (57). These investigators therefore evaluated human milk therapy, but it was not effective.

However, recent studies demonstrate that in contrast to zinc (87) and iron (39), no low-molecular-weight complexes of copper occur in human milk (84). The lack of such a low-molecular-weight copper complex may perhaps explain, at least in part, the failure of human milk as a therapeutic treatment for Menkes' disease. It is also possible this disease should be treated prenatally (42, 68). If copper could be added to milk in a form that would produce a low-molecular-weight complex, it might increase the bioavailability of copper for these patients. The bioavailability of copper complexed with the high-molecular-weight copper-binding proteins is not known, even for normal infants. Recent data show that copper is to some degree bound to casein, whereas the major copper-binding protein is serum albumin, as it is for zinc (82a).

In cow's milk, King et al (70) have shown that copper, like iron, is bound partially to the fat fraction (~15%). The remainder is bound to casein and to some constituent in the whey fraction. Added copper is bound predominantly to the casein.

Manganese

Unlike iron, zinc, and copper, there have been very few examinations of the molecular localization of manganese in milk. Manganese has been reported to be present partly in the fat globule membrane (102). Also, lactose synthase, present in milk, is a manganese-dependent enzyme (119). Some years ago it was reported that the development of an oxidized flavor in cow's milk could be retarded by addition of manganese (43). One explanation for this observation is that manganese may bind to some sites to which iron and copper bind, and their displacement then leads to a reduction of fat oxidation. Whether such a situation exists in vivo remains to be investigated.

Comments

Various trace elements in milk bind to different compounds. The major trace element complexes found thus far include enzymes, metalloproteins, and low-molecular-weight compounds. The localization of these elements to their respective ligands appears to be quite specific.

It is apparent that one of the areas requiring more attention is the molecular localization of copper and manganese in milk. When attempting to identify trace element complexes naturally occuring in milk, the analytical conditions, if not carefully controlled, can lead to spurious results. Improper use of the gel filtration technique (often an initial step in molecular localization of trace elements) is, in our opinion, a major source of error. Gel filtration is a separation method based on the size of molecules. However, gels (supposedly charge free) actually contain small but significant surface charges. Such gels act as ion exchangers and cause ligand exchange (80, 86). We have shown that this problem can be eliminated by treating the gels with a reducing agent (80). However, in the majority of the reports untreated gels were used in molecular localization of trace elements. Thus, the data must be evaluated with caution.

BIOAVAILABILITY OF TRACE ELEMENTS IN MILK

In the previous sections the concentrations of trace elements in milk and their molecular localization were discussed. In the light of this knowledge this section addresses the bioavailability of the trace elements.

Iron

Iron has received most attention in pediatric nutrition. The interest has focused on the relative bioavailability of iron in human milk and in cow's milk, as most infant formulas are based on cow's milk. The concern has been to prevent the iron deficiency anemia earlier often found in childhood.

Studies in man of iron absorption from human milk have been very limited. In an early study that used the conventional balance technique, absorption values of 45 and 75% were found for two infants (33). Later work (41, 46) also indicated a very high absorption of iron from human milk: between 50 and 100% during the first months of life. With adults, McMillan et al (96) found iron absorption values around 19%. By making calculations from the amount of iron incorporated into red blood cells and estimating the iron absorption of infants to be twice the rate of adults, these authors believe an absorption rate of 50% may be reasonable. This figure agrees with the results of Saarinen et al (126), who found the iron absorption of infants to be 49%. These investigators used an extrinsic tag and studied iron absorption in infants by using very low doses of radioactivity and a sensitive whole body counter. Using another approach, the same investigators (125) estimated the iron absorption to be around 70%. In this case the figure was based on changes in total body iron during infancy.

Iron absorption by infants from cow's milk has also been studied by using radioisotopes. Considerably lower absorption figures have been found, usually around 10% (95, 126, 132). To compensate for the relatively low bioavailability of iron in cow's milk, formulas are usually supplemented with iron in various forms. Since the amount of iron absorbed is dependent on the iron content of the food, a high iron content will decrease the percentage of iron absorption (55). Thus, only 3-4% of the iron is absorbed from infant formulas supplemented with high doses of iron (12 mg/liter) (96, 121). Levels of 6–12 mg of iron per liter of formula have been found to be adequate and are frequently recommended (15, 16, 124).

The reason for the exceptionally high bioavailability of iron in human milk is not yet known. It has been suggested that the major iron-binding protein in human milk, lactoferrin, is at least partly responsible for the bioavailability of iron. However, recent reports do not seem to support this hypothesis. In fact, the study of McMillan et al (96), as well as that of de Vet & van Gool (23), indicates a negative effect of lactoferrin on iron absorption. This observation may also be related to the finding that only a minor portion of the iron in human milk is bound to lactoferrin (39). However, it should be noted that Cox et al (18) have reported that lactoferrin may facilitate iron transport in vitro across the brush border by delivering it to specific protein binding sites at the cell surface. The apparent contradiction between these observations needs to be resolved.

It is often discussed whether the iron content of human milk is sufficient for the breast-fed infant, or whether these infants should be supplemented (15, 16, 36, 83). However, in those studies where the iron status of breast-fed infants has been carefully evaluated by several criteria and compared to infants receiving supplemented formulas, iron status parameters were similar after 6 months of breast-feeding (95, 123, 152). These studies were performed on normal, full-term infants. However, caution should be taken with regard to preterm infants. Dauncey et al (20) have shown that preterm infants are in negative iron balance and therefore may have greater needs for iron. There are very few reports on the composition of milk from mothers who delivered prematurely (5, 48); the trace element content of this milk has not yet been characterized.

