Trace Metals and Hemoglobin Metabolism

ADOLFO D. GARNICA, M.D.

Department of Pediatrics, University of Florida, College of Medicine, Gainesville, FL 32610

ABSTRACT

Hemoglobin is composed of two pairs of globin chains to which are attached four iron-containing metalloporphyrins. Factors regulating hemoglobin synthesis include the availability of iron and the presence of heme. Heme production occurs by enzymatic synthesis and includes a step mediated by aminolevulinic acid dehydrase, which is zinc-dependent and, thus, susceptible to the toxic effects of other metals. The intake of iron, copper, zinc, cobalt, manganese, cadmium or lead may affect hemoglobin levels by an influence on availability of iron or heme.

Introduction

Hemoglobin consists of the basic protein, globin, complexed with four ferroprotoporphyrin, or heme, moieties. Metalloporphyrins, or hemes, consist of divalent cations coordinately bound within protoporphyrin IX rings. One of the most important functions of iron is the synthesis of heme, which combines with specific proteins to form compounds capable of binding oxygen reversibly or heme enzymes that make oxygen available for intracellular oxidation. Heme and globin synthesis and their attachment to one another are thought to occur simultaneously in the bone marrow during the later stages of erythropoiesis. The most critical determinants of hemoglobin concentration are availability of iron and the presence of heme. Thus, anything affecting the availability of iron might also affect the synthesis of heme and hemoglobin. Copper, zinc, cobalt, manganese, and cadmium, because of their similarity in physiochemical characteristics to iron, are able to interfere with normal iron metabolism and, consequently, heme and hemoglobin synthesis.

Hemoglobin Synthesis

Heme type b is the most ubiquitous metalloporphyrin among animals and constitutes the prosthetic component of such diverse hemoproteins as hemoglobin, myoglobin, catalase, mitochondrial cytochromes P-450 and b5, mitochondrial cytochrome b, and the cytosol enzyme tryptophan pyrrollase. Oxidized iron bound to protoporphyrin IX is known as hemin.

The initial step in porphyrin synthesis is the formation in mitochondria of aminolevulinic acid...
levulinic acid (ALA) by the condensation of glycine and succinyl-coenzyme A, mediated by the enzyme ALA synthetase, which is the rate-limiting enzyme in heme synthesis.\textsuperscript{19,64} The final steps in heme synthesis, which are mediated by coproporphyrinogen oxidase and heme synthetase, also occur in the mitochondria and allow heme synthesis to be regulated by end-product inhibition of heme synthetase and end-producer repression of its synthesis.\textsuperscript{19,35,63} Bone marrow culture studies have shown that erythropoietin stimulates ALA synthetase prior to an effect on globin synthesis.\textsuperscript{26,46}

The role of heme in the regulation of hemoglobin synthesis has not been completely defined. A mutual interdependence of heme and globin synthesis has been postulated.\textsuperscript{5,19,38,39,57,61,63} Heme synthesis is partially controlled by ALA synthetase activity. High intracellular heme levels result in decreased heme synthesis; low heme concentrations cause a diminution in globin synthesis.\textsuperscript{5,19,29,57} Heme (as hemin) has been shown to be necessary for globin synthesis in intact reticulocytes and their cell-free preparations.\textsuperscript{5,19,29,30} Intact cells incubated without hemin and rendered iron-deficient by iron chelation demonstrate an impairment of globin synthesis, which implies an inhibition of globin synthesis resulting from an inhibition of heme synthesis.\textsuperscript{5,18,19,45} Hemin and ferrous-iron/transferrin mixture prevent and reverse this effect in intact cells.\textsuperscript{43} Studies utilizing reticulocyte cell-free lysates indicate that the impairment of globin synthesis associated with a lack of hemin apparently consists of a failure in the first step of protein synthesis mediated by the formation of hemin-controlled translational repressor of initiation formed in the absence of hemin.\textsuperscript{19,29,30,35,38} A pro-inhibitor is normally present in the cytoplasm but is inactivated by hemin during the early stages of its formation.\textsuperscript{19,29,30,38}

**Heme Oxygenase**

Intracellular heme levels are affected by the activity of heme oxygenase, a catabolic enzyme whose activity is partially iron-dependent.\textsuperscript{4} The heme oxygenase system is present in the microsomal membranes of liver, kidney, brain, spleen, and bone marrow and has been shown to possess substrate specificity for heme, the $\alpha$ and $\beta$ chains of hemoglobin, methemalbumin, and methemoglobin, but not for free porphyrins.\textsuperscript{32,69} In mammals, heme is metabolized by oxidative degradation, releasing the central metal.

