Some Indicators of Manganese and Copper Adequacy in Some Infant Formulas for Baby Pigs and Infants

Robin H. Marcus

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SOME INDICATORS OF MANGANESE AND COPPER ADEQUACY IN SOME INFANT FORMULAS FOR BABY PIGS AND INFANTS

by

Robin Hope Marcus

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE in

Nutrition and Food Sciences

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1964
ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to Dr. Deloy Hendricks for his help and encouragement. My thanks and sincere appreciation are extended to my committee members, Dr. Arthur Mahoney and Dr. Stanley Allen, for their time and energies, and very constructive criticism. Many thanks to Dr. Allen for helping me understand the end-points of the histopathological results.

Special thanks to Susan Collinge and Dennis Bouchier for helping me with laboratory technique and analysis. Much appreciation to Dr. Daren Cornforth for his help with picture taking of slides.

I would also like to thank Gary Chan, M.D. and Jean Hollis, whom I have never met, for providing the infant serum samples and infant data.

My thanks to Dr. Carol Bocan and Flora Bardwell for their input at the start of this study.

I am deeply grateful to my parents and family for their continued support.

My deepest thanks to Robert Alan Watts for without him I would have never attended Utah State University. I am indebted to him for many things. It is with his help and encouragement that I have made it through this program.

Robin H. Marcus
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ABSTRACT

Some Indicators of Manganese and Copper Adequacy in Some Infant Formulas for Baby Pigs and Infants

by

Robin H. Marcus, Master of Science Utah State University, 1984

Major Professor: Dr. Deloy G. Hendricks
Department: Nutrition and Food Science

The objective of the study was to ascertain whether some infant formulas provide sufficient amounts of manganese and copper for adequate nutrition. Twelve piglets, within the first week of life, were divided by sex, litter and weight into two treatment groups. Both groups received concentrated Isomil, a soy-based infant formula and distilled water ad libitum. Isomil was analyzed and found to contain 1.05ppm manganese and .98ppm copper. The supplemented group received an additional 30.6mg manganese and 6.76mg copper per can (388ml) of concentrated Isomil. Weight gains were not significantly different. Manganese and copper levels were significantly greater in the livers of the supplemented group. The supplemented group also had significantly higher manganese and zinc in femurs. These were determined by atomic absorption spectrophotometry.

Serum copper and manganese were determined using an atomic absorption spectrophotometer equipped with a graphite furnace.

The supplemented group had a significantly higher serum manganese
The supplemented group had a significantly higher serum manganese and copper at the end of the study than the unsupplemented group. Within a group, the unsupplemented group had a significantly higher serum manganese and the supplemented group had a significantly higher serum copper in week five versus week one.

No significant differences or lesions were found upon histopathological examination of spinal cord and rib tissue from the piglets.

Birth weight, two month and six month weight, and serum levels of copper and manganese from human infants fed breast milk versus Similac were studied. Serum copper was significantly greater at two months, and serum manganese was significantly greater at six months in the breast-fed infants.

Although no clinical deficiency symptoms were observed in the infant pigs, tissue levels were significantly decreased in the unsupplemented group. The significantly greater serum copper and manganese levels of the breast-fed infants may be related to different bioavailability of these minerals from breast milk versus Similac or other infant formulas. Many marginal deficiencies probably go undetected. It is recommended that infant formulas contain a minimum concentration of manganese and copper which will provide infants of 0 - 6 months of age with .5 - .7mg/day, to meet the current estimated safe and adequate recommendations.
CHAPTER I

INTRODUCTION

BACKGROUND

The importance of trace elements in both animal and human nutrition has been widely recognized (Murthy and Rhea, 1971). The requirements for trace elements which are essential to maintain life are increased during periods of growth and stress (Underwood, 1977; Shaw, 1979; Shaw 1980; Hurley, 1979). Nutrient requirements are most critical during the first few months of life which is characterized by a period of rapid physical growth and mental development (Committee on Nutrition, American Academy of Pediatrics, 1976).

Considerable variation in concentrations of trace elements exists among infant formulas (Belavady, 1978; Casey, 1977). In addition to the wide variation in absolute amounts of the trace elements in formulas, Lonnerdal et al. (1983) have found large variations in the ratios of the trace elements.

Brostrom (1981) states that "neither the form in which the trace minerals are present in human milk nor the interaction between them is known. Although both may have physiological significance, especially when human milk values serve as a reference for fortification of infant formulas." (p. 55)

There is increasing evidence that the requirements for several
trace minerals are dependent to a considerable degree on the type of milk feeding. This has been demonstrated repeatedly for iron and zinc. The bioavailability of iron and zinc have been found to be considerably higher from human milk than from cow's milk (Casey et al., 1981; Sandstrom et al., 1983).

The concentration of manganese in human milk is low, ranging from 4 to 8 µg/liter. Lonnerdal et al. (1983) report the manganese concentrations of several formulas were 100 to 1000 times higher than that of human milk (Vuori, 1979), while some had undetectable concentrations. Seven formulas had manganese levels less than those recommended by the Committee on Nutrition of the American Academy of Pediatrics (Barness, 1981).

Lonnerdal et al. (1983) report a range of 0.31 - 0.65 mg/liter of copper in infant formulas in the United States. Looking at all infant formulas studied from eight different countries the range was 0.01 - 1.35 mg/liter of copper. Copper in human milk has been reported to range from 0.2 - 0.3 mg/liter and in cow's milk to range from 0.1 - 0.2 mg/liter (Lonnerdal et al., 1981).

The bioavailability of copper and manganese from milk and milk products has not been well studied. According to recent reports there may be differences in bioavailability of these trace minerals in human milk versus cow's milk and infant formulas due to differences in protein binding ligands (Lonnerdal et al., 1982; Chan et al., 1982). A ligand has been defined as "an atom, group, ion, radical, or molecule which forms a coordination complex with a central atom or ion." (Webster's New World Dictionary (p. 817, 1976).
While there are no documented cases of manganese deficiency in human infants, manganese deficiency in experimental animals has severe effects on normal prenatal and postnatal development (Hurley, 1981). Dupont et al. (1977) have reported that some children with epileptic seizures who have low serum manganese levels improve with manganese supplementation.

The ability of infant formulas to meet the nutrient needs of the infant is crucial since formula is frequently the sole source of nutrition. Failure to meet nutrient requirements during this period can have lasting effects on growth and development (John and Lamy, 1975; Hambidge, 1977; Ohtake, 1977; Oberleas and Prasad, 1969) and may well affect an individuals' mental and or physical performance for the remainder of his life.

**STATEMENT OF THE PROBLEM**

To date, many studies have been conducted to determine the nutritional value and adequacy of infant formulas. The majority have been concerned with overall observable findings such as weight gain and length increases. A number of studies have been conducted to determine serum proteins, nitrogen retention, and calcium and phosphorus adequacy of the formulas.

Most recently studies have been conducted on iron and zinc bioavailability and trace mineral content of infant formulas and breast milk.
Although intake levels of manganese and copper have been studied, the actual amounts of manganese and copper incorporated into body tissues and blood levels have received limited attention.

Considerable variation exists in concentrations of trace elements among infant formulas. It has been shown that requirements for several trace minerals are partially determined by a number of factors which affect their bioavailability. These factors include the ratios of trace elements to each other, processing of the formula, presence of phytate and lactose, type of protein-binding ligands, and maturity and state of the physiological system which is consuming the product. The bioavailability of copper and manganese from cow's milk and infant formulas may be affected by these factors.

Estimated safe and adequate intakes for manganese and copper have been established by the Committee on Dietary Allowances of the National Research Council (National Academy of Sciences, 1980). These estimates are substantially higher than infants would receive in their "normal" diet (prior to the introduction of Beikost) in the first six months of life.

Stave (1978) states "that because of the breadthness of its metabolic involvements and particularly its role in cartilage and skeleton production the importance of manganese in the perinatal period must be underscored." (p. 445) Burch et al. (1975) reports that although manganese is a small integral part of total nutrition the vital role it plays in metabolic processes cannot be over emphasized. Because of the importance of these trace minerals it is essential to determine if our infant formulas are meeting the needs of the infant.
OBJECTIVES

1. To ascertain whether Isomil provides sufficient amounts of manganese and copper for adequate nutrition. The judgement of adequate nutrition will be made by looking for observable differences between the group of infant pigs fed Isomil or Isomil supplemented with manganese and copper at levels adequate for the young pig. Adequate nutrition will also be evaluated by looking at the livers, femurs and blood levels of manganese and copper and looking for significant differences between the groups.

2. As a second criteria in judging whether Isomil provides enough manganese and copper for adequate nutrition, porcine neurol and rib tissue will be histologically examined for significant lesions and any differences among the supplemented and unsupplemented groups.

3. As a third criteria for judging adequacy of infant formula in providing manganese and copper, blood samples from human infants fed Similac versus breast milk, will be analyzed for these minerals. Samples will be analyzed at two months and six months of age. In this case, because of the limited sample size, trends in serum values will be looked at.
LIMITATIONS OF THE STUDY

1. Nutritional status of the sow's during gestation was not controlled. This should have equal affect on both treatment groups because they were balanced for litter.

2. Nutritional status of the mother's of human infants during gestation and lactation was not controlled. This may affect the mineral stores and overall nutritional status of the infant.

3. Trace minerals in household water supplies of infants studied is not known. This may have been a source of additional minerals in the diet.

4. Beikost may have been introduced to some infants during the period studied. This may have provided additional copper and manganese in the diet.
CHAPTER II

LITERATURE REVIEW

FUNCTIONS OF MANGANESE

The involvement of manganese in metabolism covers a very wide spectrum (Stave, 1978). Although there are very few manganese containing metalloenzymes, there are many enzymes which are activated by manganese. Manganese is a component or activator of enzymes such as pyruvate carboxylase, arginase, leucine aminopeptidase, alkaline phosphatase and of enzymes which participate in oxidative phosphorylation (Shenkin and Wretlind, 1978; Hull, 1974; Burch et al., 1975).

Because metal activation of enzymes is usually nonspecific it is difficult to relate enzymatic activity to deficiency symptoms. An exception to this general occurrence is the relationship between manganese and the class of enzymes called glycosyltransferases. The ability of manganese to activate these enzymes appears to explain the abnormalities in glycosaminoglycan metabolism that are associated with manganese deficiency (Leach et al., 1969; Leach, 1971).

The activity of two enzymes (polysaccharide polymerase and galactotransferase) involved in chondroitin sulfate synthesis are decreased with manganese deficiency. Changes in mucopolysaccharides are what cause the skeletal abnormalities (Leach and Maenster, 1962; Leach, 1967). Burch et al. (1975) indicated these changes seem to
be specific for manganese deficiency. Manganese is a necessary cofactor for the enzymes involved in chondroitin sulfate synthesis. The chondroitin sulfate-protein complex is necessary to maintain the rigidity of connective tissue.

Asling and Hurley (1963) reviewed the influence of trace elements on the skeleton. They reported manganese is necessary for optimal growth in mice, rats, and some other species. Swine, guinea pigs, and calves do not show impaired growth with manganese deficiency. He stated usually skeletal development is impaired, resulting in shortened and often deformed limbs.

Role of manganese in the synthesis of protein. Manganese appears to be involved in the synthesis of protein, DNA and RNA. A DNA-manganese complex was first reported by Wiberg and Neuman (1957). They concluded from its dissociation constant that manganese binds to DNA more strongly than do other metals.

The effect of manganese when compared to other ions showed a significant increase in protein biosynthesis, attributable to manganese, in isolated rat liver nuclei (Weser and Koolman, 1970). Burch et al. (1975) reports a correlation between decreased hepatic manganese content and decreased hepatic protein content was found in protein-calorie malnutrition. Although direct evidence of the in vivo role of manganese in mammalian protein biosynthesis is still limited, in vitro evidence indicates that manganese is involved in protein synthesis (Burch et al., 1975).

Role of manganese in carbohydrate metabolism. The role of manganese in carbohydrate metabolism has been broken down into two general
areas: 1) the stabilization and/or activation of gluconeogenic enzymes, and 2) the relation between manganese deficiency and diabetic-like symptoms.

An important preliminary step in the gluconeogenic pathway, the carboxylation of pyruvate to oxaloacetate, is catalyzed by the manganese metalloenzyme pyruvate carboxylase. Although, under conditions of manganese deficiency, magnesium was found to replace manganese as the bound metal in the enzyme isolated from deficient chicks.

Manganese has been reported to influence glucose utilization. Studies with a diabetic patient who was relatively insensitive to insulin led to the suggestion of a possible relationship between manganese and diabetes. A consistent fall in blood glucose levels, resulted after administration of a manganese chloride solution. The time interval between administration of manganese and the decrease in blood glucose level was consistent with an enzyme activation theory (Levin et al., 1962 and Belyaev, 1938).

Everson and Shrader (1968) found reduced glucose utilization in the manganese-deficient guinea pig. In their studies of the effects of low dietary manganese on the prenatal and postnatal development of the guinea pig they found that the pancreas in neonatal animals was often absent or disproportionately reduced in size.

In another study by Shrader and Everson (1968) pancreatectomy and diabetes have been correlated with decreased manganese levels in blood and tissues. When glucose tolerance tests were performed on
deficient and control guinea pigs using both oral and intravenous glucose administration they found the deficient guinea pigs showed decreased utilization of glucose which resulted in a diabetic-like glucose curve in response to glucose loading. It appeared from this study that subsequent manganese supplementation completely reversed the reduced glucose utilization.

Role of manganese in seizures. In a study by Papavasilou et al. (1979) of manganese tissue levels in treated epileptics they report that reduced manganese availability at the neuronal level, where manganese stabilized membrane excitability, may affect epileptogenic lesions to increase the likelihood of seizure activity.

Dupont et al. (1977) report that blood manganese levels were lower in children with convulsive disorders than in healthy controls, which suggests that a manganese deficient state might exist in these disorders.

MANGANESE IN THE BODY

Burch et al. (1975) reported that manganese is widely distributed in body tissues and fluids. In the human, the brain, kidney, pancreas, and liver, in that descending order, show relatively higher manganese concentrations than do other organs (Sandstead et al., 1970). In animals, the bones, the liver, kidney and especially the pituitary gland are richer in manganese.

Widdowson et al. (1972), in a study of accumulation of Cu, Zn, Mn, Cr, and Co in the human liver before birth reports that the fetal
liver does not appear to store manganese as it does copper and iron. They found the concentration of manganese in the liver was similar all through gestation and on into adult life. Schroeder et al. (1966) and Stave (1978) reported similar findings.

Hudnik et al. (1983) report means of 12.3 and 12.6 mg/gram manganese in pig livers. The age of the pigs was not reported. Whether the means are on a dry or wet basis was not reported.

Gamble et al. (1971) report that the end of the ribs of fetal pigs had a much higher concentration of manganese than other fetal tissues and than the same part of the rib of the mother. It is possible that the bones constitute the fetal store.

Keen et al. (1983) report that low blood levels of manganese in manganese deficient animals reflected low levels of the element in soft tissue. They report that the differences in liver manganese levels appear to be functionally important, since the activity of the metalloenzyme manganese superoxide dismutase was lower in livers from deficient animals than in controls (Marklund and Marklund, 1974).

