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Trace Metals in Hematopoiesis

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The trace metals represent an extremely small part of the total mass of the organism. In spite of this, these elements appear to play major roles in metabolism. At the level of the hematopoietic system, specific roles can be identified for iron, copper, zinc, and cobalt. Other trace metals appear to play less clearly defined roles in hematopoiesis but clearly are involved by virtue of a number of interactions at the level of absorption, intermediary metabolism, or modulation of toxicity. Still other trace metals, such as lithium, appear to have a therapeutic role in altering production of granulocytes, by an as yet unidentified mechanism.

Key words: trace metals, hematopoiesis, copper, zinc

INTRODUCTION

The availability of very sensitive quantitative techniques has resulted in the demonstration of most of the elements of the periodic table in man [1]. Identification of minute quantities of any given element, however, does not provide evidence of a defined biologic activity or significance, since these trace elements may be mere contaminants of other nutrients or biological materials. Since most of the elements recognized in minute quantities have been found in the formed elements of the blood [2–8], and since blood sampling provides a convenient site for study, considerable quantitative hematologic information has become available. In addition, our ability to evaluate regulatory factors in hematopoiesis by measuring the products of the reaction (ie, circulating levels of blood cells or their contents, such as hemoglobin) has led to extensive evaluation of these elements in blood cell formation.

The consideration of a role for "trace" elements in human hematopoiesis was stimulated by the early recognition of the importance of iron in the production of red cells. Al-

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though the total body iron content is small (in terms of total body weight), the role of iron as a major structural component of hemoglobin immediately separated it from the other elements present in "trace" amounts [9]. Indeed, the term "trace elements" has been considered to imply an element present in "minute" quantity but, nevertheless, important as a cofactor in physiological or biochemical reactions, and therefore functionally noteworthy in spite of the "trace" amount.

The recognized biologic importance of trace elements has been highlighted by a classification defining them as essential, possibly essential, and nonessential (present presumably as a biologic contaminant of another moiety and with no known biologic function) [1, 10]. The known "essential" elements include chromium, cobalt, copper, fluorine, iodine, iron, manganese, molybdenum, nickel, selenium, silicon, tin, vanadium, and zinc. Since it is the purpose of the current review of trace elements to focus upon their identified role in hematopoiesis, we will consider those elements whose deficiency produces an alteration in the blood elements (copper and zinc), those whose role is still somewhat speculative (cobalt and lithium), and finally, those in which activity is recognized by virtue of interactions with required nutrients (eg, iron), and thereby define some of the current information concerning cadmium and nickel, and essentially all of the divalent trace elements.

TRACE ELEMENTS ESSENTIAL FOR HEMATOPOIESIS

Although iron is quantitatively the most critical element required for erythropoiesis, it serves as more than a "trace" moiety. Furthermore, the large literature available on iron metabolism permits us to delete iron from this review.

Copper

Copper was recognized a century ago as essential for normal plant growth and development. Characterization of its role in animal metabolism and function is relatively recent. Copper is an essential structural component of a number of metalloenzymes. The first such enzyme to be discovered was cytochrome oxidase, shown by Cohen and Elvehjem in 1934 to be essential for the elaboration of heme A [11]. Because of the key role of the cytochromes in mitochondrial electron transport, this cuproenzyme influences nearly every energy-requiring pathway in the body. Lysyl oxidase, another cuproenzyme, is required for normal collagen cross-linkages, and copper deficiency states in several animal models have been associated with fatal cardiac and vascular defects [12, 13]. Copper's structural role in dopamine β-decarboxylase and tyrosinase is responsible for the abnormalities seen in catecholamine and melanin formation in copper-deficient laboratory animals [14]. Although these functions have been clearly defined in animal models, a specific role for copper in man and the definition of the deficiency state have been only recently recognized [15, 16].

The hematologic effects of copper were among the first recognized physiologic roles of this trace element. In 1928, Hart et al [17] observed that rats on a diet of cow's milk developed an anemia unresponsive to the administration of iron. The anemia was corrected with liver extract, but only when iron was concomitantly administered. Subsequent studies demonstrated that copper sulfate was the active factor in the liver extract that corrected this anemia [17]. During the next three decades, copper deficiency states were shown to be associated with anemia in a variety of animal studies. The expression of this anemia, however, showed considerable variation in different species. In all nonhuman species the anemia of copper deficiency was hypochromic, and in laboratory rats and swine the red

cells were characteristically microcytic [18–19]. By contrast, a copper deficiency state occurring naturally in Australian sheep was macrocytic and was associated with increased iron stores in the reticuloendothelial system and hepatic parenchymal cells [1]. Marston and coworkers provided evidence that the repair of the anemia in sheep could be effected by the administration of copper salts alone [20, 21]. The differences in erythrocyte morphology and in the repair requirements between rodents and sheep with copper deficiency suggested that multiple pathophysiologic mechanisms were important in the production of anemia in the presence of copper deprivation.

In spite of these studies describing the hematologic sequelae of copper deficiency in experimental animal models as well as the "spontaneous" occurrence in range sheep, copper was not believed to have a role in hematopoiesis in man. In 1930, Josephs recognized an anemia in premature infants that was unresponsive to iron but did repair with the concomitant administration of iron and copper salts [22]. The enthusiasm for this new clinical observation was modest, and it was more than three decades later that clinical studies from Peru identified a group of malnourished infants on a diet of cow's milk prepared with deionized water who developed clinical evidence of copper deficiency characterized by anemia and neutropenia [15]. The severity of the anemia was variable and was characterized by a dual population of erythrocytes consisting of both hypochromic macrocytic and hypochromic microcytic red cells [16]. It was particularly striking that when the infants were moved into a facility where the water supply was conducted by copper pipes, the anemia "spontaneously" corrected [23]. Furthermore, it was shown that supplementation of the infant diet with copper also promptly corrected the anemia and neutropenia.

The relevance of copper deficiency to alterations in hematopoiesis is now well established. Anemia has been demonstrated in animal models [18–21, 24–26], malnourished premature infants [15, 16, 23, 32], infants maintained on prolonged total parenteral nutrition in the absence of copper supplementation [27], adults with short bowel syndrome [28–30], and sickle cell anemia patients treated with pharmacologic doses of zinc [31]. The most troublesome feature of the anemia is the heterogeneity of its expression in the various species. This poses uncertainty regarding the pathophysiologic mechanisms and confuses dissection of the potential molecular mechanisms involved. Thus, in the laboratory studies in rodents [17] and the very extensive examination of swine [18, 19, 24–26] by Cartwright and coworkers, the anemia was shown to be hypochromic and microcytic. By contrast, the anemia seen in the Australian sheep is macrocytic, albeit hypochromic [1, 20, 21]. Heterogeneity is similarly expressed in the human studies. The anemia in malnourished copper-deficient Peruvian infants was hypochromic with marked anisocytosis, suggesting a dual population of small cells and macrocytes [15, 16]. Yet, recent reports of anemia resulting from copper deficiency in man have noted significant evidence of megaloblastic and/or sideroblastic features [28, 29, 32].