The influence of trace metals on heme oxygenase synthesis was first observed in 1974 as an increase in hepatic heme oxygenase activity in rats caused by divalent cobalt.\textsuperscript{49,50,52} An increase in heme oxygenase activity resulting in a depletion of cellular hemoproteins can also be induced by chromium, manganese, iron, copper, zinc, and lead.\textsuperscript{50} Studies utilizing inhibitors of nucleic acid or protein synthesis indicate that the increase in heme oxygenase activity is the result of true induction.\textsuperscript{52,54} None of the metals increase enzyme activity when added directly to the incubation mixture, but all form stable complexes with sulphydryl groups.\textsuperscript{52} An accentuation of the inductive effect by prior reduction of cellular glutathione suggests that the metals react directly with sites involved in heme oxygenase regulation.\textsuperscript{51} Thus, Maines has postulated that the metals are physiologic regulators of heme oxygenase activity whose inductive effect results from the action of the central metal ion released from the metalloporphyrin molecule.\textsuperscript{52}

Cobalt, however, has also been reported to cause an increase in liver heme oxygenase activity and an increase in the degradation of heme and hemoproteins by a mechanism requiring the formation of cobalt protoporphyrin.\textsuperscript{68} Cobalt heme (cobalt-protoporphyrin IX) has been reported to possess the same heme
Iron Metabolism

An adequate supply of iron is essential for heme synthesis and, under some circumstances, may constitute the limiting factor. An adequate supply of iron is essential for heme synthesis and, under some circumstances, may constitute the limiting factor. 

Thus, anything affecting iron absorption or utilization will also affect hemoglobin synthesis. Iron absorption may be affected by iron nutritional status, the chemical form of iron ingested, or dietary composition. Hill has suggested that the biological antagonism between two elements is determined by their physicochemical properties. One type of interaction involves elements with similar valence shell electron structures, such as iron, cobalt, or manganese. Animal studies have demonstrated antagonism between ferrous or ferric iron, trivalent cobalt, and divalent manganese. Thus, divalent manganese and trivalent cobalt cause a decrease in hemoglobin in animals fed cobalt- or manganese-supplemented diets by interfering with iron absorption. Moreover, in iron-deficient animals, the intestinal absorption of iron, manganese, cobalt, nickel, and zinc is increased. The absorption of the essential element, zinc, and the toxic metal, cadmium, are also increased in iron-deficient animals. Copper, zinc, and cadmium, moreover, interfere with iron absorption and metabolism, although their valence electron structures are different from iron. Thus, the intestinal transport system for iron is not specific, although there are indications that specific proteins mediate the intestinal absorption of iron, as well as other metals.

The intestinal uptake and transfer of zinc in iron deficient animals has been reported to be significantly greater than in iron-loaded ones. This increase in zinc uptake has been interpreted to reflect an activation of binding sites by the low iron diet. Conversely, the increase in iron uptake seen in zinc-deficient animals implies an activation of binding sites also by a low zinc intake. Thus, analogous binding sites for the intestinal uptake of zinc and iron have been postulated, which are activated by a deficient iron intake. Iron deficiency produced by bleeding also increases iron absorption. However, bleeding does not increase zinc absorption, and dietary zinc deficiency does not increase iron transfer. These findings are thought to infer the existence of mutually exclusive binding sites for the transfer of iron and zinc from the intestinal mucosa into the bloodstream. Animal studies indicate that cobalt and manganese share transfer binding sites with iron, while zinc shares binding sites with chromium. Cobalt inhibits the intestinal iron absorption by a mechanism which is not completely defined. The similarity in the subcellular distribution patterns of cobalt and iron in the intestinal mucosal cells implies an affinity of cobalt for iron-binding proteins. However, cobalt does not displace iron from its binding sites, even when present in quantities several times that of iron. Thus, cobalt apparently does not decrease iron absorption by interfering with binding sites on or in the luminal membranes of mucosal cells but, instead, by interfering with the release process on the contraluminal side.