In Perinatal Physiology, edited by Stave (1978), Linder reports the serum concentration of manganese in the term fetus to be .034 mg/ml. Linder also reports that manganese is attached to the mineral portion in bone which does not appear to be a storage form since it is not readily available to the soft tissues of the body. Plantin and Meurling (1980) report manganese concentration in the serum from full-term healthy newborns to be .058 mg/ml. Mean weight was 3.4 ± 0.3 kg. Chan et al. (1982) report the serum manganese of full-term newborns to be .01835 ± 0.009 mg/ml.
The mechanism of absorption of manganese is unknown, although Sandstead et al. (1970) reported that maximal absorption occurs in the duodenum.

In a study by Shils (1972) on minerals in total parenteral nutrition it was reported that about 10% of the ingested element is absorbed. The percentage of manganese reported to be absorbed has varied considerably from different sources.

Low (1976) stated that manganese is rather poorly absorbed from the small intestine. Casey and Hambidge (1980) report that absorption of manganese is quite low at 3-4% and claim this appears to be unaffected by dietary levels. The apparent absorption of manganese in the small intestine of two pigs was found to be 20% by Savic and Zebrowska (1972).

Burch et al. (1975) report that slight over dosage is probably not harmful because there appears to be a very effective homeostatic mechanism which makes manganese one of the least toxic of the trace elements.

Manganese is excreted mainly through the bile, although some is excreted via pancreatic juice and trace amounts appear in the urine (Shils, 1972; Sandstead et al., 1970; Burch et al., 1975) Some is reabsorbed in the lumen of the duodenum, jejunum, and ileum. It has been reported that if an animal is given a loading dose of manganese or if there is biliary obstruction the excretion rate through the
gastrointestinal route is increased. The combination of biliary excretion and auxiliary gastrointestinal excretion are thought to be the means which provide man with an efficient method of maintaining tissue concentrations.

Balance studies conducted by Widdowson et al. (1974) showed newborn infants to be in substantial negative balance for manganese. Babies one week old were found to be excreting an average of 3.1 mg more manganese per day than they were taking in from the diet. The excretion of manganese in the feces was more than 5 times as high as the intake in the milk. This was true for all the infants who were full term, male, 6 days old, fully breast fed, and received no supplements. The intake in the milk and the excretion in the feces did not have any relation to each other. It was concluded that the manganese excreted was probably derived from body stores.

Some researchers have hypothesized that the large fecal loss of manganese in one week old infants may be attributed to failure to reabsorb manganese secreted by the bile.

Potter and Nestel (1976) found greater bile acid excretion with soybean than with cows milk in infants. The study used 5 infants from the first week of life to 2 or 3 months of age. They concluded that the substitution of soybean milk for cows milk leads to an increase in bile acid excretion in young infants. Signer et al. (1974) found premature infants consuming human milk excreted less bile acids in the stool than did infants fed with cows' milk formula. In both groups the fecal loss of bile was increased compared with that in older infants and children.
REPORTED MANGANESE DEFICIENCY IN HUMANS

Doisy (1973) reported the first recognized case of human manganese deficiency which was observed while studying vitamin K deficiency in a volunteer under metabolic ward conditions. The patient showed signs of weight loss, transient dermatitis, occasional nausea and vomiting, changes in hair and beard color, and slow growth of hair and beard. These findings occurred due to the accidental failure to add manganese to the purified diet mixture. Because sterility seems to accompany manganese deficiency, the study could not be repeated.

FUNCTIONS OF COPPER

Copper is known to be a component of a number of metalloenzymes (Evans, 1973; O'Dell, 1976; Ulmer, 1977). These metalloenzymes are essential for a variety of functions including mitochondrial energy generation, cross linking of collagen and elastin, and melanin formation.

Cytochrome oxidase is the terminal oxidase of the electron-transport chain. This enzyme is necessary for the reduction of molecular oxygen to water which is tied to energy metabolism through synthesis of ATP (Shaw, 1980; Walravens, 1980).
Ceruloplasmin, another copper containing enzyme, functions in the oxidation of the ferrous iron of body stores to the ferric form (Frieden and Hsieh, 1976). For normal hemoglobin synthesis iron must be transported from storage sites in the liver, reticuloendothelial system and intestine to the bone marrow by transferrin. Copper plays a role in the release and transfer of iron from storage cells to plasma transferrin. Ceruloplasmin also plays a role in copper transport and in regulation of biogenic amines (Osaki et al., 1964; Osaki et al., 1966).

Lysyl oxidase, an amine oxidase, catalyzes the cross linking of the polypeptide chains of collagen and of elastin (Partridge et al., 1964; Chou et al., 1968). The collagen and elastin of copper deficient animals has been found to be abnormal with reduced cross linking and consequent reduction in strength and elasticity. Aneurysms of major vessels readily develop and may cause death from aortic rupture (Starcher et al., 1964; Carnes, 1971). In addition to lysyl oxidase there are other amine oxidases which are present in connective tissues. It is believed that these function in the deamination of norepinephrine, serotonin and histamine (Osaki et al., 1964).

Tyrosinase facilitates the hydroxylation of tyrosine to form dihydroxyphenylalanine (DOPA), a step in the production of melanin (Fitzpatrick et al., 1961).

Superoxide dismutase catalyzes the transformation of two superoxide anions in the presence of two hydrogen ions to molecular oxygen and peroxide. As a result superoxide dismutase helps protect
the cell from the damaging effects of oxygen toxicity (McCord and Fridovich, 1969, 1970).

**COPPER IN THE BODY**

Hambidge (1977) reports the liver, brain, heart and kidneys contain the highest concentration of copper. Muscle and bones contain about one-half of the total body content of copper because of their large mass. The liver of the term neonate contains about 50% of the total body copper compared with only 10% in the adult.

Casey and Robinson (1978) report the liver at term may contain up to 80% of the total body content in concentrations up to 10 times that in adult livers. Liver stores of the full-term neonate are usually adequate to supply the infants' copper needs for four to six months by which age foods other than milk are generally introduced. Because copper stores are mainly accumulated in the last three months of gestation the premature infant is born with very meager stores (Shaw, 1973). In a study by Ohtake (1977) on serum zinc and copper levels in healthy Japanese infants it was found that the concentration of serum copper in newborn infants was significantly lower than in healthy children (six to twelve years of age), but had risen by five months of age to the same levels. No significant differences in serum copper concentration were found between male and female infants.
There was a tendency for serum copper levels in exclusively or partially breast-fed infants to be higher than those in bottle-fed infants at one month of age. Forty-five healthy Japanese infants with ages ranging from five days to twelve months were included in the study. Ohtake (1977) reports a range serum copper of 0.47 to 1.04 ug/ml in infants from five days to five months of age. At seven days of age, Sann et al. (1980) determined serum copper to be 0.79 ± 0.08 ug/ml in full-term, appropriate for gestational age infants.

Serum copper concentrations reported by Henkin et al. (1973) were similar to those observed by Sann et al. Henkin et al. (1973) in a study of total plasma zinc and copper in infancy found there were no significant differences in mean total diffusable or non-diffusible zinc or copper among males and females, Caucasians or blacks, or between infants who were breast-fed or bottle-fed during the first two to three months of life. The probability that infants are still utilizing liver stores of copper at this stage may be the reason.

**ABSORPTION AND EXCRETION OF COPPER**

Absorption of copper is regulated at the level of the intestinal mucosa and excretion is mainly through the intestinal tract either via the bile (Ulmer, 1977) or as nonabsorbed copper. The amount excreted in the urine is negligible in normal healthy individuals, amounting to about 1 to 2% of the intake.
In man the site of maximal absorption is in the stomach and duodenum. Mason (1979) reports it seems reasonable to assume an absorption of 40-60% of the oral intake of copper accepting the fact that there is wide individual variation. Hambidge (1977), and Casey and Hambidge (1980) report that about 40% of ingested copper is absorbed via the stomach and upper small intestine. Strickland et al. (1972) and King et al. (1978) report similar mean values for copper absorption of 56% and 57% respectively, in human adults.

In studies of experimental animals, many factors have been found to interfere with copper absorption. These include competition for binding sites by zinc and possibly cadmium, interactions between molybdenum, sulphates and copper, the effects of dietary phytates and the influence of ascorbic acid intake (Mason, 1979). There is a need to determine the extent to which findings in experimental animals have application to the problem of copper absorption and metabolism in man.

REPORTED COPPER DEFICIENCY IN INFANTS

Reported symptoms of copper deficiency include anemia, hypopigmentation and changes in the texture of hair, abnormalities in bone structure, failure of myelination and central nervous system defects in animals (Pike and Brown, 1975; Rucker et al., 1969; Robinson and Lawler, 1977). A lack of rigidity in the leg joints has also been reported (Teague and Carpenter, 1951).

Graham and Cordano (1969) and Karpel and Peden (1972)
described several instances of copper-dependent anemia which have occurred in children. In one of these cases, skeletal deformities occurred that were similar to those found in animals with copper deficiency.

Dietary copper deficiency has been described in premature infants, neonates, and previously malnourished children, and has resulted in anemias, neutropenia, and skeletal demineralization (Cordano et al., 1964; Cordano and Graham, 1966; Graham and Cordano, 1969; Griscom et al., 1971; Al-Rashid and Spangler, 1971; Seely et al., 1972; Ashkenazi et al., 1973). About one-third of infants and children admitted to a hospital in Peru because of malnutrition and chronic diarrhea had evidence of copper deficiency (Graham and Cordano, 1969).

Karan and Pathak (1975) reported prematurity, low birth weight, and maternal undernutrition as factors in the development of copper deficiency. Stave (1978) agrees that prenatal stores of copper may not be sufficient to provide for the suckling period if the mother has been deficient during gestation or if the infant is born prematurely.

Casey and Hambidge (1980) note that several areas of concern may be identified with respect to problems of copper nutrition. These include prematurity, generalized malnutrition, and prolonged diarrhea, problems associated with intestinal malabsorption, patients on long term total parenteral nutrition, and long term use of chelating agents.

Solomons (1979) reports that depressions in circulating
copper have been observed in human copper deficiency states and that administration of supplemental copper by oral or parenteral routes restored normal circulating copper levels.

The majority of reported cases of copper deficiency in human infants have been in premature infants. Recently a case of copper deficiency has been reported in an infant of 38 weeks gestation (Bennani-Smires et al., 1980). Copper deficiency has also been reported in a few cases where infants were fed cow's milk (Naveh et al., 1981) or formulas that had a low copper content (Al-Rashid and Spangler, 1971; Tanaka et al., 1980).

INFANT FEEDING PRACTICES AND RECOMMENDATIONS

The convenience of formulas led to a decline in breast-feeding, a trend which has recently begun to reverse in the United States. (Martinez and Dodd, 1983; Hirschman and Butler, 1981; Hendershot, 1981).

The majority of nutritionists and physicians will recommend breast-feeding over formula feeding for normal healthy infants for a variety of reasons, including immunological reasons, differences in protein content, carbohydrate and fat composition (The Committee on Nutrition of the American Academy of Pediatrics, 1976; The Council on Scientific Affairs of the American Medical Association, 1979).

Concern and attempts to provide substitutes for human milk for newborn infants have been made as early as 1919 by Gerstenberger and Ruh. The goal has been that artificial milks should resemble as
closely as possible the milk they are intended to replace. Barltrop (1974), however, has pointed out that breast milk itself varies greatly in composition, and that data for breast milk constituents are usually given as average values which can be misleading.

Factors which affect the composition of an individual mother's milk include the stage of lactation and maternal diet (Vuori et al, 1980). Although differences are small, composition may also vary from feed to feed, and even between each breast (Picciano and Guthrie, 1976). This kind of variation makes it difficult to determine the optimal intake of any particular constituent and should be taken into account when comparing breast milk with substitutes.

Casey (1977) in a study conducted in New Zealand found that there is a wide variety of types of milk and milk substitutes commercially available for infant feeding. He also found that among these products is a wide range of concentrations for most of the trace elements, even among similar types of foods.

John and Lamy (1975) have divided the commercially available infant formulas into three groups based on their composition and/or intended use. They are standard formulas, soy-based formulas and specialized formulas. The standard formulas are cows' milk-based formulas intended to replace or supplement human milk for feeding normal, healthy infants. The soy-based milk free formulas were developed for use in cases where: 1) infants are allergic or suspected of being allergic to milk protein, 2) infants with galactosemia, and 3) infants with congenital or acquired lactase deficiency. From an economic viewpoint, they may also be
advantageous for use in areas where animal protein is scarce (Jung and Carr, 1977). Soy-based formulas are used for an estimated 10-15% of all formula fed infants (Committee on Nutrition, American Academy of Pediatrics, 1983).

The Infant Formula Act of 1980 (United States Congress, 1980) specified nutrient requirements for infant formulas. The minimum copper and manganese requirements for infant formulas are 402 ug/liter and 34 ug/liter, respectively.

In close agreement are the recommended minimum and maximum concentrations of copper and manganese in infant formulas by the Committee of the American Academy of Pediatrics. They recommend 420 ug/liter and 35 ug/liter for copper and manganese, respectively (Barness, 1981). The World Health Organization (1973) report recommended 0.08/mg/kg body weight/day of copper for infants. The estimated safe and adequate daily dietary intakes for manganese and copper, as specified by the Committee on Dietary Allowances of the National Research Council (National Academy of Sciences, 1980), are 500 - 700 ug for 0 - 6 months of age. The recommended minimum levels of manganese and copper in infants formulas and the estimated safe and adequate daily intakes are summarized in Table 1 below (page 24).

If the minimum nutrient levels for copper and manganese are compared with the recommended safe and adequate daily dietary intakes it is evident that many infants will not be receiving the recommended intakes of manganese and copper.

Fomon et al. (1979) suggests that even though most infants seem to grow normally there are a great variety of feeding practices in
technically developed countries. He comments further that although we may not detect differences in health as a result of such wide differences in feeding practices this does not necessarily mean that the choice of feeding practice is of no consequence. The consequences may be too subtle to be detected by casual observation or may be of a long-term rather than short term nature.

MANGANESE IN MILK, INFANT FORMULAS AND INTAKES OF INFANTS

Refer to Table 1 for a summary of manganese and copper content in human milk and infant formulas, and intakes of infants. The literature cited to formulate the table is described in more detail below.

It has been reported that the range of manganese intake during the first 6 months of life varies widely, depending on the nature of the food, intakes between 0.01 and 0.294 mg/day, corresponding to 0.0025 to 0.075 mg/kg of body weight have been reported (McLeod and Robinson, 1972).

Murthy and Rhea (1971) found wide variations in trace elements in formulas they analyzed. The analyzed formulas included infant foods such as evaporated milk, modified milk and formulas containing soya flour and lamb meat products. The average manganese in milks and formulas varied from 0.089 to 1.74 ppm. These same researchers found manganese in cow's milk produced in the United States varied from 0.040 to 0.155 ppm averaging 0.090 ppm.