In the adult rat, iron absorption is normally 5–15% of intake (75). In contrast, during the first 14 days of postnatal life, the infant rat can absorb almost 100% of the iron ingested; this value drops to about 25% at 30 days of age (30, 75). The high absorptive capacity of the neonatal rat may be due to pinocytosis, an active process in the intestinal mucosa during the first 2 weeks of postnatal life (30).

Although the concentration of rat milk iron is high, and its absorption is virtually total, it is not clear if the resulting amounts ingested are optimal for the developing rat, since liver iron stores are depleted during the suckling period (63, 92, 93). It has recently been shown that the iron content of rat milk can be increased by dietary supplementation, and furthermore, that this increase is reflected in various tissues of the suckings (66). Such an increase has also been demonstrated in tissues from mice whose dams were given iron supplements (12). It is not known, however, if such an increase is beneficial to the young.

Zinc

The interest in bioavailability of zinc in milk was aroused by the findings in Colorado that zinc supplementation of an infant formula led to improved growth of male infants (150). The rationale for the supplementation was that some "normal" children had very low hair zinc levels, similar to those reported for dwarfs in the Middle East, suggested to be zinc deficient (115). It was found that the zinc content of the formulas used was less than 2 mg/liter, considerably less than the 3–4 mg/liter normally present in whole cow's milk. In a double-blind controlled study it was found that unsupplemented male infants grew less and had lower plasma zinc levels than those who received the zinc supplement. However, even with levels of 5.8 mg of zinc/liter (which is considerably higher than that of human milk, about 1–3 mg/liter in mature milk), plasma zinc values were significantly lower than in breast-fed infants (50). Similar findings of higher serum zinic levels of breast-fed infants than those of infants fed a formula supplemented with zinc have also been reported in Japan (108). Thus, it appears that zinc in breast milk has higher bioavailability than zinc added to a formula in the form of a salt. It is also apparent that zinc in cow's milk is less bioavailable than zinc from human milk.

Zinc citrate has been shown to be very easily absorbed (45, 145). Very little is known about the magnitude of zinc absorption from various milks in the human. Using the rat as a model, Johnson & Evans (60) studied the relative zinc availability in human milk, cow's milk, and infant formulas. A zinc absorption value of 59% was given for human milk, 42–50% for cow's milk, and 27–39% for infant formulas. However, it should be noted that the zinc levels of the milks and formulas used were very different, ranging from 0.4 to 6.7 mg/liter. As with iron the total amount of zinc present will affect the percentage absorbed (128); therefore, the values presented by Johnson & Evans (60) should be interpreted with caution. Furthermore, as it has been demonstrated that the rat is quite different from the human with regard to total zinc content in the milk as well as in its binding compounds (86), the use of the rat as a model for zinc bioavailability in infants must be questioned.

In the rat, zinc, in contrast to iron, was not found to be increased in the milk by dietary supplementation (66). Whether this was due to differences in binding characteristics for the two elements of the vehicle of supplementation or to a difference in homeostatic control remains to be investigated. In mice, it is believed that the bioavailability of zinc is high, particularly in the early neonatal period. Nishimura (107) has reported that when newborn mice were deprived of zinc-rich colostrum and fostered onto dams in mid- or late lactation, they developed severe zinc deficiency. This phenomenon is not found in the rat, suggesting either a difference in the bioavailability of milk zinc between the two species or of zinc metabolism in the neonate (63).

Copper and Manganese

Information regarding the bioavailability of the elements copper and manganese is very limited. For copper this is most likely explained by the lack of a convenient radioisotope; ^{64}Cu has a very short half-life, which makes absorption studies almost impossible. However, the observations in rat and mouse (116, 139) that liver copper concentration may increase in the neonate and that levels remain constant in the young dog liver suggest that bioavailability of milk copper is high (65). The very low levels of manganese in milk and milk products and the resulting difficult analytical problems have undoubtedly limited research on this element. In our opinion, increased emphasis should be given to bioavailability studies of these two elements, especially since copper deficiency is reported in formula-fed infants (1, 137), and since studies have indicated suboptimal plasma manganese levels in infants that could be improved by supplementation (109).

INFANT FORMULAS

This review has so far dealt with milks of various species. Although homologous milk is generally regarded as the most appropriate food for the neonate, milk substitutes are often given. For the human infant, formulas are usually based on cow's milk or soy protein. The composition of the cow's milk or soy protein formula is modified and adjusted ("adapted" or "humanized") so that the substitute will more closely fit the metabolic needs of the infant. Thus, for example, the osmolarity and the protein content are reduced, whereas the lactose content is increased (51). Similar approaches have been taken with regard to the trace element content. As discussed above, the bioavailability of trace elements is not the same from cow's milk as from human milk, or when they are added as inorganic salts. Therefore, higher amounts of trace elements are given to compensate for the lower availability. At present, the Academy of Pediatrics (15–17) recommends that iron and zinc be added to formulas; this is now customary, as is additions of copper and manganese. However, the amounts added vary considerably and we have found several formulas that have very low levels of trace elements (65a, 85a). Several types of iron supplementation have been used (35). Inorganic iron salts such as ferrous sulfate and sodium iron pyrophosphate are now most commonly used, but elemental iron has also been used.