The heterogeneity of expression of the hematologic changes has led to a variety of explorations of the mechanisms whereby copper affects erythropoiesis or granulopoiesis in an attempt to confirm the causal basis for the changes and characterize the interaction of copper with better-understood factors in hematopoiesis. A variety of postulates regarding the role of copper in the development of anemia have been studied. For obvious reasons, these studies have been largely confined to animals and, therefore, do not clarify the recently observed morphologic findings in man. However, since exploration of these postulated mechanisms has provided insight into the role of copper in hematopoiesis, they will be briefly reviewed. The postulated mechanisms for copper's role in erythropoiesis have emphasized the accompaniments of copper deficiency-associated inhibition of gastroin-

testinal absorption of iron, and impairment of reticuloendothelial handling of iron, inadequate ceruloplasmin activity, ineffective heme synthesis, and shortened erythrocyte survival.

Inhibition of gastrointestinal absorption of iron. The very earliest studies of copper [7] posed evidence that suggested a critical interrelationship between aspects of iron metabolism and copper. These led to the extensive and elegant studies in the swine model by the Utah group [18, 19, 24–26] on aspects of iron absorption in copper-deficient swine. They demonstrated that when copper deficiency was induced in swine then maintained on a "normal" content of iron in the diet, the animals developed reductions in total body iron similar to that seen in severe iron deficiency [18]. In addition, when gastrointestinal absorption was studied in normal swine with radiolabeled iron, 6% of the administered dose was absorbed, whereas similar studies in copper-deficient swine showed the absorption to be less than 2% [18, 25]. Simple copper replacement to the swine with longstanding copper deficiency was ineffective in the repair of the anemia, whereas concomitant administration of iron and copper, or even the administration of iron after copper supplementation, did result in a prompt return of plasma iron to normal and subsequent correction of the anemia [18, 25, 26].

Cartwright and his coworkers also investigated the effects of copper deficiency in newborn swine who were on an iron supplementation program by either the oral or intramuscular route [25]. Regardless of the route of iron supplementation, the copper-deficient young swine developed a severe microcytic hypochromic anemia. A three-phased pattern of changes in the circulating iron pools was defined based upon measurements of plasma iron concentrations. In the copper-deficient animals on oral iron supplementation, an initial phase of about 6-weeks duration occurred, during which the plasma iron levels were normal. In an intermediate phase between 6 and 12 weeks, the plasma iron concentration fell precipitously. Finally, late in the course, the plasma iron rose slightly higher, still well below control levels, and persisted at that level. These three phases also were seen in those animals receiving parenteral iron, although during the terminal phase the plasma iron reached nearly normal values. The total iron binding capacity in the group supplemented with oral iron was increased throughout the study, whereas the swine maintained on intramuscular iron had normal total iron binding capacity measurements throughout. Studies to evaluate the compartmentalization of iron revealed that the oral iron group was profoundly iron deficient. As expected, the total body iron content in the intramuscular iron group was normal; however, there was a marked reduction in the erythrocyte iron to less than half that of the control group.

These studies suggested that an important component of the anemia in copper-deficient swine was impaired gastrointestinal absorption of iron. Studies of the proximal duodenum of the copper-deficient swine fed supplemental iron demonstrated large iron granules within the macrophages of the lamina propria, as well as increased amounts of fine granular iron deposits within the columnar epithelial cells. This was in contrast to normal swine who had only trace amounts of iron in these sites. The copper-deficient animals who were given intramuscular iron did not develop these duodenal deposits [25, 26]. Parallel study of the proximal duodenum after oral administration of radiolabeled iron to the copper-deficient animals showed excessive retention of the radioiron in the mucosa, which was shown to persist at that site for long periods of time. Analysis of subcellular fractions from the duodenal mucosa revealed most of the radiolabeled iron was in the ferritin fraction. These studies led to the hypothesis that an abnormality develops in copper-deficient swine which affects the transfer of iron from the mucosal cells [25]. This transport defect was

particularly striking and dramatically depicted by the finding of excess iron within the mucosal cells when all other parameters of (total) body iron deficiency were present.

Impaired reticuloendothelial iron metabolism. As mentioned above, the induction of copper deficiency in swine results in a reduction of erythrocyte iron even when the total body iron content is normal [25, 26]. In addition, these studies also showed dense aggregations of iron in the macrophages of the gastrointestinal mucosa. A viable consideration of the relationship of copper to iron physiology is that copper depletion has an effect on the handling of iron by the reticuloendothelial cells.

Studies of iron kinetics in the copper-deficient swine model by the Utah group have defined some of the characteristics of this handling. They have shown that the amount of iron entering newly produced erythrocytes (as determined by red cell iron incorporation rates) is only slightly increased from that seen in non-copper-deficient swine [33]. When copper-deficient animals were given parenteral iron adequate to achieve normal total body iron stores, hypoferremia was observed [25, 26]. The implication that impaired release of storage iron occurs in copper deficiency in swine was broadened by similar observations in a murine model [34]. An important confirmation that altered reticuloendothelial handling of iron occurs in copper deficiency was achieved by administration of damaged erythrocytes both to control and to deficient swine. In the normal animals there was a prompt rise in the plasma iron. By contrast, this was not seen in the copper-deficient swine [25].

Further evidence of tenacious retention of iron by the macrophage was shown when the liver and spleen of copper-deficient animals were examined. It was found that both copper deficiency and iron deficiency produced nearly identical (negligible) total body iron stores [25]. Copper-deficient animals receiving oral iron supplementation, however, had nearly three times the concentration of iron in their livers as did the iron-deficient animals. Similarly, splenic iron concentrations also were elevated in copper-deficient swine, and the degree of iron excess was greater in the animals which had received their iron supplementation by an intramuscular route. In addition, these latter animals frequently had excessive iron deposition in hepatic parenchymal cells, as well.

Thus, these animal studies provide evidence that, in some manner, the reticuloendothelial cells have an unusual retention of iron, resulting in a pathophysiologic expression of altered iron metabolism similar to that seen in the "iron-reutilization" defect that characterizes the "anemia of chronic disease" both in animals and in man.

Inadequate ceruloplasmin activity in iron metabolism. Another mechanism that has been explored to explain the relationship of copper to iron metabolism is the role of the copper transport protein ceruloplasmin. It is well known that the plasma enzyme ferroxidase catalyzes the oxidation of ferrous iron to ferric iron. Osaki et al have shown that ceruloplasmin is the most effective ferroxidase in human serum [35]. Relevant to that finding is the evidence that as copper deficiency develops in swine, the known reduction in plasma iron is actually preceded by a marked fall in ceruloplasmin concentration [29, 30]. Thus, even before hypoferremia was observed, the average level of ceruloplasmin activity was only 0.5% of normal [36]. The most sensitive measure of ceruloplasmin activity was found to be the measure of paraphenylene diamine (ppd) oxidase activity. When inorganic copper was administered to copper-deficient swine, the plasma ppd oxidase activity began to increase within 15 min and continued to rise over the next 3 hr. In addition, the reduced plasma iron concentration values, characteristic of established copper deficiency in swine, did not increase until the oxidase activity exceeded 1%. After that, the plasma iron values rose in a linear pattern. Both human and porcine ceruloplasmin were administered to copper-deficient swine. The rate of rise in plasma iron was found to be proportionate to the

amount of ceruloplasmin administered, regardless of species source. Rat ceruloplasmin is known to be a poor source of ferroxidase activity, and its administration failed to produce a change in plasma iron values [37]. It is particularly noteworthy that swine made hypoceruloplasmic by the administration of ceruloplasmin-poor plasma had a similar decrease in plasma iron levels, which was also reversible by the administration of ceruloplasmin. By contrast, copper sulfate administration to those animals with ceruloplasmin depletion was ineffective in reversing the hypoferremia [38]. In addition, when asialoceruloplasmin, which is quickly cleared from the circulation by the liver, was administered to copper-deficient swine, no increase in plasma iron concentration was noted [36].