McLeod and Robinson (1972) found the manganese concentration in
Table 1. Manganese and copper in milk, infant formulas, and intakes for infants 0–6 months of age, and recommended minimum levels of copper and manganese in infant formulas (a)

<table>
<thead>
<tr>
<th></th>
<th>Estimated Safe &amp; Adequate Amounts</th>
<th>Infant Formula Intake</th>
<th>Infant Intake</th>
<th>Amount in Breast Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ug/day)</td>
<td>Act 1980 (minimum) (ug/day)</td>
<td>(ug/liter)</td>
<td>(ug/liter)</td>
</tr>
<tr>
<td>Copper</td>
<td>500–700#</td>
<td>402</td>
<td>1–294###</td>
<td>89–174*</td>
</tr>
<tr>
<td>Manganese</td>
<td>500–700#</td>
<td>34</td>
<td>180–920$</td>
<td>10–2140</td>
</tr>
</tbody>
</table>

(a) Based on the 1980 Recommended Dietary Allowances a 6 kg infant would need an average of 600 calories/day (115 kcal/kg). This corresponds to approximately .9 liters of breast milk and 1 liter of infant formula/day. Therefore, for mineral intake of infants per day, amounts per liter of formulas can be multiplied by .9 and 1 respectively, for breast-fed and formula-fed infants.

# National Academy of Sciences (1980) These are estimated safe and adequate intakes recommended for infants 0–6 months of age

## McLeod and Robinson (1972)

### American Academy of Pediatrics recommendations

*Murthy and Rhea (1971), **Vuori (1979), ***Lonnerdal et al. (1983)

$Waslein (1976), $$Picciano and Guthrie (1976)

breast milk to range from 12.0 to 20.2 ug/liter with a mean of 15.

They report the manganese concentration in pasteurized cow's milk to have a range of 32 to 52 ug/liter with a mean of 40.

Vuori (1979) conducted a longitudinal study of manganese in human milk. The concentration of manganese in milk reported by McLeod and Robinson (1972) is a great deal higher than that reported by Vuori. In the Vuori study the median manganese concentration was 5.9 ug/liter in the second week of lactation. 50% of the values ranged between 4.9 and 7.0 ug/liter before the second month of lactation and remained at that level up to the fifth to sixth month of lactation, after which they tended to increase. Casey (1977) found similar values for breast milk (0.02 ug/ml) and homogenized cow's milk (0.06 ug/ml) to those found by McLeod and Robinson (1972).
Lonnerdal et al. (1983) reports manganese in infant formulas ranges from 0.0 to 7.8 ug/liter.

The reported concentrations of manganese in human and cow's milk have varied considerably. Whether this is the result of an actual variation, contamination, or insensitive analytical technique remains a question.

**COPPER IN MILK, INFANT FORMULAS AND INTAKES OF INFANTS**

Copper content (and manganese content) in human milk and infant formulas, and intakes of infants are summarized in Table 1 (above). The literature cited to formulate the table is described in more detail below.

Waslein (1976) reports that data collected on the copper content of the diet of 377 babies from the United States indicated a wide range from .18 to 0.92 mg/day or 7 to 170 ug/kg/body weight. Total daily intakes of copper ranged from an average of 0.16 for one month old infants to 0.38 mg for six month old infants, 50 to 60% of the intake coming from milk.

Picciano and Guthrie (1976) report that copper content of milk varied considerably among women and within the same women in a study of copper, iron, and zinc contents of mature human milk. Fifty women in their 6th to 12th week of lactation participated in the study. They report a range of .09 to 0.63 ug/ml noting a large proportion of low values. Their calculations indicate fully breast-fed infants under 3 months of age receive about .05 mg/kg per day of copper.
In a later study, Picciano and Deering (1977) report that three month old totally breast-fed infants receive an average of 0.03 mg/kg/day of copper. The study included 26 full term infants who were being breast fed exclusively. The quantity of milk consumed was measured by test weighing at each feeding for three consecutive days, at months 1, 2, and 3. The daily mean volume of milk ingested was 604, 628, and 644 mls. at 1, 2, and 3 months, respectively. They found that the earlier reported data on the average trace element content of human milk overestimate the actual levels in prolonged lactation.

In Fomon's book (1974) on infant nutrition it is reported that mature human milk contains 0.4 mg/liter of copper. Vuori and Kuitunen (1979) found that 50% of the mothers in their study had a copper content in their milk less than this value after seven weeks of lactation. All of them had a copper content in their milk less than .4 mg/liter after fourteen weeks of lactation.

Hambidge (1977) reports the mean copper content of mature breast milk (analyzed in his lab) to be 480 ug/liter and the average copper concentration in cow's milk to be about 150 ug/liter.

Walravens (1980) found the mean copper content of twenty-three samples of homogenized cow's milk to be 135 ug/liter; much lower than that of breast milk during any of the various stages of lactation and similar to other reported values.

The content of human colostrum has been reported to be two to three times that of later milk (Lahey and Schubert, 1957; Munch-Petersen, 1950). Although Nassi et al. (1974) report that the
concentration of copper seems to be no higher in colostrum than in mature milk. The copper content of human milk is two to three times higher than that of cow's milk.

Cow's milk alone would provide only 20 to 25 ug of copper per 100 kilocalories. Data from a balance study by Kleinbaum (1962) of full-term, breast-fed infants appears to indicate that healthy, full-term infants during the first month of life require approximately 0.5 mg/copper/day to keep in positive balance. The intake recommended by the Food and Nutrition Board, National Academy of Sciences (1980), of 500 to 700 ug/day for infants 0 to 6 months of age would not be met by cow's milk alone.

Mason (1979) points out that in evaluating this type of reported data one must consider the differences in methods of assay, care against contamination, and also differences in sample preparation. Factors which influence availability and absorption of dietary copper must also be considered.

Reports of copper in human milk range from 0.15 to 1.34 mg/liter (Anon, 1977; Belavady and Gopalan, 1959; Cavell and Widdowson, 1964; Murthy and Rhea, 1971; Nacci et al., 1974; and Picciano and Guthrie, 1976). The extent to which this variation is due to individual differences, different stages of lactation, or to different methods of sampling is not known.

Vuori and Kuitunen (1979) conducted a study of the concentrations of copper and zinc in human milk. The study included 27 healthy, Finnish mothers. The results of their longitudinal study suggest that no single value can be given for the level of trace
elements in human milk without relating it to the stage of lactation. Copper was determined by atomic absorption spectrophotometry. They report the median copper concentration decreased during the course of lactation from about 0.6 mg/liter to 0.25 mg/liter. As a result of their findings they stress the importance of considering the stage of lactation in the evaluation of the trace element content of breast milk.

In a study of 59 healthy infants ranging from newborn to one year of age, Sorenson and Butrum (1983) found dietary copper intake averaged .52 mg/day. This falls in the low end of the range of the estimated safe and adequate amounts recommended by the Committee on Dietary Allowances (National Academy of Sciences, 1980). The 6 to 12 month age group consumed significantly more copper than the 0 - 5.9 month age group. Average copper intake values for each group were not reported.

Picciano and Guthrie (1976) compared the levels of copper in human milk with those in cow's milk and formula preparations commonly used in infant feeding. The range of reported values of copper of cow's milk is .05 to .30 ug/ml. Formulas based on cow's milk were found to contain from 0.13 to 0.25 ug/ml. In general the soy-based formulas contained relatively high amounts, values ranging from .32 to .5 ug/ml.

Ranges of copper in infant formulas reported by Lonnerdal et al. (1983) were .01 to 2.14 mg/liter. They studied 53 regular infant formulas and 41 special infant formulas used for clinical disorders.

In agreement with other investigators, they point out that it is difficult to assess the adequacy of these levels because little is
known about their availability. They also state that quantitative interrelationships among various trace elements may play a more important role nutritionally than the absolute amount of a particular element.

BIOAVAILABILITY OF NUTRIENTS IN MILK AND INFANT FORMULAS

It has been realized over the years that the mere presence of nutrients in a food does not ensure that they are present in a form which will be available for absorption into the body (Weingartner and Erdman, 1978; Cheryan, 1980).

Although a nutrient may be added to a formula in an available form, if it is in the presence of certain compounds, complexes may form which result in unavailability to the body.

In recent years it has become apparent that quantitative interrelationships among various trace elements may play a more important role nutritionally than the absolute amount of a particular element (Picciano and Guthrie, 1976; Priev, 1965; Widdowson et al., 1974; Walravens and Hambidge, 1975). It has been suggested that iron and zinc (Mason, 1979), zinc and copper (Solomons and Jacob, 1981), and iron and manganese (Gruden, 1979) can be mutally antagonistic. It is possible that a high intake of one element may reduce the absorption of the other element.

Soy protein, phytate and bioavailability. Jaffe (1981) reports that in the processing of soy isolates, the formation of tightly bound protein-phytic acid-mineral complexes may reduce the availability
of some minerals. In reports of Erdman and Forbes (1981) and Jaffe (1981), zinc and iron in soy have appeared to be less well absorbed than from other sources in the diet. Although supplemental zinc absorption did not appear to be impaired.

In a study of phytate and trace metal utilization, Davies and Nightengale (1975) reported that the mechanism by which phytate reduces the availability of copper and manganese probably results from the ability of these trace metals to form metal-phytate complexes which are stable within the intestinal tract. Vohra et al. (1965) found that at a pH of 7.4 sodium phytate forms complexes with metal in the following decreasing order: Cu, Zn, Ni, Co, Mn, Fe, and Ca.

Davis et al. (1962) in a study on the interference of soybean proteins with the utilization of trace minerals found that isolated soybean protein contains a component which combines with zinc, manganese, and copper. In the study chicks were fed diets containing three levels of zinc, manganese, copper, and iron with and without ethylenediaminetetraacetic acid (EDTA) to measure the effect of isolated soybean protein on the availability of these elements. They found that the chicks' dietary requirements for these minerals are increased when isolated soybean protein serves as a dietary constituent without a chelating agent. Chicks develop the respective deficiency symptoms because of the unavailability of these minerals. Davies and Nightengale (1975) agree with speculations of Oberleas (1973) that it appears likely that phytate, under the appropriate dietary conditions, may promote a nutritional deficiency of Zn, Fe,
Cu, Mn, Ca, or Mg depending upon which first becomes limiting in the diet.

Erdman (1979) reports that prediction of mineral bioavailability from phytate-containing foods is complicated by several factors. These include the complex interactions between the minerals and phytic acid contained in the foods, intestinal and meal phytase activities, previous food processing conditions, digestibility of the foods, and the physiological status of the consumer of the foods.

The Committee on Nutrition of the American Academy of Pediatrics (1983) stated that the amount of phytic acid in infant formulas and its effect on mineral and trace elements need to be more clearly delineated.

Lactose and bioavailability. In experiments with infant formula containing soy, Weisberg (1974) has indicated that the absence of lactose in soy formulas may be another reason for the lower percentage absorption of minerals from soy-based formulas as compared to milk-based formulas. Most of the soy-based formulas use sucrose. Weisberg (1974), Theurer et al. (1971) and Theurer et al. (1973) reported that lactose has been shown to be effective in improving the absorption of several minerals. They also report that the processing to which the formula is subjected plays a role in mineral availability.

Several researchers have reported the bioavailability of trace elements for infants to be higher from human milk as compared to cow's milk (Saarinen et al., 1977; McMillan et al., 1977; Hambidge et
al., 1979). Because of this fact, the American Academy of Pediatrics has recommended that infant formulas be supplemented with adequate amounts of these essential nutrients (Committee on Nutrition, American Academy of Pediatrics, 1976a, 1976b, 1978). Trace elements are usually supplemented in the form of inorganic salts, although the bioavailability of these elements is low in this form (Fransson and Lonnerdal, 1982). Because of this, formulas are supplemented with trace elements at levels higher than those reported in human milk (Fomon et al., 1979).

Bioavailability of iron and zinc. There is increasing evidence that the requirements for several trace minerals are dependent to a considerable degree on the type of milk feeding. This has been demonstrated repeatedly for iron and zinc. The bioavailability of iron and zinc have been found to be considerably higher from human milk than from cow's milk (Johnson and Evans, 1978; Casey et al., 1981; Sandstrom et al., 1983).

Lonnerdal et al. (1983) found absorption of zinc was 41 ± 9% from human milk and 28 ± 15% from cow's milk. Absorption from humanized cow's milk formula was 31 ± 7% and soy-based formula was 14 ± 4%. One explanation proposed, attributes the degree of bioavailability to differences in major zinc binding ligands between human milk and cow's milk (Eckbert et al., 1977; Lonnerdal et al., 1980).

Plasma zinc levels of breast-fed infants have been found to be higher than those of infants fed a zinc-supplemented cow's milk formula. The fact that acrodermatitis enteropathica can be treated
with breast milk appears related to the enhanced zinc absorption from human milk versus cow's milk due to different zinc binding ligands (Hambidge et al., 1977; Walravens, 1980).

Iron bioavailability from human milk has been approximated at 50% while bioavailability of iron in formulas is around 5-10% (Saarinen et al., 1977; McMillan et al., 1977). Some of the studies of iron bioavailability are confounded by the fact that human milk has much less iron than the formulas to which it is compared. Lonnerdal et al. (1983) report the average ratio of zinc/iron in human milk ranges from 2.5 to 10. In formulas the range was from .02 to 40. The large variation in ratios may affect the availability of these minerals.

Bioavailability of copper and manganese. Very little is known about the differences in bioavailability of copper in human milk versus cow's milk and infant formula (Lonnerdal et al., 1981). Lonnerdal et al. (1982) have reported that copper in cow's milk is predominantly bound to casein, while copper in human milk is bound to other ligands. Lonnerdal et al. (1982) report the difference in copper binding may result in a different bioavailability of copper to the infant. The somewhat lower ratio of zinc to copper in human milk may significantly enhance copper absorption in breast-fed infants. Widdowson et al. (1974) reported a ratio of about 4:1. Based on breast milk composition other researchers report a ratio of 6:1 as recommended for infant feeding (Fomon, 1974; Johnson and Evans, 1978). Lonnerdal et al. (1983) reported large variations in the ratios of trace elements in formulas they studied. The average
zinc/copper ratio in human milk was 3.3 to 10 while in infant formulas the range was .4 to 74.

The manganese contents of milk and milk products are very low (Vuori, 1979; Murthy and Rhea, 1971; McLeod and Robinson, 1972; Lonnerdal et al., 1981). Lonnerdal et al. (1981) indicate little is known regarding the molecular localization and bioavailability of manganese. Forbes and Erdman (1983) report that a high lactose content of a practical versus purified diet might accentuate manganese absorption.

Flanagan et al. (1980) report absorption of a number of metals, including manganese, is enhanced in mice fed a low-iron diet. Lonnerdal et al. (1983) report the average iron/manganese ratio in human milk was 25 to 100 versus .04 to 425 in formulas studied. Reported ranges of ratios of zinc/iron, iron/manganese and zinc/copper in human milk versus infant formulas are summarized in Table 2.

In a study by Chan et al. (1982) a difference in ligand binding of manganese was found among human milk and cow's milk and infant formula. Infant formula was very different from both human milk and cow's milk in that there was no high molecular weight manganese-binding species in infant formula. This could be very important.

Table 2. Ranges of ratios of zinc/iron, iron/manganese and zinc/copper in human milk and infant formulas*.