It has been suggested that lactoferrin in human milk, which is present in a largely unsaturated form, has a bacteriostatic function (11). The strong affinity of lactoferrin for iron would enable this protein to sequester iron from its surroundings and thus prevent the growth of harmful bacteria that require iron. That this postulated mechanism does, in fact, work to some extent has been demonstrated in vitro with several strains of bacteria such as *Escherichia coli, Streptococcus mutans,* and *Vibrio cholerae* (4, 11). However, definitive in vivo evidence is still lacking. Some workers have argued that the abundance of iron in the intestine of iron-supplemented breast-fed infants could actually promote bacterial growth by saturating lactoferrin.

This problem could presumably be even more serious in the case of infants fed formulas, whose concentration of lactoferrin is negligible. It is interesting to note that the incidence of diseases such as gastrointestinal infections is far lower in breast-fed than in bottle-fed infants (19, 32). A bacteriostatic function of lactoferrin may play a role in these infection defense mechanisms, but certainly other factors such as immunoglobulins and enzymes are also part of these mechanisms (51).

Zinc, in the form of a salt, is now generally used to supplement cow's milk formulas at a level of 3–4 mg/liter, and larger supplements are added to soy-based formulas (17).

Since the report of Al-Rashid & Spangler in 1971 (1) of copper deficiency in a premature infant receiving a low-copper formula, formulas are supplemented with copper to raise the content to between 0.4–0.6 mg/liter (149). Even higher concentrations are present in some special formulas for premature infants (149). However, this matter requires additional attention since cases of copper deficiency are identified in infants fed on some formulas (137).

Manganese is added to some infant formulas in the form of inorganic salts. However, a wide variation in manganese content occurs among formulas, and from the extremely low content in some of them it is obvious they are not supplemented (65b, 85a). Since no strong arguments proposing manganese supplementation have been advocated and the bioavailability of added salts remains unknown, further research in this area is needed.

Supplementation of infant formulas with trace elements is now common and data supporting supplementation are strong for iron and zinc. However, as so little is known about the biological availability of these elements, the optimal levels have not yet been adequately determined. There is a risk that too high levels may compromise the infant in other ways, as discussed, or by competitive inhibition of the absorption of one element with another, such as can occur with zinc and copper (14). An interesting question is whether the supplements could be given in other forms, thereby making them more available, and/or thus circumventing the potential negative effects an excess might produce (83). All these aspects should be investigated thoroughly before high levels of trace elements are routinely added to infant formulas.

CONCLUDING REMARKS

We have summarized current knowledge concerning concentrations of iron, copper, zinc, and manganese in milks of different species and how they change during the course of lactation. It is apparent that great variability exists among species in the absolute concentrations as well as in the developmental changes in concentration of these elements. Furthermore we have shown differences in molecular localization of these elements in the various milks and have suggested that such differences may affect bioavailability.

The composition of the maternal diet has been shown to influence to some extent the trace element composition of milk in experimental animals. However, the very limited data available for humans suggest that human milk may be relatively unaffected by the diet of the lactating women. Whether this apparent discrepancy between milk of human and other species is due simply to a paucity of information, or is in fact a function of different homeostatic mechanisms, remains to be investigated.

The concentration in infant formulas of the trace elements iron, copper, zinc, and manganese has also been reviewed. Research in this area has been very limited; little is know about optimal levels, optimal modes of supplementation, or potential interaction effects of these elements in infant formulas. These problems constitute important and potentially fruitful avenues of future research.

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Literature Cited

1. Al-Rashid, R. A., Spangler, J. 1971. Neonatal copper deficiency. *N. Engl. J. Med.* 285:841–43
2. Archibald, J. G. 1958. Trace elements in milk: A review—Pt. II. *Dairy Sci. Abstr.* 20:325–30
3. Archibald, J. G., Lindquist, H. G. 1943. Manganese in cows' milk. *J. Dairy Sci.* 26:325–30
4. Arnold, R. R., Cole, M. F., McGhee, J. R. 1977. A bactericidal effect for human lactoferrin. *Science* 197:263–65
5. Atkinson, S. A., Radde, I. C., Chance, G. W., Bryan, M. H., Anderson, G. H. 1980. Macro-mineral content of milk obtained during early lactation from mothers of premature infants. *Early Human Develop.* 4:5–14
6. Belavady, B. 1978. Lipid and trace element composition of human milk. *Acta Paediatr. Scand.* 67:566–71
7. Belavady, B., Gopalan, C. 1959. Chemical composition of human milk in poor Indian women. *Ind. J. Med. Res.* 47:234–45
8. Bennetts, H. W., Chapman, F. E. 1937. Copper deficiency in sheep in Western Australia: A preliminary account of the aetiology of enzootic ataxia of ewes. *Aust. Vet. J.* 13:138–49
9. Berfenstam, R. 1952. Studies on blood zinc: A clinical and experimental investigation into the zinc content of plasma and blood corpuscles with special reference to infancy. *Acta Paediatr.* 41: Suppl. 87, pp. 43–49
10. Brock, A., Wolff, L. K. 1935. The quantity of copper, zinc, and manganese in cows' milk. *Acta Brevia Neerland. Physiol. Pharmacol. Microbiol.* 5:80–81
11. Bullen, J. J., Rogers, H. J., Leigh, L. 1972. Iron-binding proteins in milk and resistance to *Escherichia coli* infection in infants. *Br. Med. J.* 1:69–75
12. Carmichael, D., Hegenauer, J., Lem, M., Ripley, L., Saltman, P., Hatlen, L.