The implication of these studies is that ceruloplasmin is required for the release of iron from reticuloendothelial cells to the plasma and that, at least in part, this interaction requires the oxidation of ferrous iron, presumably on the reticuloendothelial cell membrane [37]. A serious issue with this conceptual formulation is the fact that patients with Wilson disease who have exceedingly low levels of ceruloplasmin do not have an evident defect in iron transport or exchange. Classical iron deficiency has been described in Wilson disease with no unusual features [39]. A proffered explanation for this discrepancy is that there are adequate ceruloplasmin levels in patients with Wilson disease to prevent the defect in iron handling at the reticuloendothelial level [36].

Ineffective erythrocyte handling of iron. Cartwright and coworkers [24] demonstrated that the synthesis of heme and porphyrin were normal in red blood cell precursors obtained from copper-deficient swine. When copper-deficient pigs were supplemented with intramuscular iron to raise the plasma iron concentration to normal, the circulating hemoglobin and hematocrit values were just as low as in those animals with uncorrected combined copper and iron deficiency. When 20 copper-deficient pigs were given oral iron supplementation for 3–4 months, the animals could be segregated into two groups on the basis of the serum iron concentration; in 13 (of the 20) the plasma iron was low and the reticulocyte count was elevated. In the second group (of 7), the plasma iron was normal, as was the reticulocyte count. As evidence of altered iron metabolism and erythropoiesis, a marked increase in marrow sideroblasts was seen in the copper-deficient animals supplemented by intramuscular iron. Since the sideroblasts seen had numerous large iron granules in comparison to the control animals or the copper-deficient animals supplemented by oral iron, it was believed that impaired intracellular handling of iron was an acquired effect of copper deficiency.

Goodman and Dallman investigated the localization of iron in the erythroblasts of copper-deficient Wistar rats [40]. The erythroblasts had peripheral vesicles containing electron-dense particles; the particles were shown to be ferritin. When the copper-deficient rats were supplemented with intramuscular iron, the erythroblasts and reticulocytes were shown to contain increased amounts of iron, which was segregated within these vesicles. The mitochondria were not laden with iron. Furthermore, the administration of lead, which is known to produce intramitochondrial iron deposition in normal animals, resulted in a mild anemia and sparse iron accumulations limited to the vesicles. Thus, lead administration and iron loading in copper-deficient animals produced only a modest anemia, and the expected mitochondrial iron accumulation was not seen. These studies suggest that copper deficiency interferes with the intracellular accumulation of iron. Several hypotheses have been proposed to explain how copper may mediate the appropriate handling of iron. These include the possible existence of an intracellular copper-containing enzyme, analogous to ceruloplasmin ferroxidase, which is required for the transfer of ferric iron into the mitochondria. However attractive such a mechanism, evidence for such an enzyme is lacking. An alternative explana-

tion is depressed activity of the copper-containing enzyme cytochrome oxidase C, since copper-deficient animals have reduced levels of cytochrome oxidase in many tissues [41, 42]. It has been postulated that the energy requirement for the entry of iron into the mitochondria of copper-deficient animals exceeds that available from oxidative phosphorylation; hence, in the setting of copper deficiency there would be a block in the intracellular handling of iron. The block is proposed at the interface of the cytosol and the outer mitochondrial membrane [26].

Decreased red cell survival in copper deficiency. A fifth mechanism of hematopoietic injury has been proposed by Bush et al [33]. Their ferrokinetic studies showed that the plasma clearance of radiolabeled iron expressed as a T 1/2 was significantly reduced in the copper-deficient swine (0.3 hr as compared to 1.2 hr in normals), and the plasma iron turnover rate was slightly increased from 1.1 to 1.8. The expressed erythrocyte life span was markedly reduced from 63 days in normal animals to 13 days in copper-deficient swine. When red cells obtained from copper-deficient erythrocytes were labeled and given either to deficient or to normal swine, their survival also was reduced. When normal sheep erythrocytes were given to copper-deficient animals, there was no abnormality in red cell survival. These studies stand alone, and the mechanism whereby this defect occurs has not been further investigated.

Copper deficiency states in man. In the past few years, copper deficiency states in man have begun to be recognized. Copper is ubiquitous and, unlike iron, it is easily absorbed from the gastrointestinal tract. It has been estimated that as little as 2 mg of copper per day will maintain copper balance in the adult [43]. An average American diet contains anywhere from 2–5 mg of copper per day. Newborns have a daily requirement of 80 μg/kg of body weight [44, 45]. Copper deficiency in infancy was first described in premature infants raised solely on cow's milk diets [22]. Such children have severe hypochromic anemias associated with hypoferremia and hypocupremia. Morphologically, the erythroid elements have been reported to range from microcytic to macrocytic [15, 16]. The supplementation of the diet with iron alone is insufficient to correct the anemia. Therapy both with iron and with copper is required for complete recovery.

The recent studies of Al-Rashid [46], Seely [47], and Ashkenazi [32] further characterized the syndrome of neonatal copper deficiency. This syndrome was first noted in premature infants who had been raised on skimmed milk diets. The syndrome which developed was characterized by a sideroblastic anemia with marked vacuolization of erythroid and myeloid precursors, neutropenia, hypopigmentation of the hair, osteoporosis with cupping of the metaphyses, disruption of the elastin in arterial walls, mental retardation, and impaired visual acuity. Similar abnormalities, particularly with a microcytic hypochromic anemia and neutropenia, have now been reported in infants with prolonged malnutrition [15, 16, 46, 47], infants with gastrointestinal malfunction maintained on copper-poor hyperalimentation [27], adolescents or adults with "short bowel syndrome" [28–30], and sickle cell anemia patients receiving zinc [31]. In most cases, copper supplementation resulted in a marked reticulocytosis and prompt correction of the anemia and neutropenia.

Mechanisms for the abnormalities observed in human copper deficiency are the same as those noted for copper-deficient swine. No abnormalities of B_{12} or folate have been noted in copper deficiency in man. Zidar observed inappropriate levels of serum and urinary erythropoietin in a patient with hypocupremia secondary to short bowel syndrome [29]. Copper supplementation resulted in a rise in erythropoietin activity which paralleled the rise in the reticulocyte count. Again, the possible responsible mechanisms have been inability to absorb iron, inappropriate handling of iron by the reticuloendothelial system, and inef-

fective intracellular handling of iron [18, 19, 24–26]. Demonstration of these defects in man has been difficult. Parenthetically, evidence of actual shortened erythrocyte survival has not yet been documented in copper deficiency in man.