<table>
<thead>
<tr>
<th>Ratios</th>
<th>Human milk</th>
<th>Infant Formulas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc/Iron</td>
<td>2.5-10</td>
<td>.02-40</td>
</tr>
<tr>
<td>Iron/Manganese</td>
<td>25.0-100</td>
<td>.04-425</td>
</tr>
<tr>
<td>Zinc/Copper</td>
<td>3.3-10</td>
<td>.40-74</td>
</tr>
</tbody>
</table>

*Reference used: Lonnerdal et al., 1983
depending on whether the high molecular weight protein enhanced or retarded the absorption of manganese.
Piglet. Twelve piglets, from two litters, were obtained from the Utah State farm within the first week of life. They did not receive iron injections. They were weighed and placed in two stainless steel cages. The bottom of the cages were slatted. Cages were three feet in length by one and one-half feet in width. Depth was two feet. There were four pigs from litter one with a mean weight of 2.46 kg and a standard deviation of .39. There were eight pigs from a second litter with a mean weight of 2.61 kg and a standard deviation of .32. There were eight females and four males.

Heat lamps were placed above the cages to provide warmth. Room temperature was kept at 80°F for the first 12 days and then lowered to 75°F. Heat lamps were turned off on the 18th day of the experiment. At this time the room temperature was increased to 85°F. Distilled demineralized water and concentrated Isomil diluted 1:1 with distilled demineralized water was provided for two days, ad libitum, to allow adjustment to the formula. A few drops of antibiotic (Terramycin) was added to the formula and the water during the first week of the study to prevent and or minimize diarrhea. Terramycin has been used as a feed supplement. The recommended dosage is 5 - 20 grams per ton. The recommended dosage by mouth is 5 - 15 mg/lb body
Six piglets had experienced some diarrhea, piglets in pens 1, 2, 6, 7, 8, and 12. This corresponds to three piglets from each treatment group. All cases of diarrhea had disappeared by the fourth day.

On the third day piglets were divided into two groups. Groups were balanced for litter, sex and body weight. From this point piglets were housed, individually, in stainless steel cages. Piglets were watched daily for any observable signs of copper or manganese deficiency.

Piglets continued to receive the diluted formula ad libitum. Isomil diluted 1:1 with distilled demineralized water was fed to piglets in pens 1 - 6. Isomil diluted 1:1 with distilled demineralized water and supplemented with a manganese and copper supplement was fed to piglets in pens 7 - 12. Starting with week three the Isomil was fed to both groups in concentrated form with piglets in pens 7 - 12 continuing to receive the manganese and copper supplement. Isomil lot #76704 was used in this study. The label on Isomil states that concentrated Isomil contains .4 ppm manganese and 1 ppm copper. Analysis in the lab indicated concentrations of 1.05 ug/ml and .98 ug/ml for manganese and copper, respectively. This is equal to .41 mg and .38 mg of manganese and copper, respectively, per can (388 ml) of concentrated formula. The supplemented group was also provided with .5 ml of solution containing 30.6 mg manganese and an additional 6.76 mg copper, per can of concentrated formula, than that contained in the Isomil. On a fluid basis (1:1 dilution) this
corresponds to a total (including manganese and copper in the Isomil) of 40 ppm and 9 ppm, manganese and copper, respectively. The supplements were in the form of manganese sulfate monohydrate and copper sulfate. Copper sulfate has been used in other studies to supplement copper intake of both piglets and infants (Whitehair and Miller, 1975; Casey and Hambidge, 1980). Whitehair and Miller (1975) report copper sulfate is more effective than copper sulfide in producing a growth response in pigs. Copper sulfate and manganese sulfate monohydrate are listed as as common mineral sources for swine by the National Academy of Sciences, National Research Council (1979). Cupric sulfate and manganese sulfate are ingredients in both Isomil and Similac at the present time.

Piglets learned to eat very quickly (within one to two days) from stainless steel trough-type feeders. Isomil and water was provided every six hours or sooner except through the evening. Formula intake was estimated by subtracting amount of formula remaining in the feeder from amount given to the piglet. Water intake was not recorded. Although piglets sometimes walked into feeders and on occasion splashed some formula out of feeders, it is felt formula intakes recorded are close to actual intakes. The feeders were washed with very hot tap water, detergent was not used.

Piglet weights were obtained on a weekly basis, at the same time each week, prior to providing the piglets with fresh formula in the morning. Blood samples were taken immediately before piglets were divided into treatment groups, at the end of week two, end of week three and four, and also at the end of the 5th week. Serum was
analyzed for copper and manganese. At the end of five weeks the pigs were killed. Livers and femurs were removed. These were analyzed for copper, manganese, and zinc.

Infant. With parental consent, blood samples were collected by a pediatrician from infants fed either breast milk or Similac. Approximately 0.2 cc of blood was obtained at the physicians' discretion. A heel prick was used to obtain the samples. Samples were obtained at two months and six months of life. Serum levels of manganese and copper were determined. Information obtained about the infants also included length of gestation, birth weight and length, and weights at two months and six months. Complete data were obtained for five breast-fed infants and four infants fed Similac. Partial data were obtained for an additional four breast-fed and four Similac-fed infants. Similac fed to infants was not analyzed in the laboratory. This was obtained by the infants' mothers on an individual basis. A comparison of calories, protein, main source of carbohydrate, manganese, and copper content of Isomil, Similac, and breast milk is shown in Table 3.

PROCEDURES

Feces. Feces and urine were collected during the third week of the study. Formula intake was recorded as usual. Water intake was not recorded. Feces were collected using a stainless steel pan which fit beneath the cages. Urine was collected via a hole in the center of the pan used to collect the feces. The urine collection was not
Table 3. Calories, protein, main source of carbohydrate, manganese and copper content of Isomil, Similac, and breast milk*

<table>
<thead>
<tr>
<th></th>
<th>Calories</th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>Manganese</th>
<th>Copper</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liter gms/liter</td>
<td></td>
<td>Main Source</td>
<td>ug/liter</td>
<td>ug/liter</td>
</tr>
<tr>
<td>Isomil</td>
<td>680</td>
<td>20</td>
<td>corn syrup</td>
<td>200</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>and sucrose</td>
<td>(525)**</td>
<td>(490)**</td>
</tr>
<tr>
<td>Similac</td>
<td>680</td>
<td>15.5</td>
<td>lactose</td>
<td>340</td>
<td>410</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(600)#</td>
<td></td>
</tr>
<tr>
<td>Breast-milk</td>
<td>747##</td>
<td>10.6##</td>
<td>lactose##</td>
<td>4.9-20$</td>
<td>90-1340$$</td>
</tr>
</tbody>
</table>

*Values listed are for standard dilution (as fed to infants), if no reference listed information taken from product label
**Values listed are those stated on the can when purchased, values listed in parentheses are those found in the lab of the Isomil fed to the piglets.
#During the study copper content of Similac was 410 ug/liter, it has since been increased to 600 ug/liter (standard dilution)
##George and Lebenthal (1981)
$Murthy and Rhea (1971), Vuori (1979)
$$Picciano and Guthrie (1976)

complete because some urine "leaked" out from the sides of the cages.

Feces were analyzed for manganese and copper. Urine was not analyzed.

Feces were oven dried at 98C. They were stored in plastic containers. Feces were finely ground in a blender with stainless steel blades to obtain homogeneous samples. Two gram samples were weighed into acid washed porcelain crucibles. Triplicate determinations were made. They were ashed in a muffle furnace for approximately 36 hours at 550C. The cooled ash was brought into solution with 0.6N HCL which was heated to facilitate the process. The crucibles were rinsed a minimum of three times with distilled demineralized water to retrieve all of the minerals. Samples were diluted to 100 grams into acid washed polyethylene bottles. Samples from the supplemented group were diluted a second time. They were
then read along with standards on an Instrumentation Laboratory aa/ae Spectrophotometer, Model 457. Standards were prepared from Fisher stock solutions with concentrations of 1000 mg/liter of copper and manganese and distilled demineralized water. A complete set of standards was read before and after 18 samples were run on the atomic absorption to check for instrument drift. Concentrations of standards used for determination of copper in feces were a blank of distilled demineralized water, 1, 2, 3, and 4 ppm. Concentrations of standards used for determination of manganese in feces were a blank of distilled demineralized water, 0.5, 1, 2, and 3 ppm. Correlation coefficients calculated from a linear regression of concentrations of standards versus absorption were never less than .99. Instrument parameters used were those specified by Instrumentation Laboratory Inc., Analytical Instrument Division, February 1981. These were the same as those specified in Table 3 with the following exceptions. Spectral band width for copper was 1 nm and for manganese was .5 nm. Lamp current for copper was 5 mA. Sensitivity for copper and manganese was .03 and .02 ug/ml, respectively. The working range for copper and manganese is linear up to 4 and 3 ug/ml, respectively.

Serum. Serum was diluted with a 5% Triton X-100 solution. Serum samples were analyzed all at one time. They were not randomized for analysis. Pig serum samples were diluted with either a 1 in 10 (Initial blood sample and two week sample) or 2 in 10 dilution (all other blood samples obtained). Baby serum samples were diluted 1:1 with 5% Triton X-100.
Samples were read using a Jarell Ash Atomic Absorption Spectrophotometer Model 810 equipped with a FIA-10 graphite furnace by an experienced lab technician. Determinations were made for copper and manganese. Single determinations were made for serum samples of pigs and duplicate determinations were made for infants when sample size permitted. A carbon tube atomizer furnace with a pyrolytic carbon coating was used. Instrument parameters used for determination of serum copper concentration were as follows: samples diluted with 5% Triton-X 100 were injected into the carbon rod and dried at 120°C for 60 seconds, ashed at 600°C for 60 seconds and atomized at 1975°C for 10 seconds. Determinations for manganese were dried at 120°C for 60 seconds, ashed at 800°C for 60 seconds and atomized at 2050°C for 10 seconds. A manganese absorbing line (2795 lambda) was used for channel A and a Pb non-absorbing line (2820 lambda) was used for channel B as recommended by the manufacturer. A ramp atomize mode equal to 2 and argon gas at 2.5 liters/minute were used for determination of both copper and manganese.

Livers. Livers were weighed immediately upon removal from the pigs, then placed in plastic bags and stored frozen until analysis. After thawing at room temperature, approximately 2 to 4 gram samples of liver were weighed into acid washed porcelain crucibles. Samples were taken from as close to the center of the livers as possible. Triplicate determinations were made on two different occasions. Samples were charred on a small hot plate for approximately one hour. They were then ashed in a muffle furnace at 1150°F (625°C) for 48 hours. The cooled ash was brought into solution with 0.6N HCL which
was heated to facilitate the process. The crucibles were rinsed a minimum of three times with distilled demineralized water to retrieve all of the minerals. They were diluted to 25 grams into acid washed polyethylene bottles. One ml of this solution was then diluted with distilled water to 10 ml. They were then analyzed along with standards on Varian techtron D1-30 digital indicator atomic absorption 120. Standards were prepared from Fisher stock solutions with concentrations of 1000 mg/liter of the minerals being determined and distilled demineralized water. A complete set of standards was analyzed before and after 12 samples were run on the atomic absorption. Concentrations of standards used for determination of copper in livers and bones were a blank of distilled demineralized water, 0.5, 1, 2, 3, and 4 ppm. Concentrations of standards used for determination of manganese in bones and livers were a blank of distilled demineralized water, 0.2, 0.5, 0.7, and 1 ppm. Standards used for zinc determinations were a blank of distilled demineralized water, 0.4, 0.7, 1, 1.2, 1.5, and 2.0 ppm. Correlation coefficients calculated from a linear regression of concentrations of standards versus absorption was never less than .99. Periodic checking of standards was also performed so that any corrections needed for instrument drift could be made. Instrument parameters used were those specified by the Varian techtron manual, indicated in Table 4.

Femurs. Femurs were removed from piglets, placed in plastic bags and stored frozen until analysis. Femurs were scraped clean of excess tissue. They were oven dried at 98C for approximately 48 hours and weighed. They were then refluxed with petroleum ether for 24 hours.
Table 4. Instrument parameters for atomic absorption

<table>
<thead>
<tr>
<th></th>
<th>Copper</th>
<th>Manganese</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength, nm</td>
<td>324.7</td>
<td>279.5</td>
<td>213.9</td>
</tr>
<tr>
<td>Spectral band width, nm</td>
<td>0.5</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Lamp current, mA</td>
<td>3</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Flame</td>
<td>Air acetylene</td>
<td>Air acetylene</td>
<td>Air acetylene</td>
</tr>
<tr>
<td>Sensitivity, ug/ml</td>
<td>0.04</td>
<td>0.021</td>
<td>0.009</td>
</tr>
<tr>
<td>Optimum working range, ug/ml</td>
<td>2-8</td>
<td>1-4</td>
<td>0.4-1.6</td>
</tr>
<tr>
<td>Detection limit, ug/ml</td>
<td>.002</td>
<td>.002</td>
<td>.001</td>
</tr>
</tbody>
</table>

Femurs were ground in a Wiley mill and 2 gram samples were weighed into acid washed porcelain crucibles and ashed in a muffle furnace. Minerals were brought into solution by the same procedure as that for liver samples. Determinations were made in two runs, one with duplicate and one with triplicate determinations. Porcine necropsy. Samples of porcine spinal cord and rib tissue were sent to the Utah State University Veterinary Diagnostic Laboratory, Department of Animal, Dairy, and Veterinary Science, Utah State University, Logan, Utah for histopathological examination. Transverse sections of spinal cord and longitudinal sections of rib tissue were made into slides using hematoxylin and eosin staining. Types of abnormalities that were looked for included demyelination, particularly of the dorsal spino-cerebellar tract (McGavin et al., 1962). In the case of demyelination the axons or nerve fibers eventually disappear. This also occurs to much of the interstitial tissue. In sections stained with hematoxylin-eosin, demyelination is diagnosed by the presence of an excessive number and size of holes in an area of white matter (Smith et al., 1972). Signs of demyelination include the presence of irregular fat globules or deposits surrounding the neurons or in the interstitial tissue. In normal
myelination the myelin sheath is usually similar in thickness to the nerve fiber it is surrounding. In cases of demyelination the ratio of myelin to the nerve fiber it surrounds is disproportionate (personal communication with Dr. Stanley Allen, Department of Animal, Dairy, and Veterinary Science, Utah State University, Logan, Utah). Unfortunately with hematoxylin-eosin stains this is not always detectable with certainty. Part of the uncertainty results from difficulty in deciding whether demyelination has occurred or if the spaces are due to edema or to excessive shrinkage of the tissue in the preparation of the stain (Smith et al., 1972 and personal communication with Dr. Stanley Allen). Had the author had more foresight the slides would have been stained with Marchi's method, a usual stain for fats or myelin sheath staining methods. Myelin sheath staining methods directly color normal myelin while leaving demyelinated areas blank.

Neher et al. (1956) found thinning of the epiphyseal cartilage in the radiuses of manganese-deficient pigs. Thinning of the epiphyseal plate of the rib tissue was looked for in this study.

**DATA ANALYSIS**

Statistical evaluation was performed on data collected using the Student's test (Ott, 1977).
**CHAPTER IV**

**RESULTS**

**PIGLET**

**Formula intake.** Piglet formula intake was recorded when piglets had been divided into treatment groups (for 35 days). The first two days of the study were used to allow piglets to adjust to the formula. Total intake of formula, protein, manganese and copper (for the 35 days) is shown in Table 5.