1977. Iron supplementation of the lactating mouse and suckling neonates. *J. Nutr.* 107:1377–84

13. Cavell, P. C., Widdowson, E. M. 1964. Intakes and excretions of iron, copper, and zinc in the neonatal period. *Arch. Dis. Child.* 39:496–501
14. Chu, R. C., Cox, D. H. 1970. Excessive dietary zinc during lactation and nutritive value and mineral composition of rat's milk. *Nutr. Rep. Int.* 2:179–84

14a. Clegg, M. S., Keen, C. L., Lönnerdal, B., Hurley, L. S. 1981. Influence of ashing techniques on the analysis of trace elements in animal tissues. I. Wet ashing. *Biol. Trace Elem. Res.* Submitted for publication

14b. Clegg, M. S., Keen, C. L., Lönnerdal, B., Hurley, L. S. 1981. Influence of ashing techniques on the analysis of trace elements in animal tissues. II. Dry ashing. *Biol. Trace Elem. Res.* Submitted for publication

15. Committee on Nutrition, American Academy of Pediatrics. 1976. Iron supplementation for infants. *Pediatrics* 58:765–68
16. Committee on Nutrition, American Academy of Pediatrics. 1978. Relationship between iron status and incidence of infection in infancy. *Pediatrics* 62:246–50
17. Committee on Nutrition, American Academy of Pediatrics. 1978. Zinc. *Pediatrics* 62:408–12
18. Cox, T. M., Mazurier, J., Spik, G., Montreuil, J., Peters, T. J. 1979. Iron-binding proteins and influx of iron across the duodenal brush border. *Biochim. Biophys. Acta* 588:120–28
19. Cunningham, A. S. 1979. Morbidity in breast-fed and artificially fed infants. II. *J. Pediatr.* 95:685–89
20. Dauncey, M. J., Davies, C. G., Shaw, J. C. L., Urman, J. 1978. The effect of iron supplements and blood transfusion on iron absorption by low birth weight infants fed pasteurized human breast milk. *Pediatr. Res.* 12:899–904
21. De Maria, C. G. 1978. Trace element content in colostrum of different ruminant species at various post-partum intervals. *Ann. Rech. Vet.* 9:277–80
22. Department of Health and Social Security. 1977. *Report on Health and Social Subjects: The composition of mature human milk*, p. 18. London: Her Majesty's Stationery Off. 47 pp.
23. de Vet, B. J. C. M., van Gool, J. 1974. Lactoferrin and iron absorption in the small intestine. *Acta Med. Scand.* 196:393–402
24. Eckhert, C., Sloan, M., Duncan, J. R., Hurley, L. S. 1977. Zinc binding: A difference between human and bovine milk. *Science* 195:789–90
25. Eden, A., Green, H. H. 1940. Microdetermination of copper in biological material. *Biochem. J.* 34:1202–08
26. Elvehjem, C. A., Herrin, R. C., Hart, E. B. 1926. Iron in nutrition. III. The effects of diet on the iron content of milk. *J. Biol. Chem.* 71:255–62
27. Elvehjem, C. A., Steenbock, H., Hart, E. B. 1929. The effect of diet on the copper content of milk. *J. Biol. Chem.* 83:27–34
28. Evans, G. W., Johnson, P. E. 1980. Characterization and quantitation of a zinc-binding ligand in human milk. *Pediatr. Res.* 14:876–80
29. Ezekiel, E. 1965. The iron-binding proteins in milk and the secretion of iron by the mammary gland in the rat. *Biochim. Biophys. Acta* 107:511–18
30. Ezekiel, E. 1967. Intestinal iron absorption by neonates and some factors affecting it. *J. Lab. Clin. Med.* 70: 138–49
31. Ezekiel, E., Morgan, E. H. 1963. Milk iron and its metabolism in the lactating rat. *J. Physiol.* 165:336–47
32. Fallott, M. E., Boyd, J. L. III., Oski, F. A. 1980. Breast-feeding reduces incidence of hospital admissions for infection in infants. *Pediatrics* 65:1121–24
33. Feuillen, Y. M. 1954. Iron metabolism in infants. II. Absorption of dietary iron. *Acta Paediatr.* 43:181–87
34. Feuillen, Y. M., Plumier, M. 1952. Iron metabolism in infants. I. The intake of iron in breast feeding and artificial feeding (milk and milk foods). *Acta. Paediatr.* 41:138–44
35. Fomon, S. J. 1974. *Infant Nutrition.* Philadelphia: Saunders 575 pp.
36. Fomon, S. J., Filer, L. J. Jr., Anderson, T. A., Ziegler, E. E. 1979. Recommendations for feeding normal infants. *Pediatrics* 63:52–59
37. Forsum, E., Lönnerdal, B. 1979. Variation in the contents of nutrients of breast milk during one feeding. *Nutr. Rep. Int.* 19:815–20
38. Forsum, E., Lönnerdal, B. 1980. Effect of protein intake on protein and nitrogen composition of breast milk. *Am. J. Clin. Nutr.* 33:1809–13
39. Fransson, G.-B., Lönnerdal, B. 1980. Iron in human milk. *J. Pediatr.* 96:380–84
40. Fransson, G.-B., Lönnerdal, B. 1980. Distribution of iron in human milk fat. *Näringsforskning* 24:66–67 (Abstr.)