Iron and copper have been reported to share a common affinity for intestinal
transferrin and metallothionein.\textsuperscript{11,12} Starcher has implicated metallothionein in copper absorption.\textsuperscript{68} El-Shokabi et al have observed also the binding of iron by metallothionein but did not speculate on its functional significance.\textsuperscript{12} In normal rats, the majority of enteral doses of radioactive copper were found in the transferrin fraction of mucosal homogenates. Significant quantities of metallothionein-bound copper were observed only in iron-deficient animals, along with an increase in mucosal transferrin. The increase in metallothionein-bound copper and decrease in transferrin-bound copper following the simultaneous administration of iron and copper suggest that copper is displaced from transferrin by iron. In iron-deficient animals, however, iron absorption and the amount of iron bound by supernatant proteins could be decreased by the simultaneous administration of excess copper. El-Shokabi et al postulate, therefore, that mucosal transferrin is a determinant of iron absorption in iron deficiency, but copper, because of its affinity for transferrin and metallothionein, may compete for binding sites when administered in sufficient quantities.\textsuperscript{12}

**Lead Poisoning**

Although lead is neither essential nor a trace metal, a discussion of lead intoxication in this context seems appropriate because of its relationship to iron, copper, and zinc metabolism.\textsuperscript{22,32,41,67} In lead poisoning, nearly all blood lead is found in erythrocytes, bound intracellularly, and on the reticulocyte membrane.\textsuperscript{41} Lead increases the production of immature erythrocytes, shortens erythrocyte survival time, may inhibit globin synthesis, and impairs iron metabolism by inhibiting ferrochelatase and interfering with the uptake of iron by the reticulocytes. Ferrokinetic studies in lead-intoxicated patients demonstrate decreased hemoglobin synthesis associated with an inhibition of iron release from reticulocytes and an accumulation of iron-containing particles in the red blood cell precursors of the bone marrow.\textsuperscript{22}

Animal studies suggest that lead-induced anemia might be caused by an interference with iron and copper metabolism.\textsuperscript{22,32,41,67} Klauder and Petering have postulated an impairment of copper-directed iron utilization as a consequence of which copper nutrition becomes an important determinant of the clinical manifestations of lead-induced anemia.\textsuperscript{41} Lead intoxication is most often associated with anemia and low serum iron levels.\textsuperscript{32,67} Indeed, a decrease in circulating hemoglobin is one of the first signs of lead poisoning.\textsuperscript{22} However, unlike iron-deficiency anemia, the anemias of lead poisoning and copper deficiency are sideroblastic. Since the greatest demand for iron is for heme synthesis in the bone marrow, lead may affect the component of copper metabolism which promotes the utilization of iron for heme synthesis.\textsuperscript{41} The decreased ceruloplasmin activity seen in copper deficiency is associated with a decrease in tissue iron mobilization and an increase in tissue ferritin and hemosiderin.\textsuperscript{23,45,56} It is unlikely, however, that ceruloplasmin activity is the lead-sensitive component of copper metabolism responsible for the impaired iron utilization, since ceruloplasmin activity is not affected by lead. Klauder and Petering suggest, alternatively, that there may be an intracellular copper compartment at the bone marrow level essential for the mobilization of iron for incorporation into heme, and the function of this compartment is adversely affected by lead.\textsuperscript{23,41,56}