**Fecal excretion of manganese and copper.** Manganese and copper intake, fecal excretion, and apparent digestion are shown in Tables 6 and 7. Isomil intake and weight of feces are also included in Tables 6 and 7, respectively. Data in these tables are totals for the third week of the study. Apparent digestion of minerals is equal to intake minus amount excreted in the feces. Linear regressions of intake versus apparent digestion (mg) were determined for both minerals (refer to table 8).

**Weight gain.** Initial and final weights of each piglet, and weight gains with means and standard deviations of each treatment group are shown in Table 9. Mean weights attained at the end of the study were 11.88 kg ± 2.64 and 11.73 ± 1.93, for supplemented and unsupplemented groups, respectively. Mean weight gains per day were .25 kg ± .07 and .25 kg ± .05 for the supplemented and unsupplemented groups, respectively. No significant differences were found between groups using Student's t test.

**Pig serum.** Values for manganese and copper content of pig serum are shown in Tables 10 and 11. A graph of mean serum manganese versus
Table 5. Total intake (35 days) of Isomil, protein, manganese, and copper, and mean weight gain per day of piglets fed Isomil versus Isomil supplemented with manganese and copper during the first five weeks of life*

<table>
<thead>
<tr>
<th>Piglet</th>
<th>Isomil (liters)</th>
<th>Protein (grams)</th>
<th>Manganese (mg)</th>
<th>Copper (mg)</th>
<th>Weight gain day (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplemented</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>76.25</td>
<td>3050</td>
<td>6043.7</td>
<td>1404.7</td>
<td>.39</td>
</tr>
<tr>
<td>2</td>
<td>44.43</td>
<td>1777</td>
<td>3521.5</td>
<td>818.5</td>
<td>.21</td>
</tr>
<tr>
<td>3</td>
<td>43.18</td>
<td>1727</td>
<td>3423.0</td>
<td>795.6</td>
<td>.20</td>
</tr>
<tr>
<td>4</td>
<td>48.22</td>
<td>1929</td>
<td>3822.4</td>
<td>888.4</td>
<td>.22</td>
</tr>
<tr>
<td>5</td>
<td>62.52</td>
<td>2501</td>
<td>4955.0</td>
<td>1151.7</td>
<td>.28</td>
</tr>
<tr>
<td>6</td>
<td>58.49</td>
<td>2340</td>
<td>4636.5</td>
<td>1077.6</td>
<td>.30</td>
</tr>
<tr>
<td>Mean</td>
<td>55.52</td>
<td>2221</td>
<td>4400.4</td>
<td>1022.8</td>
<td>.27</td>
</tr>
<tr>
<td>S.D.</td>
<td>12.76</td>
<td>511</td>
<td>1012.5</td>
<td>235.3</td>
<td>.07</td>
</tr>
<tr>
<td>Unsupplemented</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>59.51</td>
<td>2380</td>
<td>23.8</td>
<td>59.5</td>
<td>.26</td>
</tr>
<tr>
<td>8</td>
<td>58.78</td>
<td>2351</td>
<td>23.5</td>
<td>58.8</td>
<td>.30</td>
</tr>
<tr>
<td>9</td>
<td>61.97</td>
<td>2479</td>
<td>24.8</td>
<td>62.0</td>
<td>.29</td>
</tr>
<tr>
<td>10</td>
<td>72.57</td>
<td>2903</td>
<td>29.0</td>
<td>72.6</td>
<td>.33</td>
</tr>
<tr>
<td>11</td>
<td>42.50</td>
<td>1700</td>
<td>17.0</td>
<td>42.5</td>
<td>.19</td>
</tr>
<tr>
<td>12</td>
<td>43.41</td>
<td>1736</td>
<td>17.4</td>
<td>43.4</td>
<td>.19</td>
</tr>
<tr>
<td>Mean</td>
<td>56.46</td>
<td>2258</td>
<td>22.6</td>
<td>56.5</td>
<td>.26</td>
</tr>
<tr>
<td>S.D.</td>
<td>11.58</td>
<td>463</td>
<td>4.6</td>
<td>11.6</td>
<td>.06</td>
</tr>
</tbody>
</table>

* For average intake per day divide by 35
Table 6. Third week manganese intake, fecal excretion, apparent digestion of manganese, and Isomil intake of piglets fed Isomil versus Isomil supplemented with manganese and copper during the first five weeks of life

<table>
<thead>
<tr>
<th>Piglet</th>
<th>Mn Intake mg</th>
<th>Fecal Mn mg ± S.D.</th>
<th>Apparent Mn Digestion, mg</th>
<th>Apparent Mn Digestion, %</th>
<th>Isomil Intake*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplemented</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1303.2</td>
<td>1087.0±14.7</td>
<td>+216.2</td>
<td>+16.6</td>
<td>16.31</td>
</tr>
<tr>
<td>2</td>
<td>676.0</td>
<td>387.7±1.1</td>
<td>+288.0</td>
<td>+42.6</td>
<td>8.46</td>
</tr>
<tr>
<td>3</td>
<td>752.2</td>
<td>469.5±5.8</td>
<td>+282.7</td>
<td>+37.6</td>
<td>9.41</td>
</tr>
<tr>
<td>4</td>
<td>736.7</td>
<td>555.1±5.2</td>
<td>+181.6</td>
<td>+24.7</td>
<td>9.22</td>
</tr>
<tr>
<td>5</td>
<td>937.0</td>
<td>771.2±2.9</td>
<td>+165.8</td>
<td>+17.7</td>
<td>11.77</td>
</tr>
<tr>
<td>6</td>
<td>1032.1</td>
<td>996.4±3.3</td>
<td>+ 35.7</td>
<td>+ 3.5</td>
<td>12.92</td>
</tr>
<tr>
<td>Mean</td>
<td>906.2</td>
<td>711.2</td>
<td>+195.0</td>
<td>+23.8</td>
<td>11.35</td>
</tr>
<tr>
<td>S.D.</td>
<td>236.6</td>
<td>287.6</td>
<td>92.9</td>
<td>14.5</td>
<td>2.96</td>
</tr>
</tbody>
</table>

| Unsupplemented |          |                     |                          |                          |                  |
| 7      | 12.8        | 7.9± .1             | + 4.9                   | +38.3                    | 12.19            |
| 8      | 11.4        | 6.8± .1             | + 4.6                   | +40.4                    | 10.81            |
| 9      | 13.4        | 6.5± .1             | + 6.9                   | +51.5                    | 12.72            |
| 10     | 16.1        | 8.6± .9             | + 7.5                   | +46.6                    | 15.28            |
| 11     | 7.9         | 4.6± .1             | + 3.3                   | +41.8                    | 7.51             |
| 12     | 8.1         | 3.5± .1             | + 4.6                   | +56.8                    | 7.70             |
| Mean   | 11.6        | 6.3                 | + 5.3                   | +45.9                    | 11.04            |
| S.D.   | 3.2         | 1.9                 | + 1.6                   | 7.1                      | 3.03             |

* Isomil intake for third week, liters
Table 7. Third week copper intake, fecal excretion, apparent digestion of copper, and weight of feces of piglets fed Isomil versus Isomil supplemented with manganese and copper during the first five weeks of life.

<table>
<thead>
<tr>
<th>Piglet</th>
<th>Cu Intake mg</th>
<th>Fecal Cu mg ± S.D.</th>
<th>Apparent Cu Digestion, mg</th>
<th>Apparent Cu Digestion, %</th>
<th>Feces* Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplemented</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>300.1</td>
<td>139.5±.9</td>
<td>+160.6</td>
<td>+53.5</td>
<td>126.3</td>
</tr>
<tr>
<td>2</td>
<td>155.7</td>
<td>51.7±.8</td>
<td>+104.0</td>
<td>+66.8</td>
<td>42.4</td>
</tr>
<tr>
<td>3</td>
<td>173.2</td>
<td>64.2±1.0</td>
<td>+109.0</td>
<td>+62.9</td>
<td>55.3</td>
</tr>
<tr>
<td>4</td>
<td>169.6</td>
<td>76.8±.1</td>
<td>+ 92.8</td>
<td>+54.7</td>
<td>67.5</td>
</tr>
<tr>
<td>5</td>
<td>216.5</td>
<td>121.1±2.9</td>
<td>+ 95.4</td>
<td>+44.1</td>
<td>129.9</td>
</tr>
<tr>
<td>6</td>
<td>237.7</td>
<td>145.8±1.6</td>
<td>+ 91.9</td>
<td>+38.7</td>
<td>121.9</td>
</tr>
<tr>
<td>Mean</td>
<td>208.8</td>
<td>99.9</td>
<td>+109.0</td>
<td>+53.5</td>
<td>90.6</td>
</tr>
<tr>
<td>S.D.</td>
<td>54.5</td>
<td>40.6</td>
<td>26.2</td>
<td>10.7</td>
<td>39.8</td>
</tr>
<tr>
<td>Unsupplemented</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>12.0</td>
<td>14.4±1.7</td>
<td>- 2.4</td>
<td>-20.0</td>
<td>135.5</td>
</tr>
<tr>
<td>8</td>
<td>10.6</td>
<td>13.0±.1</td>
<td>- 2.4</td>
<td>-22.6</td>
<td>112.3</td>
</tr>
<tr>
<td>9</td>
<td>12.5</td>
<td>13.0±.2</td>
<td>- .5</td>
<td>- 4.0</td>
<td>106.0</td>
</tr>
<tr>
<td>10</td>
<td>15.0</td>
<td>18.2±4.7</td>
<td>- 3.2</td>
<td>-21.3</td>
<td>149.9</td>
</tr>
<tr>
<td>11</td>
<td>7.4</td>
<td>7.4±.4</td>
<td>0.0</td>
<td>0.0</td>
<td>57.3</td>
</tr>
<tr>
<td>12</td>
<td>7.5</td>
<td>7.4±.1</td>
<td>+ .1</td>
<td>+ 1.3</td>
<td>59.0</td>
</tr>
<tr>
<td>Mean</td>
<td>10.8</td>
<td>12.2</td>
<td>- 1.4</td>
<td>-11.1</td>
<td>103.3</td>
</tr>
<tr>
<td>S.D.</td>
<td>3.0</td>
<td>4.2</td>
<td>1.4</td>
<td>11.3</td>
<td>38.4</td>
</tr>
</tbody>
</table>

* Total weight of feces for third week, dry weight, grams
Table 8. Linear regression of intake versus apparent digestion (mg) of manganese and copper of piglets fed Isomil versus Isomil supplemented with manganese and copper during the first to the fifth week of life

<table>
<thead>
<tr>
<th>Treatment</th>
<th>X-axis</th>
<th>Y-axis</th>
<th>Correlation coefficient</th>
<th>Slope</th>
<th>Y-intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplement</td>
<td>Manganese</td>
<td>Manganese</td>
<td>-.411</td>
<td>-0.161</td>
<td>+341.308</td>
</tr>
<tr>
<td>Unsupplement</td>
<td>Manganese</td>
<td>Manganese</td>
<td>+.882</td>
<td>+0.438</td>
<td>+ .214</td>
</tr>
<tr>
<td>Supplement</td>
<td>Copper</td>
<td>Copper</td>
<td>+.703</td>
<td>+0.337</td>
<td>+ 38.486</td>
</tr>
<tr>
<td>Unsupplement</td>
<td>Copper</td>
<td>Copper</td>
<td>-.784</td>
<td>-0.377</td>
<td>+ 2.680</td>
</tr>
<tr>
<td>Both treatments</td>
<td>Manganese</td>
<td>Manganese</td>
<td>+.729</td>
<td>+0.173</td>
<td>+ 20.728</td>
</tr>
<tr>
<td>Both treatments</td>
<td>Copper</td>
<td>Copper</td>
<td>+.969</td>
<td>+0.532</td>
<td>- 4.696</td>
</tr>
</tbody>
</table>

Table 9. Initial and final weights, and weight gains of piglets fed Isomil versus Isomil supplemented with manganese and copper from first week to fifth week of life

<table>
<thead>
<tr>
<th>Piglet</th>
<th>Initial Weight, kg</th>
<th>Final Weight, kg</th>
<th>Weight Gain, kg</th>
<th>Group Weight Gain Mean, kg</th>
<th>S. D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplemented</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.70</td>
<td>16.54</td>
<td>13.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.96</td>
<td>10.13</td>
<td>7.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.12</td>
<td>9.07</td>
<td>6.95</td>
<td>9.32</td>
<td>2.62</td>
</tr>
<tr>
<td>4</td>
<td>2.84</td>
<td>10.64</td>
<td>7.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.31</td>
<td>12.27</td>
<td>9.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.41</td>
<td>12.63</td>
<td>10.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unsupplemented</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2.31</td>
<td>11.42</td>
<td>9.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1.98</td>
<td>12.36</td>
<td>10.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2.75</td>
<td>12.89</td>
<td>10.14</td>
<td>9.16</td>
<td>1.95</td>
</tr>
<tr>
<td>10</td>
<td>2.85</td>
<td>14.47</td>
<td>11.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>2.50</td>
<td>9.25</td>
<td>6.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>3.00</td>
<td>9.98</td>
<td>6.98</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

time is shown in Figure 1. A graph of mean serum copper versus time is shown in Figure 2.

Comparisons of week one and week five were made for both groups for serum manganese and copper. When comparing week one with week five, significant differences were found in serum values in two of the
Table 10. Manganese content of pig serum (ug/ml), from piglets fed Isomil versus Isomil supplemented with manganese and copper from first week to fifth week of life

<table>
<thead>
<tr>
<th></th>
<th>Mean ± standard deviation</th>
<th>* indicates significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supplemented</td>
<td>Unsupplemented</td>
</tr>
<tr>
<td>Week 1</td>
<td>.065±.038</td>
<td>.103±.066</td>
</tr>
<tr>
<td>Week 2</td>
<td>.122±.044</td>
<td>.089±.072</td>
</tr>
<tr>
<td>Week 3</td>
<td>.326±.275</td>
<td>.555±.221</td>
</tr>
<tr>
<td>Week 4</td>
<td>.036±.059</td>
<td>.023±.032</td>
</tr>
<tr>
<td>Week 5</td>
<td>.031±.009</td>
<td>.018±.017</td>
</tr>
</tbody>
</table>

* indicates significance

Table 11. Copper content of pig serum (ug/ml), from piglets fed Isomil versus Isomil supplemented with manganese and copper from first week to fifth week of life

<table>
<thead>
<tr>
<th></th>
<th>Mean ± standard deviation</th>
<th>* indicates significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supplemented</td>
<td>Unsupplemented</td>
</tr>
<tr>
<td>Week 1</td>
<td>1.16±.361</td>
<td>.95±.135</td>
</tr>
<tr>
<td>Week 2</td>
<td>.80±.280</td>
<td>.59±.207</td>
</tr>
<tr>
<td>Week 3</td>
<td>.52±.469</td>
<td>.75±.261</td>
</tr>
<tr>
<td>Week 4</td>
<td>1.18±.341</td>
<td>1.02±.307</td>
</tr>
<tr>
<td>Week 5</td>
<td>1.56±.416</td>
<td>.76±.410</td>
</tr>
</tbody>
</table>

* indicates significance

four comparisons. The unsupplemented group had a significantly lower serum manganese in week five when compared with week one. The supplemented group had a significantly higher serum copper level in week five when compared with week one. No significant differences were found when weeks one and five were compared for serum copper of the unsupplemented group and serum manganese of the supplemented group.