41. Garby, L., Sjölin, S. 1959. Absorption of labelled iron in infants less than 3 months old. *Acta Pediatr. Scand.* 48: Suppl. 117, pp. 24–28
42. Garnica, A. D., Fries, J. L., Rennert, O. M. 1977. Menkes' kinky hair syndrome: Is it a treatable disorder? *Clin. Gen.* 11:154–61
43. Garrett, O. F. 1941. Some factors affecting the stability of certain milk properties. V. Inter-relation of certain metals and metallic ions and the development of oxidized flavor in milk. *J. Dairy Sci.* 24:103–9
44. Garry, P. J., Anaokar, S. G. 1980. Rat milk iron levels compared to pups' iron status. *Fed. Proc.* 39:894 (Abstr.)
45. Giroux, E., Prakash, N. J. 1977. Influence of zinc-ligand mixtures on serum zinc levels in rats. *J. Pharm. Sci.* 66:391–95
46. Gorten, M. H., Heppner, R., Workman, J. B. 1963. Iron metabolism in premature infants. I. Absorption and utilization of iron as measured by isotope studies. *J. Pediatr.* 63:1063–71
47. Grebennikov, E. P., Soroka, V. R., Sabadash, E. V. 1963. Trace elements in human and animal milk. *Voprosy. Pitanyia* 22:87
48. Gross, S. J., David, R. J., Bauman, L., Tomarelli, R. M. 1980. Nutritional composition of milk produced by mothers delivering preterm. *J. Pediatr.* 96:641–44
49. Gruden, N. 1979. Transduodenal iron transport in rats fed milk diet supplemented with iron and/or manganese. *Nutr. Rep. Int.* 19:69–74
50. Hambidge, K. M., Walravens, P. A., Casey, C. E., Brown, R. M., Bender, C. 1979. Plasma zinc concentrations of breast-fed infants. *J. Pediatr.* 94:607–8
51. Hambraeus, L. 1977. Proprietary milk versus human breast milk in infant feeding. *Pediatr. Clin. N. Am.* 24:17–36
52. Hegenauer, J., Saltman, P., Ludwig, D. 1979. Effects of supplemental iron and copper on lipid oxidation in milk. 2. Comparison of metal complexes in heated and pasteurized milk. *J. Agric. Food Chem.* 27:868–71
53. Hegenauer, J., Saltman, P. Ludwig, D., Ripley, L., Bajo, P. 1979. Effects of supplemental iron and copper on lipid oxidation in milk. 1. Comparison of metal complexes in emulsified and homogenized milk. *J. Agric. Food Chem.* 27:860–67
54. Hegenauer, J., Saltman, P. Ludwig, D., Ripley, L., Ley, A. 1979. Iron-supplemented cow milk. Identification and spectral properties of iron bound to casein micelles. *J. Agric. Food Chem.* 27:1294–301
55. Heinrich, H. C., Gabbe, E. E., Whang, D. H. 1969. Die Dosabhängigkeit der intestinalen Eisenresorption bei Menschen mit normalen Eisenreserven und Personen mit Pralatentem/latentem Eisenmangel. *Z. Naturforsch. Teil B* 24:1301–10
56. Hurley, L. S. 1979. *Developmental Nutrition.* Englewood Cliffs, NJ: Prentice-Hall. 335 pp.
57. Hurley, L. S., Keen, C. L. 1979. Teratogenic effects of copper. In *Copper in the Environment. Pt. 2. Health Effects,* ed. J. Nriagu, pp. 33–56. New York: Wiley. 489 pp.
58. Hytten, F. E. 1954. Clinical and chemical studies in human lactation. II. Variation in major constituents during a feeding. *Br. Med. J.* 1:175–82
59. Jelliffe, D. B., Jelliffe, E. F. P. 1979. *Human Milk in the Modern World.* New York: Oxford Univ. 500 pp.
60. Johnson, P. E., Evans, G. W. 1978. Relative zinc availability in human breast milk, infant formulas, and cow's milk. *Am. J. Clin. Nutr.* 31:416–21
61. Kaldor, I., Ezekiel, E. 1962. Iron content of mammalian breast milk: measurements in the rat and in a marsupial. *Nature* 196:175–76
62. Karmarkar, M. G., Ramakrishnan, C. V. 1960. Studies on human lactation. Relation between the dietary intake of lactating women and the chemical composition of milk with regard to principal and certain inorganic constituents. *Acta Paediatr.* 49:599–604
63. Keen, C. L., Hurley, L. S. 1980. Developmental changes in concentrations of iron, copper, and zinc in mouse tissues. *Mech. Ageing Dev.* 13:161–76
64. Keen, C. L., Lönnerdal, B., Clegg, M., Hurley, L. S. 1981. Developmental changes in composition of rat milk: trace elements, minerals, protein carbohydrate, and fat. *J. Nutr.* 11:226–36
65. Keen, C. L., Lönnerdal, B., Fisher, G. L. 1980. Age-related variations in liver iron, copper, zinc and selenium concentrations in Beagles. *Am. J. Vet. Res.* 42:In press
65a. Keen, C. L., Lönnerdal, B., Hurley, L. S. 1981. Evaluation of various species as animal models for human milk trace element studies. *XII Int. Congr. Nutr.* Submitted for publication
65b. Keen, C. L., Lönnerdal, B., Ohtake, M., Tamura, T. 1981. Infant formulas for clinical use: trace element and min-