Zinc is also of clinical importance in lead poisoning.\textsuperscript{71} One of the most well-characterized effects of lead intoxication is an inhibition of heme synthesis. Although nearly all enzymes are affected, those most profoundly inhibited are ALA
dehydrase and ferrochelatase.\textsuperscript{71} ALA dehydrase has been reported to be zinc-dependent, and the degree of inhibition of its activity has been related to blood lead levels.\textsuperscript{15,16} Indeed, erythrocyte ALA dehydrase activity has been proposed as a biological indicator of total body lead burden.\textsuperscript{67,70} During chelation therapy for lead poisoning, the urinary zinc excretion and the blood lead:zinc ratio increase while the erythrocyte ALA dehydrase activity blood lead decreases.\textsuperscript{70} The decrease in ALA dehydrase activity, however, can be minimized by the administration of oral zinc sulfate. These results imply that the removal of zinc during chelation therapy is at least partially responsible for the observed decrease in ALA dehydrase activity and oral zinc sulfate might serve a protective function in lead poisoning.

**Cobalt**

Cobalt is primarily involved in erythropoiesis by virtue of its being a component of cyanocobalamin.\textsuperscript{55} However, it also induces an erythropoiesis and polycythemia not related to its normal function. Cobalt, in pharmacologic doses administered either orally or parenterally, has been used as a nonspecific stimulant of erythropoiesis inducing a polycythemia and increased blood volume in animals and humans, characteristically preceded by bone marrow hyperplasia and marked reticulocytosis.\textsuperscript{42,55} The mechanism of action of cobalt as an erythropoietic stimulant has not been clarified, although its effectiveness is well established. Cobalt injections into animals subjected to repeated bleeding result in an increase in erythrogenic precursors in the bone marrow and a rapid recovery from the anemia. Apparently the induction of polycythemia does not involve the formation of vitamin $B_{12}$ or an active stimulation of the bone marrow but is the result of a passive physiologic compensation to partial anoxia.\textsuperscript{7,42,55} The underlying mechanism is still obscure but is thought to be related to an inhibition of cellular respiration within the blood-forming altitude. Cobalt feeding demonstrates that the effects of cobalt exposure and high altitude on growth and polycythemia are additive.\textsuperscript{7}

**Copper**

The clinical effects of copper deficiency were reported first in a group of post-diarrheal, malnourished infants rehabilitated with a low copper, milk-base formula.\textsuperscript{8} Copper deficiency occurs most commonly among premature infants of less than 1500 g birth weight and is thought to be related to a combination of deficient dietary copper and marginal hepatic copper stores.\textsuperscript{25} Copper deficiency has not been recorded after the age of one year. Hematologically, its first manifestation is a neutropenia, which is followed by a hypochromic, microcytic anemia. The anemia of copper deficiency is sideroblastic.\textsuperscript{42,44,67} Early in the course of the copper deficiency, the anemia responds poorly to oral iron but can be treated with intramuscular iron. After prolonged copper deficiency, an intractable anemia unresponsive to intramuscular iron develops.\textsuperscript{25,45} Thus, copper deficiency has two effects on iron metabolism: the first, occurring early, is an impairment of iron absorption; the second, occurring later, is inadequate erythropoiesis.\textsuperscript{45} Copper deficiency results in a hypochromic, microcytic anemia and an accumulation of iron in the liver, which implies an unavailability of absorbed iron for hemoglobin formation.\textsuperscript{25,34,45} Animal studies using weanling pigs demonstrate the effect of varying levels of dietary iron and copper on growth, hemoglobin, plasma copper and ceruloplasmin, hepatic copper and hepatic iron.\textsuperscript{24} At low dietary copper levels, the hemoglobin, plasma and hepatic copper, plasma and hepatic iron, and serum ceruloplasmin are
reduced. At high dietary copper levels, on the other hand, the plasma and liver iron and the hemoglobin are reduced, while the plasma and hepatic copper are increased. The anemia induced by the high copper diet, moreover, could not be completely corrected by increasing the dietary iron and was, therefore, interpreted to be the result of impaired iron utilization. On the other hand, the microcytic, hypochromic anemia and reduced plasma and tissue iron seen in copper deficiency have been postulated to be the result of reduced ceruloplasmin activity. With copper repletion, an increase in ceruloplasmin oxidase activity is associated with a decrease in liver iron and an increase in liver copper. It has been suggested that ceruloplasmin ferroxidase activity is the regulating agent in the mobilization of intracellular, transferrin-bound iron into plasma. Osaki et al, moreover, have postulated a biological role for ceruloplasmin oxidase activity, which may be necessary for the oxidation of ferrous iron prior to its incorporation into transferrin and transfer from the intestine into the blood stream. In the absence of ceruloplasmin, oxidation is impaired and the utilization of iron for hemoglobin synthesis is decreased.