Mean changes for manganese of supplemented versus unsupplemented groups, from week one to week five were also compared. The decrease in serum manganese of the unsupplemented group was significantly greater, p < .1. The mean decrease in serum manganese was 2.4 times as great as the mean decrease in the supplemented group.
Figure 1. Mean pig serum manganese versus time, piglets fed Isomil versus Isomil supplemented with manganese and copper during the first five weeks of life.
Figure 2. Mean pig serum copper versus time, piglets fed Isomil versus Isomil supplemented with manganese and copper during the first five weeks of life.
Pig livers. Copper, manganese, and zinc content of pig livers are shown in Table 12. Fresh weight of livers and per cent ash of livers are shown in Table 13.

Pig femurs. Dry weight and per cent ash of femurs are shown in Table 13. Means and standard deviations for copper, manganese, and zinc of femurs are shown in Table 14.

Correlations including manganese and copper content of pig femurs, livers, and fifth week serum concentrations were determined. The correlation coefficients, slopes, and y-intercepts for copper and manganese concentration of bone versus liver are shown in Table 15.

The linear regression analysis for zinc and copper, and zinc and manganese concentration of bone versus liver are shown in Table 16. The correlation of manganese in bone versus zinc in liver was considered to be significant.

Serum manganese (fifth week) versus liver manganese in the unsupplemented group had a correlation coefficient of +.707. The correlation in the supplemented group was -.247. Other regressions determined had correlations less than .60. These included copper in bone versus zinc in bone, manganese in bone versus zinc in bone, and copper in bone versus manganese in bone.

Table 12. Copper, manganese, and zinc content of pig livers from piglets fed Isomil versus Isomil supplemented with manganese and copper during the first to fifth week of life

<table>
<thead>
<tr>
<th>Element</th>
<th>Supplemented</th>
<th>Unsupplemented</th>
<th>* indicates significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>249±57.8</td>
<td>133±46.3</td>
<td>* p &lt; .01</td>
</tr>
<tr>
<td>Manganese</td>
<td>53±5.1</td>
<td>13±1.2</td>
<td>* p &lt; .01</td>
</tr>
<tr>
<td>Zinc</td>
<td>46±7.9</td>
<td>42±11.9</td>
<td></td>
</tr>
</tbody>
</table>
Table 13. Dry weight and % ash of femurs, and fresh weight and % ash of livers of piglets fed Isomil versus Isomil supplemented with copper and manganese during the first five weeks of life

<table>
<thead>
<tr>
<th>Piglet</th>
<th>Femur dry weight (grams)</th>
<th>Femur Mean* % ash</th>
<th>Liver Fresh weight (grams)</th>
<th>Liver Mean** % ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplemented</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>19.19</td>
<td>56.8</td>
<td>408</td>
<td>1.59</td>
</tr>
<tr>
<td>2</td>
<td>17.61</td>
<td>59.0</td>
<td>218</td>
<td>1.50</td>
</tr>
<tr>
<td>3</td>
<td>14.15</td>
<td>61.2</td>
<td>191</td>
<td>1.55</td>
</tr>
<tr>
<td>4</td>
<td>17.59</td>
<td>60.7</td>
<td>258</td>
<td>1.47</td>
</tr>
<tr>
<td>5</td>
<td>14.54</td>
<td>61.7</td>
<td>245</td>
<td>1.60</td>
</tr>
<tr>
<td>6</td>
<td>16.10</td>
<td>62.6</td>
<td>263</td>
<td>1.58</td>
</tr>
<tr>
<td>Mean</td>
<td>16.53</td>
<td>60.3</td>
<td>264</td>
<td>1.55</td>
</tr>
<tr>
<td>S.D.</td>
<td>2.00</td>
<td>2.2</td>
<td>76</td>
<td>.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unsupplemented</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>16.08</td>
<td>61.5</td>
<td>264</td>
<td>1.50</td>
</tr>
<tr>
<td>8</td>
<td>14.38</td>
<td>62.0</td>
<td>252</td>
<td>1.55</td>
</tr>
<tr>
<td>9</td>
<td>16.84</td>
<td>63.1</td>
<td>242</td>
<td>1.56</td>
</tr>
<tr>
<td>10</td>
<td>17.28</td>
<td>62.4</td>
<td>283</td>
<td>1.54</td>
</tr>
<tr>
<td>11</td>
<td>14.97</td>
<td>60.8</td>
<td>218</td>
<td>1.49</td>
</tr>
<tr>
<td>12</td>
<td>15.07</td>
<td>58.7</td>
<td>231</td>
<td>1.46</td>
</tr>
<tr>
<td>Mean</td>
<td>15.77</td>
<td>61.4</td>
<td>248</td>
<td>1.52</td>
</tr>
<tr>
<td>S.D.</td>
<td>1.10</td>
<td>1.5</td>
<td>23</td>
<td>.04</td>
</tr>
</tbody>
</table>

* Mean of 5 determinations, ** Mean of 6 determinations

Table 14. Copper, manganese, and zinc content of pig femurs from piglets fed Isomil versus Isomil supplemented with manganese and copper during the first to the fifth week of life

<table>
<thead>
<tr>
<th>Element</th>
<th>ug/gram dry fat-free weight</th>
<th>Means ± standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>补</td>
<td>Unsupplemented</td>
</tr>
<tr>
<td>Copper</td>
<td>6.8±2.9</td>
<td>6.6±1.3</td>
</tr>
<tr>
<td>Manganese</td>
<td>3.7±0.61</td>
<td>2.4±0.34</td>
</tr>
<tr>
<td>Zinc</td>
<td>109.0±2.70</td>
<td>100.0±2.70</td>
</tr>
</tbody>
</table>
Table 15. Linear regression of copper and manganese in bone versus liver of piglets fed Isomil versus Isomil supplemented with manganese and copper during the first to the fifth week of life

<table>
<thead>
<tr>
<th>Treatment</th>
<th>X-axis</th>
<th>Y-axis</th>
<th>Correlation coefficient</th>
<th>Slope</th>
<th>Y-intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplement</td>
<td>Mn bone</td>
<td>Mn liver</td>
<td>-.809</td>
<td>-6.726</td>
<td>77.162</td>
</tr>
<tr>
<td>Unsupplement</td>
<td>Mn bone</td>
<td>Mn liver</td>
<td>+.707</td>
<td>+2.408</td>
<td>7.014</td>
</tr>
<tr>
<td>Supplement</td>
<td>Cu bone</td>
<td>Cu liver</td>
<td>+.778</td>
<td>+15.654</td>
<td>142.034</td>
</tr>
<tr>
<td>Unsupplement</td>
<td>Cu bone</td>
<td>Cu liver</td>
<td>+.394</td>
<td>+8.999</td>
<td>90.1</td>
</tr>
</tbody>
</table>

Table 16. Linear regression of copper in bone versus zinc in liver, and manganese in bone versus zinc in liver of piglets fed Isomil versus Isomil supplemented with manganese and copper during the first to the fifth week of life

<table>
<thead>
<tr>
<th>Treatment</th>
<th>X-axis</th>
<th>Y-axis</th>
<th>Correlation coefficient</th>
<th>Slope</th>
<th>Y-intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplement</td>
<td>Cu bone</td>
<td>Zn liver</td>
<td>+.198</td>
<td>+0.541</td>
<td>+41.967</td>
</tr>
<tr>
<td>Unsupplement</td>
<td>Cu bone</td>
<td>Zn liver</td>
<td>-.472</td>
<td>-4.289</td>
<td>+70.048</td>
</tr>
<tr>
<td>Supplement</td>
<td>Mn bone</td>
<td>Zn liver</td>
<td>+.720</td>
<td>+9.253</td>
<td>+11.740</td>
</tr>
<tr>
<td>Unsupplement</td>
<td>Mn bone</td>
<td>Zn liver</td>
<td>-.764</td>
<td>-26.459</td>
<td>+105.610</td>
</tr>
</tbody>
</table>

manganese in bone versus copper in liver, copper in bone versus manganese in liver, copper in liver versus zinc in liver, manganese in liver versus zinc in liver, and serum copper (fifth week) versus liver copper. This was true for both groups.

Porcine necropsy. Rib and spinal cord tissues were examined histopathologically by a veterinary pathologist. The histopathological report indicated some microscopic hemorrhages in gray matter in two pigs in the unsupplemented group. This was not felt to be significant. One possible cause was the pigs death was not from natural causes.

Pictures, from slides made at the histopathological examination, are shown in Figures 3, 4, 5, and 6. Rib tissue, proximal to the epiphyseal plate, is shown in Figures 3 and 4. Spinal cord tissue,
Figure 3. Longitudinal section (100X) of porcine rib, proximal to the epiphyseal plate, from piglet fed Isomil supplemented with manganese and copper during the first five weeks of life.

Figure 4. Longitudinal section (100X) of porcine rib, proximal to the epiphyseal plate, from piglet fed Isomil during the first five weeks of life.
Figure 5. Transverse section (100X) of dorsal spino-cerebellar tract of porcine spinal cord, from piglet fed Isomil supplemented with manganese and copper during the first five weeks of life.

Figure 6. Transverse section (100X) of dorsal spino-cerebellar tract of porcine spinal cord, from piglet fed Isomil during the first five weeks of life.
from the dorsal spino-cerebellar tract, is shown in Figures 5 and 6. There did not appear to be any significant differences or lesions in either group. Types of abnormalities that were looked for included demyelination, particularly of the dorsal spino-cerebellar tract (McGavin et al., 1962). In the case of demyelination the axons or nerve fibers eventually disappear. This also occurs to much of the interstitial tissue. In sections stained with hematoxylin-eosin demyelination is diagnosed by the presence of an excessive number and size of holes in an area of white matter (Smith et al., 1972). Signs that demyelination had occurred would have been irregular fat globules or deposits surrounding the neurons or in the interstitial tissue. In normal myelination the myelin sheath is usually similar in thickness to the nerve fiber it is surrounding. In cases of demyelination the ratio of myelin to the nerve fiber it surrounds is disproportionate (personal communication with Dr. Stanley Allen, Department of Animal, Dairy, and Veterinary Science, Utah State University, Logan, Utah). Unfortunately with hematoxylin-eosin stains this is not always detectable with certainty. Part of the uncertainty results from difficulty in deciding whether demyelination has occurred or if the spaces are due to edema or to excessive shrinkage of the tissue in the preparation of the stain (Smith et al., 1972 and personal communication with Dr. Stanley Allen). Had the author had more foresight the slides may have been stained with Marchi's method, a usual stain for fats or myelin sheath staining methods. Myelin sheath staining methods directly color normal myelin while leaving demyelinated areas blank. This can be detected under a low magnification. Neher et al. (1956) found the distal cartilages of the radiusse of manganese-deficient pigs
were consistently thinner and more serrated in appearance than in controls. They found a marked thinning of the epiphyseal cartilage in the radiuses of the deficient pigs but not in the ulnas. They also found that some of the columnar arrangement of the cartilage cells was lost in the proximal growth plates in the radiuses. No significant changes were found proximal to the epiphyseal plate in the rib tissue examined in this study. Costrochondral junctions were found to be normal.

INFANT

Weight gain. Weight gains, from two months to six months, of breast-fed versus Similac-fed infants were compared. Mean weight gain of four Similac-fed infants was 2.53±.49 kg. Mean weight gain of seven breast-fed infants was 1.98±.43 kg. Similac-fed infants had a significantly greater (p < .05) weight gain. Weight gains, from birth to six months, of breast-fed versus Similac-fed infants were also compared (refer to table 17). Mean weight gain of breast-fed infants from birth to six months was 3.85 ±.93 kg. Mean weight gain of Similac-fed infants was 4.11±1.09. These are not significantly different. Linear regressions were run to determine if any relationships exist between weight gain versus serum copper or manganese change from two months to six months. No such relationships were found.

Linear regressions were also run to determine if any relationships exist between weight and serum values, i.e. two month weight versus two month serum manganese concentration. This was done for both manganese and copper at two months and six months. No relationships were found.
Table 17. Birth weights, weights, serum copper, and manganese, at two months and six months of breast-fed and Similac-fed infants

<table>
<thead>
<tr>
<th>Infant</th>
<th>Birth Wt</th>
<th>Weight, kg</th>
<th>Weight change</th>
<th>Mean Serum Cu, ug/ml</th>
<th>Mean Serum Mn, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Gestation)</td>
<td>Month 2</td>
<td>6</td>
<td>to 6 mos</td>
<td>2</td>
</tr>
<tr>
<td>W B*</td>
<td>3.74</td>
<td>5.25**</td>
<td>7.40</td>
<td>3.66</td>
<td>1.48</td>
</tr>
<tr>
<td>(42)***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JE B</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1.04</td>
</tr>
<tr>
<td>N B</td>
<td>3.51#</td>
<td>5.44</td>
<td>7.00</td>
<td>3.49$</td>
<td>0.64</td>
</tr>
<tr>
<td>(39)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DS B</td>
<td>3.52</td>
<td>5.40</td>
<td>6.92</td>
<td>3.40</td>
<td>1.06</td>
</tr>
<tr>
<td>(40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M B</td>
<td>3.35</td>
<td>4.81</td>
<td>6.46</td>
<td>3.11</td>
<td>1.00</td>
</tr>
<tr>
<td>(40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH B</td>
<td>4.34</td>
<td>6.10</td>
<td>7.95</td>
<td>3.61</td>
<td>0.95</td>
</tr>
<tr>
<td>(44)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FO B</td>
<td>3.69</td>
<td>5.34</td>
<td>7.60</td>
<td>3.91</td>
<td>0.96</td>
</tr>
<tr>
<td>(40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S B</td>
<td>4.12</td>
<td>7.30</td>
<td>10.00</td>
<td>5.88</td>
<td>1.21</td>
</tr>
<tr>
<td>(40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HU B</td>
<td>3.03</td>
<td>4.37</td>
<td>6.40</td>
<td>3.37</td>
<td>--</td>
</tr>
<tr>
<td>(40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V S</td>
<td>3.23</td>
<td>5.17</td>
<td>7.32</td>
<td>4.09</td>
<td>.69</td>
</tr>
<tr>
<td>(38)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H S</td>
<td>3.09</td>
<td>4.93</td>
<td>7.26</td>
<td>4.17</td>
<td>--</td>
</tr>
<tr>
<td>(42)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G S</td>
<td>4.17</td>
<td>6.03</td>
<td>8.43</td>
<td>4.26</td>
<td>--</td>
</tr>
<tr>
<td>(40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C S</td>
<td>4.68</td>
<td>5.83</td>
<td>7.96</td>
<td>3.28</td>
<td>.58</td>
</tr>
<tr>
<td>(40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F S</td>
<td>3.20</td>
<td>5.30</td>
<td>8.00</td>
<td>4.80</td>
<td>.86</td>
</tr>
<tr>
<td>(40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P S</td>
<td>3.23</td>
<td>4.38$</td>
<td>5.30</td>
<td>2.07</td>
<td>1.06</td>
</tr>
<tr>
<td>(--)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J S</td>
<td>2.24</td>
<td>3.50</td>
<td>6.65</td>
<td>4.41</td>
<td>.78</td>
</tr>
<tr>
<td>(42)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B S</td>
<td>3.40</td>
<td>6.00</td>
<td>9.20</td>
<td>5.80</td>
<td>--</td>
</tr>
<tr>
<td>(41)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Letters = infant, B = Breast-fed, S = Similac-fed
** 6 week weight
*** Length of gestation in weeks
# 2 week weight
$ weight change from 2 weeks to 6 months
& 7 week weight
Infant serum. Mean serum copper and manganese levels of breast-fed versus Similac-fed infants, for two months and six months, are shown in Table 18. Serum copper and manganese levels for individual infants are shown in Table 17. Serum levels for both copper and manganese were examined to note any trends within a group, and/or differences between groups.