The mechanism for the neutropenia in human copper deficiency also is obscure. Zidar's patient on two occasions demonstrated normal in vitro granulocyte maturation when mixed with normal serum. When mixed with the patient's own (copper-deficient) serum, there was impaired colony formation. The patient's copper-deficient serum did not impair colony formation from normal marrow stem cells [23]. Of interest is a reported correlation between serum copper levels and the percentage of blast cells in patients with acute leukemia [48].

Another situation in which copper deficiency occurs in man has only recently been recognized; it is in sickle cell anemia patients who are being supplemented with other trace metals. This has been best characterized in patients receiving zinc. Zinc deficiency has been shown to occur in sickle cell anemia [49, 50], and zinc repletion has been used to speed the healing of leg ulcers, a common management problem in sickle cell anemia. Zinc has also been investigated as an antisickling agent [51–55]. Although zinc administration has been shown to reduce the number of irreversibly sickled cells in the circulation [52], a clear demonstration of the clinical effectiveness of such therapy is difficult to prove. A case in point was described by Prasad [31]; on zinc supplements of 150 μg/day the patient developed neutropenia and microcytic anemia over a period of several months. Bone marrow evaluation revealed erythroid hyperplasia. Serum copper and plasma ceruloplasmin were both decreased. The addition of 1 mg of copper per day produced a return to normal ceruloplasmin levels, accompanied by a reticulocytosis, normal red cell indexes, and normal neutrophil count. Although the mechanism is not clear, one postulate is that zinc depresses copper absorption. It is of interest that similar interactions have been proposed for the combinations of copper–molybdenum [56] and copper–cadmium [57].

It is of some note that copper excess also has been associated with abnormalities of the hematopoietic system. In 1956, Roberts reported the development of a severe hemolytic anemia associated with the ingestion of copper sulfate [58]. Similar acute copper-induced episodes of hemolysis have been reported during hemodialysis utilizing water containing excessive copper salts [59], following accidental ingestion of copper [60], suicide attempts with a variety of copper salts [61], and with the application of copper sulfate to extensive burns [62]. Indeed, hemolytic anemia is a well-recognized complication of Wilson disease [63–65], generally developing early in the disease before the institution of specific therapy. It has also been reported to develop after the cessation of penicillamine therapy and has been associated with periodic increases in the circulating nonceruloplasmin-bound copper. Boulard and associates [66] have demonstrated that free copper is a potent inhibitor of a number of glycolytic enzymes, including glucose-6-phosphate dehydrogenase, red cell hexokinase, phosphofructokinase, phosphoglycine kinase, pyruvate kinase, and 6-phosphoglucuronate dehydrogenase. Cupric ion is a potent oxidizer and, as such, can affect the activity of multiple red cell enzymes and oxidize (oxy- and deoxy)-hemoglobin [67]. Other effects of copper include disulfide linkages between β-chain sulfhydryl groups and a fall in intracellular glutathione which can result in autohemolysis. Salhany et al [68] demonstrated a direct oxidation of the erythrocyte membrane sulfhydryl groups by copper. Which of these multiple factors is most responsible for the hemolytic anemia seen in Wilson disease or the acute episodes of hemolysis seen with the ingestion of or exposure to copper is not clear.

Zinc

Although zinc has been recognized as an essential trace element in mammalian systems since 1934, clear recognition of a specific deficiency state in man was not documented until Prasad recorded his studies in malnourished Egyptians in 1963 [69]. He characterized a specific syndrome of zinc deficiency consisting of dwarfism, hypogonadism, visceromegaly, and a hematologic picture that simulated iron-deficiency anemia [70, 71]. Subsequent study has shown that zinc is essential in a number of enzymatic systems, including alkaline phosphatases, carboxypeptidases, alcohol dehydrogenase, and nucleotide polymerases [72–75].

The precise role of zinc in hematopoiesis has been difficult to define. The anemia noted in underdeveloped areas among defined zinc-deficient individuals has been described as microcytic and hypochromic. It is associated with the classical findings of iron deficiency and, in most cases, has been completely reversible with the administration of iron alone [69–76]. A proposed mechanism for the concurrent development of deficiencies of both iron and zinc is the nonspecific binding of these elements by the high fiber residues of certain indigenous whole grain breads. The identification of an anemia due to zinc deficiency and responsive only to zinc has not been defined. Zinc, however, is clearly involved in several aspects of normal hematopoiesis by virtue of its role in many enzyme systems which are involved with DNA synthesis (including thymidine kinase, and the RNA and DNA polymerases) [72–75]. The implication of this relationship is that zinc deficiency severe enough to produce hematologic abnormalities may not be compatible with life.

A major role for zinc relates to its interaction with different organic molecules. Zinc, like copper, cadmium and other bivalent trace metals, tends to form chelates and bind to various ligands, particularly of the sulfhydryl group variety.

At a basic biochemical level, there are many possible interactions which appear to involve multiple bivalent elements. Thus, at normal dietary intakes of zinc, iron absorption in mice is enhanced over that seen in the absence of zinc in the diet [57]. When zinc is given in the face of copper deprivation, there is impaired handling of storage iron and the poor utilization of hepatic storage iron. Interestingly, toxic levels of zinc produce just the opposite effect in mice and rats as does zinc deficiency; in this setting there is increased utilization of storage iron. Zinc is reported to activate ALA-dehydratase and to reverse the inhibition of this enzyme by lead [77]. Other bivalent elements which bind even more strongly to sulfhydryl groups (such as cadmium) can block the effects of zinc [57–77]. The precise role of zinc in the hematologic abnormalities in man is not certain [65]. Nevertheless, it is clear that zinc interacts with the sulfhydryl units of the red blood cell membrane. This interaction has been used to define a presumed mechanism whereby zinc was implicated in the hemolytic anemia seen in patients dialyzed with fluids containing excessive zinc [78]. There has been no substantiation that the zinc induced the hemolysis in these patients [78]. Indeed, it is quite likely that other contaminant trace elements (eg, copper) were present and are even more likely causes of the hemolysis. To date, clear evidence that zinc is injurious to the red cell membrane in man has not been provided.

That zinc may have an important clinical and therapeutic role in hematopoietic lesions was proposed by Prasad and coworkers [52, 76, 79]. They recognized that a significant number of men with sickle cell anemia had impaired gonadal function, dwarfism, and abnormalities in hair growth [49], and that these changes were very similar to those reported in Egyptians and Iranians with zinc deficiency [69–71]. Studies using zinc determinations of hair in patients with sickle cell anemia documented zinc deficiency in about half the patients; plasma concentrations were less useful, perhaps because zinc is

released from hemolyzed red blood cells [49, 80]. The clinical relevance of these observations is supported by the evidence that zinc supplementation in these patients appeared to correct the hypogonadism and the hair growth abnormalities [76]. The mechanism of the zinc deficiency in sickle cell anemia appears to be the excessive urinary excretion from the chronic hemolytic anemia [49, 80]. Thus, one clear role for zinc in patients with sickle cell anemia is that of repletion of the zinc deficiency, which can result in retarded growth, hypogonadism, and abnormalities of hair growth.

A second and quite separate role for zinc relates to its use as an antisickling agent. Zinc in this setting is a therapeutic agent rather than a moiety to replete the deficient state. Brewer, in collaboration with Prasad, has extensively explored the antisickling properties of zinc [51–55] and has clearly demonstrated that its in vivo use decreases the number of circulating irreversibly sickled cells [52], and preliminary studies suggest that it may reduce the crisis frequency [52].