eral contents. *Am. J. Clin. Nutr.* In press

66. Keen, C. L., Lönnerdal, B., Sloan, M. V., Hurley, L. S. 1980. Effect of dietary iron, copper, and zinc chelates of nitrilotriacetic acid (NTA) on trace metal concentrations in rat milk and ma ernal and pup tissues. *J. Nutr.* 110:897–906
67. Keen, C. L., Lönnerdal, B., Sloan, M. V., Hurley, L. S. 1980. Effects of milking procedure on rat milk composition. *Physiol. Behav.* 24:613–15
68. Keen, C. L., Saltman, P., Hurley, L. S. 1980. Copper nitrilotriacetate: a potent therapeutic agent in the treatment of a genetic disorder of copper metabolism. *Am. J. Clin. Nutr.* 33:1789–800
69. Khurana, V., Agarwal, K. N., Gupta, S. 1970. Iron content of breast milk. *Indian Pediatr.* 7:659–61
70. King, R. L., Luick, J. R., Litman, I. I., Jennings, W. G., Dunkley, W. L. 1959. Distribution of natural and added copper and iron in milk. *J. Dairy Sci.* 42:780–90
71. Kirchgessner, M., Spoerl, R. 1975. Zur Cu-Konzentration von Rattenmilch bei unterschiedlichen Cu-Gehalten der Diäten. *Arch. Tierernährung* 25:505–12
72. Kirksey, A., Ernst, J. A., Roepke, J. L., Tsai, T.-L. 1979. Influence of mineral intake and use of oral contraceptives before pregnancy on the mineral content of human colostrum and of more mature milk. *Am. J. Clin. Nutr.* 32:30–39
73. Kleinbaum, H. 1962. Über den Kupfergehalt der Nahrungsmittel des Kindes. *Z. Kinderheilk.* 86:655–66
74. Kon, S. K., Cowie, A. T. 1961. *Milk: The Mammary Gland and Its Secretion,* vol. II. New York: Academic. 423 pp.
75. Leslie, A. J., Kaldor, I. 1971. Liver and spleen nonheme iron and ferritin composition in the neona al rat. *Am. J. Physiol.* 220:1000–4
76. Ling, E. R., Kon, S. K., Porter, J. W. G. 1961. The composition of milk and the nutritive value of its components. See Ref. 74, pp. 195–263
77. Loh, T. T. 1970. Iron in the lactating mammary gland of the rat. *Proc. Soc. Exp. Biol. Med.* 134:1070–72
78. Loh, T. T., Kaldor, I. 1973. Iron in milk and milk fractions of lactating rats, rabbits and quokkas. *Comp. Biochem. Physiol. B* 44:337–46
79. Loh, T. T., Sinnathury, T. A. 1971. Haematological data and milk iron in Malaysian women. *Aust. NZ J. Obstet. Gynaecol.* 11:254–58
80. Lönnerdal, B. 1980. Chemical modification of dextran gels for gel filtration of trace element ligands. In *Trace Element Analytical Chemistry in Medicine and Biology.* Proc. 1st Int. Workshop, ed. P. Schramel, In press. Berlin: de Gruyter

80a. Lönnerdal, B., Clegg, M., Hurley, L. S. 1981. Changes in concentration and distribution of trace elements in human milk during lactation. *Am. J. Clin. Nutr.* Submitted for publication

81. Lönnerdal, B., Forsum, E., Gebre-Medhin, M., Hambraeus, L. 1976. Breast milk composition in Ethiopian and Swedish mothers. II. Lac ose, nitrogen, and protein contents. *Am. J. Clin. Nutr.* 29:1134–41
82. Lönnerdal, B., Forsum, E., Hambraeus, L. 1976. A longitudinal study of the protein, nitrogen and lactose conten s of human milk from Swedish well-nourished mothers. *Am. J. Clin. Nutr.* 29:1127–33

82a. Lönnerdal, B., Hoffman, B., Hurley, L. S. 1981. Zinc- and copper-binding proteins in human milk. *Am. J. Clin. Nutr.* Submitted for publication

83. Lönnerdal, B., Keen, C. L., Fransson, G.-B., Hambraeus, L., Hurley, L. S. 1980. New perspectives on iron supplementation of milk. *J. Pediatr.* 96:242
84. Lönnerdal, B., Keen, C. L., Hoffman, B., Hurley, L. S. 1980. Copper ligands in human milk—a vehicle for copper supplementation in the treatment of Menkes' disease? *Am. J. Dis. Child.* 134:802–3

84a. Lönnerdal, B., Keen, C. L., Hurley, L. S. 1981. Distribution of zinc in milk from various species. *Fed. Proc.* 40:940 (Abstr.)

85. Lönnerdal, B., Keen, C. L., Hurley, L. S., Fisher, G. L. 1981. Developmental changes in the composition of Beagle dog milk. *Am. J. Vet. Res.* 42:In press

85a. Lönnerdal, B., Keen, C. L., Ohtake, M., Tamura, T. 1981. Trace element and mineral content of infant formulas. *Am. J. Clin. Nutr.* Submitted for publication

86. Lönnerdal, B., Keen, C. L., Sloan, M. V., Hurley, L. S. 1980. Molecular localization of zinc in rat milk and neonatal intestine. *J. Nutr.* 110:2414–19
87. Lönnerdal, B., Stanislowski, A. G., Hurley, L. S. 1980. Isolation of a low molecular weight zinc binding ligand from human milk. *J. Inorg. Biochem.* 12:71–78
88. Macy, I. G., Kelly, H. J., Sloan, R. E. 1950. The composition of milk: human colostrum and transitional milk; mature human, cow, and goat milks. *Nat. Res. Counc. Bull.* 119