Table 18. Mean serum copper and manganese, at two months and six months of age, of breast-fed infants versus Similac-fed infants

<table>
<thead>
<tr>
<th></th>
<th>Mean ± standard deviation</th>
<th>Mean ± standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Copper, ug/ml</td>
<td>Manganese, ng/ml</td>
</tr>
<tr>
<td></td>
<td>Month 2</td>
<td>Month 6</td>
</tr>
<tr>
<td>Breast-fed</td>
<td>1.02±.27*#</td>
<td>1.39+.46*</td>
</tr>
<tr>
<td>(# infants)</td>
<td>(6)</td>
<td>(6)</td>
</tr>
<tr>
<td>Similac-fed</td>
<td>.73+.12#</td>
<td>.99+.46</td>
</tr>
<tr>
<td>(# infants)</td>
<td>(4)</td>
<td>(4)</td>
</tr>
</tbody>
</table>

* p < .1 (Means with the same symbol are significantly different)
# p < .05, $ p < .05

In the breast-fed group serum manganese levels either remained stable or increased from two months to six months. There were two exceptions to this, in both of these cases the serum manganese dropped from 9.8 to 8.5 ng/ml. In the case of serum copper of breast-fed infants, values increased for four infants, remained stable for one infant, and showed a slight decrease for one infant (0.12 ng/ml).

Of the four infants in the Similac group, serum manganese remained stable for two, decreased substantially for one, and increased for the fourth (2.4 to 3.1 ng/ml). In the case of copper two of the infants had increases and two decreases from two to six months.
Although sample sizes were small, Student's t test was run to test for significant differences when comparing copper and manganese concentrations at two months and six months of both breast-fed and Similac-fed infants. A significant difference was found in the comparison of two month and six month serum copper levels of breast-fed infants. Six month levels were significantly higher.

A significant difference was also found when comparing manganese levels of breast-fed versus Similac-fed infants at six months. The breast-fed infants had a significantly higher serum manganese level ($p < .05$). No significant difference was found at two months.

Copper in breast-fed infants was found to be significantly higher at two months than that of Similac-fed infants ($p < .05$). No significant difference was found when comparing six month copper levels of breast-fed infants versus Similac-fed infants.
Many factors need consideration when attempting to define adequacy of infant formulas in meeting the nutrient requirements of infants. Included among these factors are several which have been reviewed, previously, in the literature review in this paper.

The demonstration of essentiality of a trace element in a variety of animal species has always led to the conclusion that the element is also essential to man (one example is selenium). Determination of what is an appropriate intake of a nutrient for man is not always straightforward.

A state of optimal nutritional status with respect to a nutrient exists somewhere between the range where no deficiency symptoms are detected and where tissue concentrations are increased to toxic levels. Unfortunately it is much easier to determine nutrient needs to prevent deficiency symptoms than it is to determine nutrient requirements for optimal nutrition.

In this country marginal nutritional status with respect to a nutrient probably affects a significant number of individuals yet goes undetected because of lack of observable, clinical symptoms. An example appears to be that sub-optimal calcium nutrition throughout growth and development may play a role in increasing the risk of osteoporosis later in life.
Determining whether the goal is to meet basic requirements of a nutrient or optimal amounts is often difficult. Even more difficult is determining what is the optimal intake of a specific nutrient. Defining what is optimal varies among researchers. Some researchers feel the establishment of a human requirement for a given substance necessitates the production of a deficiency state. Because this is ethically questionable estimates of requirements must be made for some nutrients. Many trace elements fit into this category.

The basic requirement for a trace element has been defined as that daily intake which allows the actual absorption into the organism of an amount sufficient to prevent deficiency, based upon some clear, observable symptoms that are physical, biochemical or physiological in nature. Optimal intake has been defined as the daily intake which allows absorption of an amount sufficient to maintain in near-optimal function all biochemical and physiological mechanisms in which the element is involved, under the various stress conditions of life. Meeting optimal requirements is desirable because it will prevent the more subtle and chronic symptoms of a marginal deficiency (Mertz, 1972).

**FIRST OBJECTIVE**

The first objective of this study was to determine whether Isomil provides sufficient amounts of manganese and copper for adequate nutrition. The baby pig was used as a model. In three studies by Schneider and Sarett (1966, 1969a, 1969b), it has been
shown that the baby pig is extremely useful in evaluating infant formulas.

To answer the first objective of this experiment, observable differences were looked for between the group of infant pigs fed Isomil, or Isomil supplemented with manganese and copper at levels adequate for the baby pig.

**Observable symptoms.** Observable symptoms that may have been expected were tremors, a lack of rigidity in the leg joints and the hocks becoming excessively flexed forcing some of the piglets to assume a sitting position. None of these symptoms were observed in either group.

Because nutritional status with respect to nutrients may be marginal (versus the clinical appearance of observable symptoms) tissue levels were also compared. Tissue concentrations of manganese, copper, and zinc, and serum levels of manganese and copper were determined.

**Weight gain.** Numerous studies have been done with both human infants (Glaser and Johnstone, 1952; Fomon, 1959; Omans et al., 1963; Bates et al., 1968; Widdowson, 1969; Jung and Carr, 1977). and baby pigs Schneider and Sarett (1966, 1969a, 1969b) to determine if weight gains are the same when fed a milk-based versus soy-based formula (and in infants versus breast milk). In the majority of studies no significant differences were found in weight gains (Glaser and Johnstone, 1952; Fomon, 1959; Omans et al., 1963; Bates et al., 1968; Widdowson, 1969; Jung and Carr, 1977).
A variety of studies have documented the fact that infant formulas, (both milk-based and soy-based), are capable of promoting similar weight gains and length increases to those observed in breast-fed infants. Weight gains of human infants four to six months old were found by Fomon et al. (1973) to be at least as great as those of normal full-term infants of similar age who were fed human milk.

This experiment was run for 37 days. There was no significant difference in Isomil intake of the two groups. It was expected that weight gains of piglets would be the same for both groups. There were no significant differences in weight gains of the two groups. Weights gains were similar to those reported in other studies (Julius et al., 1982; Schneider and Sarett, 1969b). Mean weight gains per day were the same as levels reported previously for baby pigs fed casein or soy protein (Miller et al, 1965), although variability was greater.

Supplementation of Isomil with manganese and copper did not influence weight gains of the piglets in this study. Final weights of piglets in this study were higher than those of piglets in a study by Whittemore and Elsley (1976) who found piglets fed sow's milk alone had attained a weight of about eight kg at five weeks of age and, piglets on sow's milk plus supplementary feed attained a weight of gain of approximately ten kg. The reason for this is not clear.

It was felt that the growth of piglets was rapid enough to provide sufficient stress so that deficiency symptoms would manifest themselves. All piglets studied had achieved a minimum weight gain
of 3.3 times their weight at the start of the experiment. The final weights had an overall mean of 4.66±.99 times the starting weights. Because the average infant is expected to double his birth weight in six months and triple his birth weight in the first year of life the stress of growth on the baby pigs was considered greater than that placed on the human infant in the first year of life.

Manganese and copper requirement of the pig. Manganese requirement for the pig has been reported to be up to 40 ppm. Underwood (1977) has reported that satisfactory growth has been reported with diets supplying only 0.5 to 1.0 -1.5 ppm manganese, however, marked tissue manganese depletion was observed. When such diets were fed throughout gestation and lactation skeletal abnormalities and impaired reproduction became apparent. All of these manifestations were prevented by supplemental manganese at a level of 40 ppm. Levels fed to the supplemented group were adequate to meet the requirements of baby pigs.

Underwood (1977) reports that no differences due to treatment were observed in baby pigs fed diets containing 6, 16, or 106 ppm copper. Therefore, it was assumed that 6 ppm is adequate for growth in such animals.

The manganese and copper concentrations in Isomil are not adequate to meet the needs of the baby pig. In addition it has been suggested that the intestinal phytase activity in the very young pig is inadequate to digest the phytate present in soy protein, causing the unavailability of cations which effectively bind with phytate. It was felt that this might decrease the bioavailability of copper
and manganese to the infant pig. It was expected that deficiency symptoms would be observable in the unsupplemented group because Isomil does not contain adequate amounts of manganese and copper for infant pigs.

**Influence of Terramycin on piglets.** Terramycin and other antibiotics have been widely fed to pigs for both growth stimulation and for disease-control measures (Hungerford, 1975). In this study Terramycin was discontinued after the first week, when diarrhea had stopped for several days. An adverse reaction which presumably was caused by the antibiotic was the development of thrush in some of the pigs. This was evidenced by appearance of a white pseudo-membrane which covered the back of the tongue. Although the specific organism causing the thrush was not confirmed in this experiment, there have been similar findings in other studies using piglets in this department. In these cases the cause was found to be Candida albicans. Signs and symptoms of candida albicans include persistent scouring, thirst, and pseudo-membranes in the digestive tract (Hungerford, 1975). The only symptom observed in the piglets in this study was the formation of a pseudo-membrane.

**Apparent digestion of manganese.** All piglets in the study were in positive manganese balance, based on apparent digestion. Only trace amounts of manganese are excreted in the urine (Shils, 1972). Results of this study lead the author to the conclusion that during the third experimental week piglets required only about 1 mg/day to maintain positive manganese balance. When both groups were looked at together, a linear regression of manganese intake versus manganese
apparent digestion (mg) had a correlation coefficient of +.729. When groups were looked at individually a correlation of -.411 was found for the supplemented group. At a high level of manganese intake young pigs may be unable to reabsorb much of the manganese secreted by the bile. The unsupplemented group had a correlation of +.882, suggesting a strong positive relationship when intake of manganese was within a relatively narrow, low, range.

Apparent digestion of copper. Piglets in the supplemented group were in substantial positive balance for copper. In the unsupplemented group pigs were essentially in negative balance. The amount of copper excreted in the urine is negligible (Ulmer, 1977). A linear regression of copper intake versus apparent digestion (mg) of copper was determined. A very strong positive relationship was found (correlation coefficient +.969) when both groups were looked at together. From the data it appears that the greater the intake of copper the greater the apparent digestion. Okonkwo et al. (1979) report similar findings. Although, fecal excretion of copper in all piglets in the unsupplemented group were a minimum of two times higher than those reported by Okonkwo et al., in piglets who had similar daily intakes of copper. The balance study by Okonkwo was conducted when piglets were 72 days of age. The younger age of the piglets in this study may explain the increased copper excretion in the feces. It is possible that these findings are related to failure of young piglets to reabsorb copper secreted by the bile. Increased bile acid excretion with a soy formula diet may also play a role in
the increased copper excretion in the feces. It must also be taken into account that the older pigs, because of increased body weight, probably have a higher total copper requirement per day. When groups were looked at individually opposite correlations were found, +.703 and -.784 for the supplemented group and the unsupplemented groups, respectively. Indicating that at the high level of copper intake, the greater the intake of copper the greater the apparent digestion. At the low level of copper intake the greater the intake the less the apparent absorption. Piglets in the unsupplemented group appear to have needed approximately 1-3 mg/day to maintain positive copper balance. These findings appear to agree with Okonkwo et al. (1979).

**Manganese, copper, and zinc in serum and tissues.** Liver, bone, and serum levels of copper and manganese were studied to help judge ability of Isomil to meet nutrient needs with respect to these minerals. Serum concentrations of some minerals are maintained within a normal range at the expense of body stores. This occurs with serum calcium and iron (in hemoglobin and hematocrit). As a result, in certain cases serum mineral concentrations are not reliable indicators of nutritional status. In other cases a decrease in serum concentration is one of the first signs of marginal deficiency. Serum copper has been reported to decrease rapidly when dietary intake is decreased.

**Serum copper.** It was expected that serum copper in the unsupplemented group would drop from week 1 to week 5. This did occur although the decrease was not statistically significant. Use
of copper from liver storage may explain why the decrease was not significant.

Five piglets from the second litter had decreases in serum copper from weeks 1 - 5 and one piglet from the first litter. Four piglets were from the unsupplemented group and one piglet was from the supplemented group. One piglet was from both the first litter and the unsupplemented group. In the case of this piglet he had the second highest weight gain (pig 10). This may have created an increased demand, or at least a greater likelihood of showing increased stress. This piglet also showed the second greatest decrease of serum manganese and was at the low end of the range for both liver and bone concentrations of copper and manganese of both groups. Of the five piglets from the second litter one was from the supplemented group. This may be coincidental or it is possible that the sow's nutritional status played a role in some of these results. Lack of control of the nutritional status of the sow's is one limitation of this study which may have contributed to piglet variation. Okonkwo et al. (1979) report a range of serum copper of 0.20 - 2.80 ug/ml in piglets receiving .13 to 5.8 mg of copper per day. These piglets were from sow's fed a low-copper (3.6ppm) diet. Although a wide range, serum copper in piglets in this study fall within this range.

**Serum manganese.** In an experiment to determine requirements of manganese for pigs Okonkwo (1977) reports serum manganese concentration merely fluctuated without showing any statistically significant response to dietary levels of manganese. The dietary levels of manganese fed ranged from .9 to 7.4 ppm. The abstract did
not indicate how manganese was determined. An explanation for the sharp increase in serum manganese in both groups, in the middle of the study cannot be explained with certainty. Several possibilities exist. The samples may have been contaminated in some way. The instrument may have malfunctioned. There may have been an error made in the calculations. Because the third week serum values are elevated by a factor of ten in both groups, the author feels this is the most likely explanation. If this is what actually occurred, serum manganese levels would have dropped consistently for both groups from week one to week five.

Manganese, copper, and zinc in livers. No significant differences were found in fresh weight or % ash of livers. No significant differences were found for zinc concentrations of livers. Liver zinc concentrations in both groups were similar to values of 40 ug/gram fresh tissue reported by Underwood (1977).

Both liver stores of manganese and copper were significantly increased in the supplemented group. The conclusion, the supplemented group was able to store copper and manganese in greater amounts than the unsupplemented group. Whitehair and Miller (1975) report the normal values of liver copper in baby pigs as approximately 200 ppm. Liver copper concentrations were 249 and 133 ug/gram in the supplemented and unsupplemented groups, respectively. Underwood (1977) reports liver copper concentrations are sensitive to low copper intakes and provide useful aids in the diagnosis of copper deficiency. McGavin et al. (1962) found histological examination of copper deficient tissue showed marked spinal demyelination affecting
mainly the dorsal spinocerebellar tracts. Liver copper values in these pigs ranged between 3 and 10 ppm. In a state of increased stress, storage of manganese and copper may mean the difference between optimal functioning of the organism and decreased activity of enzyme systems in which these minerals participate.