Several mechanisms have been postulated whereby zinc might alleviate the symptoms of sickle cell anemia. Although zinc does result in an increased oxygen affinity [53, 54], the significant role of zinc appears to be a direct one on the sickled erythrocyte's cell membrane [51, 52, 55]. Zinc improves erythrocyte deformability in a manner that appears to be antagonistic to the effects produced by calcium ion. Calcium accumulates in sickled erythrocytes and appears involved in the irreversible sickling process. Zinc antagonizes the retention of calcium and to some extent decreases the binding of hemoglobin to ghost membranes [81]. The net in vitro effect is to increase the filterability of sickle cell erythrocytes, which indicates a reduction in the amount of irreversible sickling. Some sickle cell patients have been treated with zinc (660 mg of $ZnSO_4$ orally) and had fewer episodes of pain crises [52]. It is anticipated that current controlled trials of zinc in sickle cell anemia will define the role of zinc as a therapeutic agent in this setting.

Zinc has recently been implicated in the modulation of lymphocyte function, based on immunologic studies in the zinc deficiency state [55, 82]. Humans and animals with zinc deficiency have been shown to have impaired B- and T-lymphocyte function [83–86]. It has been well demonstrated that zinc is required for lymphocyte transformation [85]. The other immunologic actions of zinc appear to involve the T lymphocytes more than B lymphocytes. Zinc deficiency in animals has been associated with thymic involution, impaired T-cell helper and killer function, and reduced mitogenic responses. In spite of these extensive changes, a defined role for zinc in human disease is not clear. One area of special interest is the possible role zinc deficiency may play in altered immune status in patients with cancer. Low serum zinc levels have been reported in a number of malignancies (including lung and gastrointestinal tumors) [87–89]. Cancer patients have also been reported to have increased urinary excretion of zinc compared to normals [90]. The relationship of zinc to antitumor therapy or the status of stability of a given tumor is not clear but is a topic of active investigation.

Cobalt

Despite cobalt's obvious structural role in the cobalamin (B_{12}) molecule, specific evidence of altered vitamin B_{12} metabolism in the presence of cobalt deficiency in man is difficult to document. Animals dying of cobalt deficiency clearly develop an anemia characterized by erythroid hyperplasia [20]. Lambs and calves with severe cobalt deficiency demonstrate a hypochromic and microcytic anemia. There are some anecdotal cases of anemia in the circumstances of cobalt deficiency in which full marrow recovery was produced by B_{12} therapy [20]. The addition of supplemental cobalt has been reported to en-

hance the rate of response to iron in the iron deficiency of pregnancy; it is not clear that supplementation with iron alone is adequate to achieve recovery of the erythron [91].

Cobalt in large amounts is capable of inducing a true erythrocytosis characterized by erythroid hyperplasia, reticulocytosis, and an increase in the red cell mass [92, 93]. As expected, the red cells from patients treated with cobalt contain normal amounts of hemoglobin and appear to have normal survival. The cobalt effect has been postulated to be due to an anoxic effect on the kidneys with a compensatory increase in the production of erythropoietin [92, 93]. The effect of cobalt lasts only as long as it is given, and there is no evidence that these patients are at any increased risk of developing hematologic malignancies. The amount of cobalt required to produce polycythemia is in the range of 20–30 mg/day. At this dose, frequent systemic toxicity is common, manifest by thyroiditis, severe gastroenteritis, and myocardiopathy [65]. Because of the toxic effects and the failure to demonstrate any marked benefit in tissue oxygenation in patients treated with cobalt, the therapeutic use of cobalt as a hematinic has been essentially abandoned.

Cadmium

Cadmium is a bivalent metallic ion for which no essential function has been identified. The accumulation of cadmium from the "wastes of industry" has exposed a number of humans to excessive quantities of cadmium and has provided information concerning the sequelae of excessive exposure [65]. In man about 1–4% of the daily intake of 30–60 μg of cadmium is absorbed, and once absorbed it has a biologic half-life of over 15 years. Toxicity following the intravenous administration of cadmium has been seen in the form of an acute hemolytic anemia [94]. Low-dose industrial exposure has been associated with chronic anemia in exposed workers. The anemia has been directly correlated with the blood cadmium level. Unfortunately, no specific bone marrow findings were reported in the 19 patients studied by Friberg [94]. In the animal model the anemia of chronic cadmium ingestion appears to be expressed as iron deficiency anemia [95, 96]. Laboratory animals given sublethal doses of cadmium develop a microcytic hypochromic anemia with a low serum iron and an elevated total iron binding capacity. Studies by Hamilton suggest that cadmium administration results in competitive inhibition of iron absorption at the level of the duodenum [95–97]. It is of interest that cadmium also affects the gastrointestinal absorption of cobalt, zinc, and copper [57]; the mechanism is unclear. There is no evidence that cadmium has a direct effect on hemoglobin synthesis [94].

Molybdenum

Xanthine oxidase is the only known molybdenum-containing metalloenzyme in mammalian systems. Molybdenum, however, has been noted to have a role in maintaining normal copper absorption and has been postulated by some to play a role in iron metabolism [56]. Much of the evidence for the latter thesis is inferential and relates to studies of the repair of iron deficiency in pregnancy using a combination of iron and molybdenum [98]. The anemia of pregnancy has been described as "slow to repair" with iron therapy alone. Dieckmann demonstrated that when molybdenum is added to the iron, a more rapid rate of repair ensues than when iron alone is used [99, 100]. Moreover, there is no good evidence to suggest that the iron deficiency of pregnancy is refractory to therapy with adequate replacement of iron alone in the setting of a normal upper gastrointestinal tract [9].

The role of molybdenum in copper absorption has been demonstrated in a number of animal studies. Molybdenum counteracts the chronic copper poisoning seen in sheep grazing on foliage excessively rich in copper [20, 21]. The reverse situation of copper's

blocking molybdenum toxicity has also been described [20, 21]. At present, the actual role of molybdenum in human hematopoiesis appears to be limited and may be largely confined to its role in the normal absorption of iron and copper in the duodenum. The occasional report of anemia developing in the setting of molybdenum excess suggests that competition for binding sites may be an important regulatory mechanism in the gastrointestinal tract.

Lithium

Lithium has been used in the treatment of manic–depressive mental illness for over a quarter of a century. About 15 years ago, it became evident that leukocytosis usually developed in patients receiving therapeutic doses of lithium carbonate as well as other lithium salts [101, 102]. The neutrophilia which develops in lithium therapy is independent of the underlying psychopathology and is not clearly dose related. This effect of lithium occurs as early as 1–2 weeks following institution of therapy and promptly disappears with cessation of lithium.