89. Martin, M. T., Licklider, K. F., Brushmiller, J. G., Jacobs, F. A. 1981. Detection oflow molecular weight copper (II) and zinc (II) binding ligands in ultrafiltered milks—the citrate connection. *J. Inorg. Biochem.* In press
90. Masson, P. 1970. *La Lactoferrine*, pp. 171–184. Bruxelles, Belgium: Editions Arscia. 232 pp.
91. Masson, P. L., Heremans, J. F. 1971. Lactoferrin in milk from different species. *Comp. Biochem. Physiol. B* 39:119–29
92. Mazur, A., Carleton, A. 1965. Hepatic xanthine oxidase and ferritin iron in the developing rat. *Blood* 26:317–21
93. McCance, R. A., Widdowson, E. M. 1951. The metabolism of iron during suckling. *J. Physiol.* 112:450–58
94. McLeod, B. E., Robinson, M. F. 1972. Dietary intake of manganese by New Zealand infants during the first six months of life. *Br. J. Nutr.* 27:229–32
95. McMillan, J. A., Landaw, S. A., Oski, F. A. 1976. Iron sufficiency in breast-fed infants and the availability of iron from human milk. *Pediatrics* 58:686–91
96. McMillan, J. A., Oski, F. A., Lourie, G., Tomarelli, R. M., Landaw, S. A. 1977. Iron absorption from human milk, simulated human milk, and proprietary formulas. *Pediatrics* 60:896–900
97. Miller, W. J., Clifton, C. M., Flower, P. R., Perkins, H. F. 1965. Influence of high levels of dietary zinc on zinc in milk, performance and biochemistry of lactating cows. *J. Dairy Sci.* 48:450–53
98. Momcilovic, B. 1979. Experimental design for the study of dietary interactions in infant rat nutrition. *Period. Biol.* 81:559–65
99. Moynahan, E. J. 1974. Acrodermatitis enteropathica: A lethal inherited human zinc-deficiency disorder. *Lancet* 2:399–400
100. Munch-Peterson, S. 1950. On the copper content in mother's milk before and after intravenous copper administration. *Acta. Paediatr.* 39:378–88
101. Murray, M. J., Murray, A. B., Murray, N. J., Murray, M. B. 1978. The effect of iron status of Nigerian mothers on that of their infants at birth and 6 months, and on the composition of Fe in breast milk. *Br. J. Nutr.* 39:627–30
102. Murthy, G. K. 1974. Trace elements in milk. *Crit. Rev. Environ. Control* 4:1–38
103. Mutch, P. B., Hurley, L. S. 1974. Effect of zinc deficiency during lactation on postnatal growth and development of rat. *J. Nutr.* 104:828–42
104. Mutch, P. B., Hurley, L. S. 1980. Mammary gland function and development: Effect of zinc deficiency in rat. *Am. J. Physiol.* 238:E26–E31
105. Nassi, L., Poggini, G., Vecchi, C., Galuan, P. 1974. Considerazioni sul contenuto di zinco, di rame e di ferro nel colostro e nel latte umano. *Minerva Pediatr.* 26:832–36
106. Newton, M. 1979. Physiology of lactation. In *Symposium on Human Lactation*, ed L. R. Waletzky, pp. 17–24. Washington, DC: US Dept. Health, Ed., Welfare Publ. No. (HSA) 79–5107
107. Nishimura, H. 1953. Zinc deficiency in suckling mice deprived of colostrum. *J. Nutr.* 49:79–93
108. Ohtake, M. 1977. Serum zinc and copper levels in healthy Japanese infants. *Tohoku J. Exp. Med.* 123:265–70
109. Papavasiliou, P. S., Kutt, H., Miller, S. T., Rosal, V., Wang, Y. Y., Aronson, R. B. 1979. Seizure disorders and trace metals: Manganese tissue levels in treated epileptics. *Neurology* 29:1466–73
110. Parkash, S., Jenness, R. 1967. Status of zinc in cow's milk. *J. Dairy Sci.* 50:127–34
111. Picciano, M. F. 1978. Mineral content of human milk during a single nursing. *Nutr. Rep. Int.* 18:5–10
112. Picciano, M. F., Guthrie, H. A. 1976. Copper, iron, and zinc contents of mature human milk. *Am. J. Clin. Nutr.* 29:242–54
113. Pond, W. G., Veum, T. L., Lazar, V. A. 1965. Zinc and iron concentration of sows milk. *J. Anim. Sci.* 24:668–70
114. Prasad, A. S., 1976. *Trace Elements in Human Health and Disease.* New York: Academic. 470 pp.
115. Prasad, A. S. 1976. Deficiency of zinc in man and its toxicity. See Ref. 114, pp. 1–20
116. Prins, H. W., Van den Hamer, C. J. A. 1978. Copper content of the liver of young mice in relation to a defect in the copper metabolism. In *Trace Element Metabolism in Man and Animals - 3*, ed. M. Kirchgessner, pp. 397–400. Freising-Weihenstephan, Germany: Inst. Ernährungsphysiologie, Tech. Univ. München. 684 pp.
117. Rajalakshmi, K., Srikantia, S. G. 1980. Copper, zinc, and magnesium content of breast milk of Indian women. *Am. J. Clin. Nutr.* 33:664–69
118. Rath, E. A., Thenen, S. W. 1979. Use of tritiated water for measurement of 24-hour milk intake in suckling lean and genetically obese (ob/ob) mice. *J. Nutr.* 109:840–47