Manganese, copper, and zinc in femurs. No significant differences were found between groups when comparing dry weight and % ash of femurs. Mean values for zinc, copper, and manganese in femurs of piglets were within the same range as reported values for bones in human fetal tissues (Casey and Robinson, 1978). Okonkwo et al. (1979) report a range of copper concentration in piglet femurs of 6.4 - 9.2 ppm, on a dry fat-free basis, when dietary copper ranged from 1.3 - 9.3 ppm. They found copper content of femurs increased as amount of copper in the diet increased. An increase in copper content of the femurs with increased copper in the diet was not observed in this study.

Values for liver manganese in this study were similar to those reported by Hudnik et al. (1983) for the unsupplemented group. The supplemented group had manganese levels in the liver four and one-half times those reported by Hudnik. The age of the pigs, manganese intake, or whether the manganese content of femurs was on a dry or wet basis was not reported. These factors may play a role in the large difference in values of the supplemented group.

It has been found that manganese is not stored in the liver as some other elements (copper, iron) and has been postulated that the bones constitute some fetal storage of manganese in baby pigs.
In this study the femurs of the supplemented group contained significantly greater levels of manganese than those of the unsupplemented group. It does not appear to be an abnormal or near toxic level because it is still within the reported range of concentration for human fetal tissue. It appears from manganese content of the femurs that the supplemented group is in a better position to handle nutritional stresses with respect to manganese status.

The purpose of zinc determinations in tissues was to show tissue concentrations of a mineral which was not altered in the diet of either group. Isomil fed to the piglets contained 10 mg zinc per liter of concentrated formula, according to the product label. The bone content of zinc in the supplemented group was found to be significantly higher that that of the unsupplemented group. The explanation for this is not clear. One explanation would be competition for storage sites in the liver displacing some zinc to the bone. A mutual antagonism has been reported between copper and zinc absorption (Hall et al., 1979). Kirchgessner et al. (1982) report increased serum, liver, and bone concentrations of copper in copper deficient animals versus controls when zinc intake was held constant. Although the difference did not appear to be significant.

If a zinc:copper interaction existed it would also be expected that the level of zinc in the bone of the supplemented group would be significantly higher as a result of the competition in the liver in the supplemented group. It is possible that the lower ratio of zinc:copper may have increased zinc absorption in the supplemented group. The fact that no significant difference was found for copper
concentrations in femurs may be related to the significant difference found with respect to zinc.

Positive correlation between manganese in bone and manganese in liver, and negative correlation between manganese in bone and zinc in liver of the unsupplemented group were of the same magnitude. The explanation for this is not clear. In the supplemented group the opposite situation exists. A negative correlation was found between manganese in bone and manganese in the liver. The reports in the literature are not consistent with respect to manganese/zinc interactions. Kirchgessner et al. (1982) report that zinc retention may increase in response to diets highly supplemented with manganese as compared to unsupplemented rations despite the zinc supply remaining the same. They also report that manganese-deficient pigs did not differ in zinc content from animals provided with adequate manganese. In this study there was a positive correlation between manganese in the bone and zinc in the liver. It is possible that in the supplemented group increased manganese in the liver displaced some zinc to the bone. This would explain the significantly higher zinc concentration in the bone of the supplemented group. This would be in addition to any influence increased copper had on zinc deposition. It is also possible that liver storage sites had reached capacity and excess manganese was displaced to the bone.
The second objective of the study was to have porcine spinal cord and rib tissue histopathologically examined for any significant differences between the groups. Observations that may have resulted from copper deficient states would include demyelination of spinal cord and fractures of ribs, thinned cortices, broadened epiphyseal cartilage, and a low level of osteoblastic activity. The ratio of the width of the myelin sheath to the nerve fibers appeared normal in both groups of piglets. There did not appear to be any abnormal fatty deposits surrounding the neurons which might be seen with demyelination. Marchi's method or a myelin sheath staining method would make the diagnosis of demyelination easier, more certain, and would have been more appropriate in this study.

Underwood (1977) reports a decreased concentration of acid mucopolysaccharides in rib tissue might be expected with manganese deficiency. Neher et al. (1956) found cartilage discs in the distal end of the radius were consistently thinner and more serrated in appearance in manganese-deficient swine than in controls. They found a marked thinning of the epiphyseal cartilage and a disarrangement of the structure of the proximal growth plate so that some of the columnar arrangement of the cartilage cells was lost. This abnormality appeared to be somewhat selective because it did not occur in the ulnas of the animals. No significant lesions or differences between groups were noted in the histopathological report in this study.
The lack of any significant lesions in the unsupplemented group indicates the provision of adequate manganese and copper in Isomil for minimum requirements of the baby pig during the five week period studied. The lack of observable, clinical symptoms reported above is in agreement with this finding.

In some pigs, manganese deficiency is first discovered at the time of reproduction. Hurley (1981) reports when pregnant animals are deficient in manganese their offspring exhibit a congenital, irreversible ataxia which is characterized by incoordination, lack of equilibrium, and retraction of the head. This indicates that minimum requirements do not always allow animals (and probably humans) to escape deficiency symptoms under the natural stress conditions of life.

**THIRD OBJECTIVE**

The third criterion for judging adequacy of infant formula in providing manganese and copper looked at blood samples from infants fed either breast milk or Similac. Similac is a cow's-milk based formula. Both breast milk and Similac contain lactose as the carbohydrate source, in contrast to the source of carbohydrate in Isomil. The carbohydrate source in Isomil is corn syrup and sucrose. This may play a role in the bioavailability of minerals.

Breast milk is reported to contain an average of 400 ug/liter of copper, (George and Lebenthal, 1981) at the time of this study Similac contained 410 ug/liter. Similac presently contains
600 ug/liter according to the product label. Breast milk contains 4.9 - 20 ug/liter manganese (Murthy and Rhea, 1971; Vuori, 1979) compared with 34 ug/liter in Similac. Differences in mineral binding ligands probably contribute to the increased copper levels in the breast-fed versus Similac-fed infants, despite the higher level of manganese in Similac versus breast milk.

The ratio of zinc:copper in breast milk has been reported at about 4 or 6:1 (Widdowson et al., 1974; Fomon, 1974; Johnson and Evans, 1978). The ratio in Similac at the time of this study was 12.2:1 and in Isomil is 10:1 (from using information on product label). The lower ratio of zinc:copper may enhance absorption of copper in the breast-fed infants.

Ohtake (1977) reports a mean range of .47 to 1.04 ug/ml in infants from five days to five months of age. Total mean range reported by Ohtake was .38 - 1.45 ug/ml. Sann et al. (1980) reports serum copper to be .79±.08 ug/ml in seven day old, full-term, infants. Mean serum copper in infants in this study ranged from .73 - 1.39 ug/ml. The reason that the values in this study are towards the upper end of the mean range reported by Ohtake may be related to the fact that serum values in this study were taken at two months and six months. Sann et al. (1980) report in full-term infants serum copper concentrations rise steeply after birth. Mason (1979) reports that the estimates of the age at which adult values are reached varies from about three to six months to nine to twelve months. Mean serum copper levels reported by Ohtake were .63 ± .17 ug/ml, with a range of .50 -1.04, and .81 ± .17 ug/ml, with a range of .50 - 1.11, for
infants one month and three months old, respectively. Mean serum copper at five months was $1.04 \pm .25 \, \text{ug/ml}$, with a range of $0.69 \text{ - } 1.45$. In this study mean serum copper levels at two months were $0.73 \pm .12 \, \text{ug/ml}$ and $1.02 \pm .27 \, \text{ug/ml}$, for the Similac-fed and breast-fed infants, respectively. Mean serum copper at six months were $0.99 \pm .46 \, \text{ug/ml}$ and $1.39 \pm .46 \, \text{ug/ml}$, for the Similac-fed and breast-fed infants, respectively.

It is also possible the higher serum copper values are related to the nutritional status of the mother's, which was not controlled, and is one of the limitations of this study. The serum copper of the breast-fed infants was significantly greater at two months than the Similac-fed infants. These results agree with findings of Ohtake (1977). Henkin et al. (1973) did not find any significant differences. Although the difference was not determined to be significant, the serum copper of the breast-fed infants was greater at six months than that of the Similac-fed infants in this study.

Casey and Hambidge (1980) report the liver stores of the full-term neonate are generally adequate to meet the copper needs of the infant for four to six months. Liver stores of copper probably play a role in these findings.

Serum manganese levels of infants in this study are considerably lower than those reported by others. The reason for this is not clear. There has been a wide range of reported concentrations of human serum manganese. D'Amico and Klawans (1976) report a range of $0.57 - 24 \, \text{ng/ml}$ in the literature.
Manganese and bile acid excretion. Manganese is excreted mainly through the bile. It has been reported that bile acid excretion is greater with soybean based formulas then with cow's milk in infants. It has also been hypothesized that the large fecal loss of manganese in one week old infants may be attributed to failure to reabsorb manganese secreted by the bile. It is possible that infants fed soybean based formulas may be excreting greater amounts of manganese as a result and therefore retaining less manganese in the body. A situation such as this might also exist when comparing cow's milk based formulas with breast milk. Signer et al. (1974) found premature infants on human milk excreted less bile acids in the stool than did infants fed cow's milk formula. This could be one explanation for the significantly increased serum manganese level of the breast-fed infants at six months when compared with the infants fed Similac. A second explanation would be the difference in binding ligands found by Chan et al. (1982).
CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

At the present time wide ranges of copper and manganese exist in infant formulas. An obvious discrepancy exists at the present time between recommendations of estimated safe and adequate intakes of manganese and actual amounts infants are receiving in the diet.

From this discrepancy it is warranted that either the current recommendations should be decreased to conform with actual intakes of healthy infants or amounts in infant formulas should be supplemented to meet current recommendations. Consideration needs to be given to differences in bioavailability of trace minerals when determining the appropriate trace element content of infant formulas. Standardization needs to exist within similar types of infant formulas.

It is indicated in the literature that manganese concentration in tissues is relatively constant throughout life and that the body does not appear to store manganese as it does some other minerals. The concentration of manganese in the bones and liver of the supplemented group was significantly greater than that in the unsupplemented group. The results of this study indicate that manganese concentrations can be increased in the tissues. This has been found in animals and appears to result in some storage of this mineral. It is possible some storage may take place in humans.
The possibility exists that as in the baby pig bones constitute some form of manganese storage in human infants. Unfortunately, this is only speculation at the present time. Therefore, reports that the fetal liver does not store manganese make it essential that infants be provided with adequate amounts of manganese because of lack of stores to rely on.

It appears levels in the supplemented group would be closer to those expected in a state of optimal nutrition than those of the unsupplemented group. With knowledge of the severe deficiency symptoms produced in animals as a result of deficiency of this mineral and the involvement of manganese in such a broad spectrum of metabolic activities, a better defined requirement for this mineral is essential.

Liver concentrations of both manganese and copper can be increased with supplementation of a soy-based diet in infant pigs. It appears that the levels of copper and manganese in Isomil are adequate to meet the minimum requirements for infants. They may not be adequate for optimal nutrition as liver and bone concentrations of animals fed Isomil were significantly lower when not supplemented with manganese. Liver copper concentrations were also significantly decreased when not supplemented with copper.

Although no clinical deficiency symptoms were observed in infant pigs, tissue levels were significantly decreased. The significantly greater serum copper and manganese levels of breast-fed versus Similac fed infants may be related to different bioavailability of these minerals from breast milk versus Similac or other infant
formulas. Sample sizes were small. This study needs to be repeated with larger numbers of infants.

Many marginal deficiencies go undetected. There are several factors the author feels need to be considered in the formation of recommended allowances for manganese and copper. These are stated below. The risk of manganese and copper toxicity has been found to be low in man. Some balance studies with copper have found .5 mg/day required for healthy, full-term infants to maintain positive balance. This is the lower end of the range of the current estimated safe and adequate recommendations (1980). Studies with manganese have found one week old healthy newborns to be in substantial negative balance. Greater bile acid excretion with cow's milk and soy-based formula than with breast milk in infants may result in decreased retention of manganese and possibly copper. Many factors have been shown to influence the bioavailability of these minerals.

With the above factors in mind it is recommended that infant formulas contain a minimum concentration of manganese and copper which will provide infants of 0 - 6 months of age with .5 - .7 mg/day, to meet the current estimated safe and adequate recommendations. It is believed it is safer to provide infants with more of these minerals than many of them are currently receiving, and that this will bring infants closer to a state of optimal nutrition with respect to these nutrients. Further research may indicate the need for levels different from the current recommendations.
REFERENCES


Belyaev, P. M. 1938. The biological role of trace elements in the human organism, V. The influence of manganese ions on blood sugar content. J. Physiol. (USSR) 25:741.


Table 19. Individual values, from two runs, for copper, manganese, and zinc content of pig livers from piglets fed Isomil versus Isomil supplemented with manganese and copper during the first to the fifth week of life*

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*Triplicate determinations were made on two different runs.*
Table 20. Individual values, from two runs for copper, manganese, and zinc content of pig femurs from piglets fed Isomil versus Isomil supplemented with manganese and copper during the first to the fifth week of life*

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Table 20. (continued)

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*Duplicate determinations were made on one run, triplicate determinations were made on a second run.
Table 21. Individual serum manganese content (ug/ml), for weeks one to five, from piglets fed Isomil versus Isomil supplemented with manganese and copper during the first five weeks of life

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Table 22. Individual serum copper content (ug/ml), for weeks one to five, from piglets fed Isomil versus Isomil supplemented with manganese and copper during the first five weeks of life

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VITA

Robin Hope Marcus
Candidate for the Degree of
Master of Science

Thesis: Some indicators of manganese and copper adequacy in some infant formulas for baby pigs and infants

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Sports and Cardiovascular Practice Group of the American Dietetic Assoc
General Clinical Nutrition Practice Group of the American Dietetic Assoc
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THIS FORM MUST ACCOMPANY EVERY MANUSCRIPT

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Major Professor

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Robyn Reed  
Main 130  
Extension 1188
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- Format of preliminary pages must be consistent with USU requirements
- Margins: left - 1 1/2 inches; right, top and bottom - 1 inch
- Spacing around headings: triple space around all forms of centered headings and before margin headings
- Headings: cross-check headings with Table of Contents for consistency in 1) wording, 2) capitalization, 3) level of headings listed in each chapter/section
- Table titles: cross-check with List of Tables for consistency in wording and capitalization, and with departmental style manual for format
- Figure titles: cross-check with List of Figures for consistency in wording and capitalization, and with departmental style manual for format
- References: cross-check reference citations and List of References for consistency with departmental style manual
- Cross-check reference citations with reference list for
  - Consistency in typing of name and year (spelling, completeness of citation)
  - Inclusion of all cited references in the reference list
  - Citations in text for all references listed in a Literature Cited or References Cited section
  - Inclusion of page numbers in citations for direct quotes

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