The observations of lithium-induced leukocytosis have evoked considerable interest in the role of lithium in granulopoiesis and its potential toxicity. Early studies suggested that the peripheral leukocytosis was the result of increased cortisol secretion; this has now been clearly disproven. Studies of the biologic effects of lithium have now shown that there is a resultant expansion of the granulocyte mass. Thus, studies of an expanded granulocyte mass with increased granulocyte turnover are well reflected in the increased vitamin B_{12} binding proteins, due to the transcobalamin binders derived from granulocytes and increased urinary and serum muramidase levels [103]. Growth and colony culture studies have shown that lithium induces a true stimulation of colony-forming activity [104, 105]. This in vitro observation is considered to be the in vivo pathophysiologic mechanism for the granulocytic hyperplasia and the leukocytosis. Lithium is known to inhibit the adenyl cyclase/cAMP axis. Recent studies [105] have indicated that lithium stimulation of granulopoiesis may occur via this mechanism. In addition, lithium inhibits erythroid colony growth in vitro and blocks the stimulatory effect of prostaglandin derivatives on erythropoiesis [105].

These observations have led to interest in the use of lithium to stimulate the marrow. Functional studies of the granulocytes have been performed in several laboratories and were recently reviewed by Friedenberg and Marx [106]. Although inconsistent effects on granulocyte function have been reported, the general view based on these in vitro studies and the extensive clinical experience with lithium in depressive states is that granulocyte function is normal. Rothstein et al [107] showed that lithium treatment of human subjects produced enlargement of the total circulating neutrophil mass and increased neutrophil production without impairing neutrophil migration into skin lesions.

The chronically stimulated state of granulopoiesis that results from lithium has raised the question of its leukemogenic potential. Studies from this laboratory in a chronically stimulated population have failed to show any evidence of increased leukemic transformation [108]. In addition, cytogenetic studies have failed to reveal any abnormalities during lithium therapy [109].

Several unanswered questions exist concerning the role of lithium in hematopoiesis. For instance, are other cell lines involved or is this only of the committed granulocyte compartment? As noted, in vitro culture data suggest that lithium inhibits erythropoiesis [105]. A mild thrombocytosis actually has been reported in some of the patients treated with lithium. Is the molecular basis for lithium's effect on granulopoiesis via inhibition of

the adenyl cyclase/cAMP axis or by another mechanism [105]? Does lithium administration offer a method for abetting granulocyte development in circumstances of marrow (or stem cell) injury, for instance following cytoreductive chemotherapy or in clinical circumstances such as Felty syndrome? Initial studies in patients with small-cell lung cancer receiving combination chemotherapy and radiation therapy indicate that lithium treatment reduces the number of severe febrile episodes, the days hospitalized with fever and neutropenia, and the number of infection-related deaths following cytotoxic drugs and radiation [110]. Nadir leukocyte and neutrophil counts were significantly higher in those cancer patients receiving lithium than in the untreated (with lithium) controls. Lithium treatment of patients with Felty syndrome has been less impressive but has shown promise [111]. Is lithium an important physiologic modulator of granulopoiesis or are the observations simply those of toxicity? Finally, does long-term lithium administration have adverse effects on hematopoiesis? The possible inhibitory effect of lithium on erythropoiesis has been noted. In addition, Levitt and Quesenberry [112] have shown that prolonged exposure to lithium (3–12 weeks) results in a dose-dependent progressive depletion of stem cells and their progeny in a murine liquid culture system. These investigators have suggested that prolonged proliferative stress induced by lithium in circumstances in which the stem cell reserve is limited may result in rapid depletion of hematopoietic cells.

TRACE METAL BIOLOGIC INTERACTIONS

Our current state of knowledge has identified only a few trace metals for which specific deficiency states provide an identifiable hematopoietic sequela or which clearly exert a defined effect on the hematopoietic system when administered in pharmacologic quantities (such as cobalt and lithium). There are a number of elements for which a specific role in the hematopoietic system is recognized by their interactions with other trace metals, thereby affecting hematopoiesis. These interactions have been difficult to recognize, characterize, and substantiate in man, the clearest window having been identified in circumstances in which trace element interactions involve either iron or copper, since these two moieties have definable roles in hematopoiesis. In spite of these limitations, the most fruitful current research in trace metal biology focuses on these important interrelationships and poses exciting new clues to rate-limiting factors in many biochemical and pathophysiologic events. These interactions have defined interesting information relative to absorptive competition, interactions in intermediary metabolism, and modulation of toxicity [113].

To a large extent, the interactions of the various trace metals are a function of the physical and chemical properties of the given species under consideration. Hill and Matrone [114] provided evidence that the primary determinant of the properties of an element in biologic reactions is the electronic structure. Electrons are distributed in a specific spatial arrangement or angular orbital distribution about the nucleus, and the angular distribution of the electronic orbitals is independent of the element studied. The orbitals of an element exist in an orderly arrangement with a maximum of one pair of electrons in an s orbital, three pairs in a p orbital, and five pairs in a d orbital. In general, the number of empty orbitals predicts the number of ligands which can be formed. Thus, the number of unpaired electrons equals the number of unpaired coordination bonds available for binding. It is now clear that for a number of divalent trace metals there are analogues with identical outer orbital electron structures, and these will behave similarly regardless of their valence. Thus, Zn^{+2} and Cd^{+2} have identical outer (10 d) shell orbital electrons, and these predictably function similarly. Cuprous (Cu^{+1}) also has the same electronic structure, in

spite of a difference in valence. The predictability of the electron interrelationship would indicate that Zn^{+2} and Cd^{+2} should be antagonistic to copper (Cu^{+1}). The validity of that competitive thesis has been documented [114]. Other expected interactions based on electronic structure would be ferric iron (Fe^{+3}) and divalent manganese (Mn^{+2}), each with five d shell electrons and a total of five outer shell electrons. Ferrous (Fe^{+2}) iron and trivalent (Co^{+3}) cobalt have six d shell electrons and six unpaired electrons in their outer shell.

The important implication of the electron concept is that ions with similar orbital structures will be antagonistic (or competitive) in their binding to ligands. This important concept has been corroborated in a variety of physiologic interactions. Thus, Hartman observed that sheep on iron-poor diets developed iron deficiency anemia more quickly when fed manganese-rich diets [115]. Matrone demonstrated similar findings in piglets and was unable to repair the anemia by a change in only the iron content of the diet [116]. The underlying mechanism for these findings appears to be competitive binding interactions in the gastrointestinal tract. This site is suggested by the observation that increasing iron supplementation was able to offset any given degree of manganese supplementation, up to the point of saturation. Other evidence of a "controller" function for the gastrointestinal mucosal cell in trace element interaction comes from Pollack's demonstration that iron deficiency was accompanied by enhanced absorption not only of iron, but of manganese and cobalt as well [117]. Subsequently, Thomas et al [118] demonstrated clear-cut competition for small bowel absorptive sites for manganese, iron, and cobalt; and Toskes et al [119] showed that mice with an inherited defect in iron absorption also had impaired cobalt absorption. Thus, these studies demonstrate that iron, cobalt, and manganese share some common absorptive sites or mechanisms in the mucosa of the small bowel. More recently, Watson et al [120] have demonstrated that iron-deficient subjects absorb 2–3 times more lead than normal when this lead is given as a carrier-free tracer dose. The number of trace elements for which such competitive mechanisms for absorption exist has now been shown to include nickel, zinc, cobalt, manganese, cadmium, and iron [114, 115, 121].