119. Richardson, R. H., Brew, K. 1980. Lactose synthase. An investigation of the interaction site of α-lactalbumin for galactosyltransferase by differential kinetic labeling. *J. Biol. Chem.* 255: 3377–85
120. Richardson, T., Guss, P. L. 1965. Lipids and metals in fat globule membrane fractions. *J. Dairy Sci.* 48:523–30
121. Rios, E., Hunter, R. E., Cook, J. D., Smith, N. J., Finch, C. A. 1975. The absorption of iron as supplements in infant cereal and infant formulas. *Pediatrics* 55:686–93
122. Romero, J. J., Canas, R., Baldwin, R. L. 1975. A technique for estimating milk production in rats. *J. Nutr.* 105:413–20
123. Saarinen, U. M. 1978. Need for iron supplementation in infants on prolonged breast feeding. *J. Pediatr.* 93:177–80
124. Saarinen, U. M., Siimes, M. A. 1977. Iron absorption from infant milk formula and the optimal level of iron supplementation. *Acta Paediatr. Scand.* 66:719–22
125. Saarinen, U. M., Siimes, M. A. 1979. Iron absorption from breast milk, cow's milk and iron-supplemented formula: An opportunistic use of changes in total body iron determined by hemoglobin, ferritin and body weight in 132 infants. *Pediatr. Res.* 13:143–47
126. Saarinen, U. M., Siimes, M. A., Dallman, P. R. 1977. Iron absorption in infants: High bioavailability of breast milk iron as indicated by the extrinsic tag method of iron absorption and by the concentration of serum ferritin. *J. Pediatr.* 91:36–39
127. Sandell, E. B. 1944. *Colorimetric Determination of Traces of Metals.* New York: Interscience
128. Sandström, B., Arvidsson, B., Cederblad, Å., Björn-Rasmussen, E. 1980. Zinc absorption from composite meals. I. The significance of wheat extraction rate, zinc, calcium and protein content in meals based on bread. *Am. J. Clin. Nutr.* 33:739–45
129. Sato, M., Murata, K. 1932. Zinc content of milk. *J. Dairy Sci.* 15:451–60
130. Sato, M., Murata, K. 1932. Manganese content of milk. *J. Dairy Sci.* 15:461–67
131. Schafer, K. H., Breyer, A. M., Karte, H. 1955. Das Spurenelement Eisen in Milch und Milchmischungen. *Z. Kinderheilk.* 76:501–13
132. Schultz, J., Smith, N. J. 1958. A quantitative study of the absorption of food iron in infants and children. *Am. J. Dis. Child.* 95:109–19
133. Sherman, A. R., Guthrie, H. A., Wolinsky, I. 1977. Interrelationships between dietary iron and tissue zinc and copper levels and serum lipids in rats. *Proc. Soc. Exp. Biol. Med.* 156:396–401
134. Sherman, A. R., Guthrie, H. A., Wolinsky, I., Zulak, I. M. 1978. Iron deficiency hyperlipidemia in 18-day-old rat pups: effects of milk lipids, lipoprotein lipase, and triglyceride synthesis. *J. Nutr.* 108:152–62
135. Siimes, M. A., Vuori, E., Kuitunen, P. 1979. Breast milk iron—a declining concentration during the course of lactation. *Acta Paediatr. Scand.* 68:29–31
136. Stevenson, J. W., Earle, I. P. 1964. Zinc in sow's colostrum and milk. *J. Anim. Sci.* 23:300 (Abstr.)
137. Tanaka, Y., Hatano, S., Nishi, Y., Usui, T. 1980. Nutritional copper deficiency in a Japanese infant on formula. *J. Pediatr.* 96:255–57
138. Tarvydas, H., Jordan, S. M., Morgan, E. H. 1968. Iron metabolism during lactation in the rabbit. *Br. J. Nutr.* 22:565–73
139. Terao, T., Owen, C. A. 1977. Copper metabolism in pregnant and postpartum rat and pups. *Am. J. Physiol.* 232:E172–79
140. Underwood, E. J. 1977. *Trace Elements in Human and Animal Nutrition.* New York: Academic. 545 pp.
141. Underwood, E. J. 1977. See Ref. 140, pp. 170–95
142. Vaughan, L. A., Weber, C. W., Kemberling, S. R. 1979. Longitudinal changes in the mineral content of human milk. *Am. J. Clin. Nutr.* 32:2301–6
143. Venn, J. A. J., McCance, R. A., Widdowson, E. M. 1947. Iron metabolism in piglet anemia. *J. Comp. Pathol.* 57:314–25
144. Versieck, J., Cornelis, R. 1980. Normal levels of trace elements in human blood plasma or serum. *Anal. Chem. Acta* 116:217–54
145. Vohra, P., Kratzer, F. H. 1966. Influence of various phosphates and other complexing agents on the availability of zinc for turkey poults. *J. Nutr.* 89:106–12
146. Vuori, E. 1979. A longitudinal study of manganese in human milk. *Acta Paediatr. Scand.* 68:571–73
147. Vuori, E., Kuitunen, P. 1979. The concentrations of copper and zinc in human milk. *Acta Paediatr. Scand.* 68:33–37
148. Vuori, E., Mäkinen, S. M., Kara, R., Kuitunen, P. 1980. The effects of dietary intakes of copper, iron, manganese, and zinc on the trace element content of

human milk. *Am. J. Clin. Nutr.* 33: 227–31
149. Walravens, P. A. 1980. Nutritional importance of copper and zinc in neonates and infants. *Clin. Chem.* 26:185–89
150. Walravens, P. A., Hambidge, K. M. 1976. Growth of infants fed a zinc supplem nted formula. *Am. J. Clin. Nutr.* 29:1114–21
151. Williams, D. M., Atkin, C. L., Seay, A. R., Bray, P. F. 1979. Failure of human milk therapy in Menkes' kinky hair disease. *Am. J. Dis. Child.* 133:218–19
152. Woodruff, C. W., Latham, C., McDavid, S. 1977. Iron nutrition in the breast-fed infant. *J. Pediatr.* 90:36–38
153. Zimmerman, A. W., Hambidge, K. M. 1980. Low zinc in mothers' milk and zinc deficiency syndrome in breast-fed premature infants. *Am. J. Clin. Nutr.* 33:951 (Abstr.)