The findings in the copper deficient infants correspond with observations in copper deficient animals. On the basis of studies in swine, Lee and associates described the following effects of copper deficiency on iron metabolism: (1) defective absorption of iron from the gastrointestinal tract; (2) restricted iron flow from the reticuloendothelial cells into plasma; (3) excessive iron accumulation in the hepatic parenchymal cells; and (4) defective incorporation of iron into heme within the normoblasts. They postulate further that the first three abnormalities might be related to a deficiency of ceruloplasmin and that the last may be secondary to a deficiency of cytochrome oxidase, which may be essential for the availability of ferrous iron for heme synthesis in the mitochondrion.

**Zinc**

Zinc is reported to be necessary for normal ALA dehydrase activity. ALA dehydrase is present in erythrocytes where it enzymatically mediates the condensation of two moles of ALA into one mole of porphobilinogen for heme synthesis. In vitro and in vivo studies suggest that zinc is required for the synthesis of ALA dehydrase and as an activator of its enzymatic activity. Fenelli et al have demonstrated an in vivo dependence of ALA dehydrase activity on dietary zinc content in rats. It has been suggested that ALA dehydrase is a zinc metalloenzyme, but Fenelli et al postulate that zinc is probably an activator of ALA dehydrase. The high in vivo and in vitro sensitivity of ALA dehydrase to low lead concentrations implicates the involvement of zinc at the enzymatically active sites and suggests a specific binding of lead to these sites. On the other hand, the requirement of relatively high zinc concentrations implies a greater affinity for lead than zinc. Copper has also been reported to be necessary for ALA dehydrase activity, but in animals fed an adequate zinc diet, copper did not affect enzyme activity. Similarly, the previously reported activation of ALA dehydrase by iron has not been confirmed.

**Cadmium**

Animal studies indicate that anemia is one of the most sensitive indicators of cadmium intoxication. Dietary cadmium supplementation also results in decreased growth and decreased tissue iron, zinc, and copper. A decrease in iron content in multiple organs and increase in urinary iron excretion have been interpreted to be indicative of a depletion...
of body iron stores. The increased cadmium and decreased iron contents observed in animals fed a cadmium-supplemented stock diet is thought to be indicative of a depletion of body stores of iron. Cadmium feeding has been reported to cause a bone marrow hyperplasia and microcytic, hypochromic anemia similar to that seen in iron deficiency and reversible by the parenteral administration of iron. These findings are similar to those found in humans exposed to environmental cadmium. The anemia and increased plasma transferrin levels found in cadmium-fed animals were thought to be due to interference with iron absorption or metabolism. Indeed, the anemia observed in quail fed cadmium-supplemented diets could be reversed by iron supplements. Studies utilizing inhalative cadmium supplementation in animals, moreover, demonstrated a decrease in liver and kidney copper but no anemia, which supports the hypothesis that the anemia seen with cadmium ingestion is the result of an inhibition of iron absorption.

Summary

1. Copper, zinc, cobalt, manganese, cadmium, and lead are all capable of altering hemoglobin synthesis, primarily through their effect on iron metabolism.
2. Copper deficiency is currently of significance in humans because of recent developments resulting in the improved survival of premature infants of less than 1500 g birth weight.
3. Zinc administration may be a useful adjunct to the treatment of lead poisoning.
4. Finally, cobalt, manganese, and cadmium are important considerations under circumstances of unusual toxic exposure.

References


49. Maines, M. D. and Kappas, A.: Cobalt induc-