Whereas the site of these absorptive interactions clearly resides in the duodenum, the exact mechanisms which control absorption of the various trace metals are still not completely defined. For instance, the absorption of iron is a two-step process involving uptake from the gut lumen to the mucosal cell and then transport from the mucosal cell to the serosal membrane. In the rat, available radiolabeled iron quickly appears in ferritin when subcellular fractions of the mucosa are studied. This ferritin-bound fraction amounts to about 90% of the absorbed iron under basal conditions [122]. In the face of increased iron demand the amount of nonferritin-bound iron in the cytosol increases. Sheehan has demonstrated that the increase in cobalt absorption in the face of iron deficiency or brisk hemolysis is not associated with the binding of cobalt to any identifiable soluble cytoplasmic fraction of the mucosa [123]. The transfer of absorbed iron and cobalt from the mucosal cell to the body appears to be increased in circumstances of enhanced iron requirements [91, 124]. The mucosal cell mechanism is clearly "rate limiting;" whether a soluble carrier molecule analogous to ferritin is a factor is not certain. One possible implication of this observation is that part of the mechanism for the control of iron and cobalt absorption is different at the intracellular level and that this difference is recognizable by the absence of a soluble cytoplasmic carrier for cobalt. The enhanced cobalt absorption that occurs in patients with iron deficiency has been proposed as a diagnostic test for the detection of iron deficiency anemia [127].

Studies of copper absorption provide evidence of a different mechanism in that iron deficiency by itself is not associated with enhanced copper absorption. Despite this fact, there is good evidence that copper has a role in the absorption of cadmium and zinc. These

relationships of copper in the absorption of zinc and cadmium have only recently begun to be defined, and the studies thus far conducted have been done in the circumstance of the model of copper deficiency. Thus, Bunn and Matrone observed that copper's influence on cadmium absorption may also secondarily alter iron absorption [57]. Cadmium when given at the level of 100 ppm to copper-deficient rats produced the classical pattern of iron deficiency-type anemia, in spite of continued adequate dietary iron intake. These same studies were performed in non-copper-deficient animals in which similar cadmium supplementation did partially ameliorate the mild anemia but did not completely abolish it. Incompletely characterized is the evidence that modulation of the anemia could be produced by supplementation with copper, zinc, or both of these. Regardless of these changes, the complete correction of the anemia required removal of all dietary cadmium.

Cadmium absorption is enhanced in the face of iron deficiency [95–97]. The mechanisms controlling the absorption of cadmium and other trace metals from the environment have been of great interest and recent study. For many of the trace metals (certainly for iron and zinc, and probably for cadmium and copper to a lesser degree), the maximum urinary excretion remains fixed regardless of the amount of oral intake. Iron balance is maintained to a large degree by mechanisms present within the duodenum [121]. Whereas excessive amounts of iron may be absorbed from the lumen, the actual transport of more iron than is required from the mucosal cell to the serosal membrane is limited. This limitation of iron transport into the body (carcass) has been the subject of extensive study and has been ennobled by the title "mucosal block," and reflects the existence of a "controller" mechanism for iron transport. Excess mucosal iron is eliminated via the desquamation of superficial mucosal cells rather than via any true secretion of iron into the lumen of the bowel. This is not the case, however, for several trace elements. Thus, biliary excretion of copper [14] and the intestinal secretion of zinc [76] appear to play significant roles in the physiologic handling of these two moieties. That this is not specific for trace metals is emphasized by the studies of the handling of cadmium, which is quite similar to that of iron [96, 97]. Mice with normal dietary intakes of iron absorb about 16% of ingested cadmium within about 4 hr. Of this, only about 1/8 or 2% of the total ingested dose reaches the circulation. The rest of the absorbed cadmium is lost into the small bowel lumen at a rate equal to that of mucosal desquamation. The absorbed cadmium is bound to low molecular weight species within the cells of the intestinal mucosa. Thus, the handling of excess cadmium is analogous to that of excess iron. Excess cadmium is bound to metallothionine-like compounds. Totally unexplained is the evidence that the duodenum is able to increase its ability to sequester excess cadmium by over twofold in the setting of iron deficiency [97]. Furthermore, when radiolabeled cadmium is administered *parenterally* to iron-deficient animals, there is increased accumulation of the isotope in the duodenum, demonstrating that cadmium kinetics are independent of the route of administration [96].

Studies which purport to show enhanced absorption of iron in the setting of increased trace metals are very difficult to assess. The matter of iron–molybdenum interactions in the anemia of pregnancy has been summarized [56, 98–100]. The data are not overly convincing, and it seems unlikely that the clinical picture of an iron deficiency anemia, not repairable by iron alone, occurs in man. At the present, this statement applies to iron–copper, iron–zinc, and iron–cobalt combinations, as well [121]. A corollary is that the use of such hematinic combinations in the clinical arena clearly is not warranted from the data at hand.

Extrapolating the observations of absorptive interactions to all trace metals is fraught with difficulties. The interactions between iron, zinc, cadmium, and cobalt seem to be similar and interrelated. However, trivalent ionic species of elements, such as chromium,

appear to have very different mechanisms for absorption, secretion, and excretion. For several elements, such as vanadium, tin, and the precious metals, our knowledge is limited by scanty studies [114]. The same mechanisms used to explain the absorptive interactions for the bivalent ions described above have been postulated. Presumably, most of these are absorbed as complexes in much the same way as iron; that is, the smaller and more soluble complexes of iron (such as amino acid complexes of iron) are absorbed preferentially to simple ionic iron [121]. This has clearly been shown to be true for copper complexes [113]. The amino acids which are themselves absorbed most easily seem to enhance the absorption of amino acids complexed to copper. Thus, the levorotatory amino acid complexes are absorbed to a greater degree than are dextrorotatory forms. It might be expected, then, that competition for binding sites with ligands of multiple types by these different ions would exist. The degree of antagonistic influences could even be maximized by the similarity in valence structure that exists. Generally, this postulate holds within the group of bivalent trace metals. Studies by Sheehan, cited above, would suggest that most of the antagonism, in fact, exists at the first step of the process, the transluminal entry into the mucosal cell. For cobalt, at least, there may exist alternate pathways for handling which may not involve direct competition with iron [123]. The second area in which trace metals appear to play some role in hematopoiesis is based upon the complex interrelationships at the level of intermediary metabolism. The best studied aspects are the effects of trace metals on heme biosynthesis and degradation. The heme pathways have focal interest by virtue of the described role of agents known to stimulate or inhibit the conversion of heme precursors to heme. Heme is involved in a number of cellular reactions, including the P450 cytochrome-dependent oxidative transport system. Multiple substances are capable of influencing the rate of heme synthesis and degradation. London observed that heme itself seems to be the most important regulator of its own synthesis via inhibition of ALA synthetase, the first and rate-limiting enzyme in the synthetic pathway [125]. For some time it appeared that this inhibition was a function of the intact heme molecule itself. Maines demonstrated that a number of metals could markedly affect the synthesis and degradation of heme in much the same fashion as the intact molecule [126, 128–130]. The first such metal noted to serve this function was iron, but the list has been expanded to include chromium, manganese, cobalt, nickel, copper, zinc, silver, cadmium, platinum, gold, mercury, lead, and tin [126]. All of these are capable of inducing heme oxygenase, the enzyme responsible for heme degradation. This induction results in a marked depletion of all the cellular hemoproteins. The inhibition of ALA synthetase by the trace metals is independent of their action on heme oxygenase [126]. Complexing of the metals with sulfhydryl agents completely blocks the effects on both ALA synthetase and heme oxygenase [126]. Chelation of the metals by the porphyrin ring structure does not appear to be necessary for this action of the mentioned trace metals.

Another site of action of the trace metals in intermediary metabolism has been investigated by Tephly [131]. The injection of rats with cobaltous chloride was found to result in a reduction in microsomal heme. This appears to be a result of inhibition of the last step in heme synthesis, the protoporphyrin IX to heme reaction which is catalyzed by ferrochetolase. It was not clear whether this was due to a direct inhibition of ferrochetolase or by inhibition via a cobalt–protoporphyrin IX complex. When multiple divalent trace metals were examined for their effect on ferrochetolase activity, it was found that cobalt and manganese were potent inhibitors of the enzyme. By contrast, copper was found to be stimulatory. Subsequent investigation suggests that copper plays a permissive role in the final insertion of iron into the protoporphyrin IX molecule. This final insertion requires

reduced glutathione. Copper can reverse the inhibitory actions of cobalt and lead on ferrochetolase. This effect is not one of competitive inhibition, since the addition of more bivalent iron does not produce the same effect. Tephly has suggested the interaction may be at another site on the ferrochetolase molecule, perhaps a site which is normally occupied by a copper ion [131]. The implication of this observation is that copper deficiency might result in reduced ferrochetolase activity with resultant reduction in heme synthesis. The implications of this mechanism for the significant ineffective erythropoiesis seen in copper deficiency are significant. It is of interest that a circulating enzyme, ceruloplasmin, is a potent ferrochetolase [35–37]. Ceruloplasmin is produced by the incorporation of cupric ion into a protein moiety. Ceruloplasmin has a half-life of 5 days, and any significant degree of total body copper depletion is accompanied by a prompt reduction in ceruloplasmin activity. Whether the intracellular enzyme ferrochetolase is identical to ceruloplasmin is not clear, but the implication is that they both are probably copper metalloenzymes and act in similar ways to catalyze the oxidation of iron [36].

A third area in which interactions between trace metals may play a role in hematopoiesis relates to modulation of toxicity. Lead is known to produce a severe sideroblastic anemia which, in part, is the result of a biochemical interference with heme synthetic activity, perhaps primarily ALA dehydratase. Komai noted that zinc is a potent inducer of this enzyme in *Ustilago sphaerogena*, and similar induction has been demonstrated in mammalian systems [132]. Finelli has described a reversal of the lead-induced inhibition of ALA dehydratase by zinc [133]. Studies by Davis and Avram demonstrated that a similar activity could be demonstrated for cadmium and that cadmium was, in fact, a much more potent stimulator of ALA dehydratase activity than zinc [77]. Because of its potent inducing activity, cadmium was shown to be useful at reversing lead-induced inhibition of the enzyme at lower concentrations than zinc. At much greater levels of cadmium, ALA dehydratase becomes inhibited, suggesting that this action is in part due to competitive inhibition. The proposed mechanism for this interaction between these divalent ions is competition for binding of the peripheral sulfhydryl groups of ALA dehydratase, as has also been suggested for the interactions of the trace metals with other heme pathway system enzymes [126, 131].

On another level, chronic administration of several of these trace metals is associated with elevations of the level of heme synthesis and breakdown [126]. This is achieved with only minor changes in the P450 cytochrome system [128]. Such chronic administration of some of these might be expected to sensitize the organism to other metal ions. The studies of Maines have demonstrated this synergistic effect on multiple enzymes of heme synthesis and breakdown with chronic iron treatment in rats secondarily exposed to gold. Another factor which modulates this activity is decreased glutathione levels. Trace metals which act in some part to deplete cellular glutathione include cobalt, nickel, platinum, and gold [128, 129].

A brief synopsis of the material covered thus far is in order. The trace metals represent an extremely small part of the total mass of the organism. Despite their low concentrations, certain of these elements appear to play major roles in metabolism. At the level of the hematopoietic system, specific roles can be identified for iron, copper, zinc, and cobalt. Other trace metals appear to play less clearly defined roles in hematopoiesis but clearly are involved by virtue of a number of interactions at the level of absorption, intermediary metabolism, or modulation of toxicity. Clearly defined physiologic roles for still others (such as lithium) are lacking.

The goal of nutrition is to provide the population with all essential nutrients while

at the same time minimizing exposure to harmful substances and preventing overexposure to those elements which, in and of themselves, are not harmful unless taken in excess. It has become clear that probably there is no such thing as an inert substance; virtually every substance can produce toxicity if given in adequate quantities. Between these two extremes of essential and toxic are a number of areas which remain the domain of trace element research.

Mertz postulated that basic laboratory research with trace metals could be described in two broad categories [134]. The first of these is the search for functions of substances for which no current function is known. The second deals with the elucidation of sites of actions for these elements. This latter process entails considerations such as biologic requirements, acquisition and excretion, and finally, interactions or interrelationships with other nutritional factors. As we noted earlier in this review, there is no argument that trace metals are a significant constituent of the cells of the hematologic system. The elements have been clearly identified, and their concentrations have been found to be remarkably constant [2–8]. This would suggest that these substances are more than simple contaminants. To this point in time, unfortunately, far too much effort has been spent in looking at these substances in terms of an "all or none" essentiality. This approach, while clearly useful in the past, fails to account for the biologic roles many of these substances appear to play by virtue of interactions and as cofactors in various reactions.

In this regard, the investigations of Hill and Matrone [114] have been particularly beneficial in providing a model which would point out (and has) new paths of investigative endeavor into absorptive interactions. Perhaps even more exciting are the studies from Maines [126, 128–130] and Tephly [131], investigating the complex interactions of even the least likely of transition elements in the reactions involving biosynthesis and degradation. Whereas it has been possible to demonstrate complex in vitro interactions of these bivalent ionic species in various enzymes of heme synthesis and degradation, further studies decumenting exactly how significant these interactions are in terms of in vivo hematopoiesis remain a subject for future investigation.

Future application of this nutritional research will have to come to grips with such issues as the precise levels of trace element activity for optimal hematopoiesis. Clearly, the era of looking at these substances in an "all or none" fashion will not answer these sorts of issues. Moreover, projection of the needs of organisms for these substances under situations of stress, pregnancy, and other states of nutritional imbalance need to be more clearly defined for these trace substances. Finally, the question of toxicity of trace elements as relates to the hematopoietic system needs to be further investigated. Clearly, consumption of gram quantities of copper sulfate can produce severe hematologic injury, as was noted in the report of Roberts in Mississippi [58]. Unaddressed issues remain in the areas of subacute toxicity to the hematopoietic system under situations of increased intake of copper, chronic administration of gold salts, and dialysis against minimally elevated zinc gradients. The effects of exposure to sublethal levels of various toxic substances are largely unknown. The similarities between the sideroblastic states seen in copper deficiency and the idiopathic refractory sideroblastic states would justify further research in trace metal interactions. These sorts of issues lead one back to the definition of essentiality as being more than the minimal requirements to sustain life. Future research activities in trace metal interactions must include particular emphasis on those interrelationships which provide the control of the hematopoietic system's regeneration, intermediary metabolism, and response to injury.

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