Nutritional Anemias

Ellen Butensky, PhD, PNP
Paul Harmatz, MD
Bertram Lubin, MD

INTRODUCTION

Anemia is defined as a circulating red blood cell mass inadequate to prevent tissue hypoxia. Anemia can be related to blood loss, decreased red cell production, increased red cell destruction, or a combination of these events. When anemia occurs as a consequence of a nutritional deficiency, any of these pathologic processes may be involved, either singly or in combination. Vitamin deficiencies that have been implicated as causes of anemia in humans include vitamin A, members of the vitamin B group [pyridoxine (B_6), riboflavin (B_2), folate (B_9), and cyanocobalamin (B_{12})], vitamin C, and vitamin E. Among minerals, iron and copper are recognized as essential for optimal erythropoiesis. Complex nutritional disturbances, such as those observed in starvation and protein/calorie deficiency states, can also result in anemia.

Hemoglobin, hematocrit, and red cell indices (mean cell volume, MCV, mean corpuscular hemoglobin, MCH, and mean corpuscular hemoglobin concentration, MCHC) are the initial laboratory assessments used to diagnose and classify an anemia. It is important to recognize and adjust for the age-related changes in these parameters when assessing anemia in the pediatric age group (Table 1).[1,2] These parameters can help classify the anemia (Figure 1)[3] as one due to decreased red cell production versus increased red cell destruction, acute blood loss, or impaired hemoglobin production. If the anemia is secondary to inhibited hemoglobin synthesis, the anemia will be characterized from these laboratory parameters as microcytic and hypochromic (decreased red cell indices). Conversely, if the anemia results as a consequence of a disturbance in cell maturation, the anemia may be described as macrocytic (increased red cell indices). If the deformability of the red cell membrane is altered, the anemia will have a hemolytic component, an elevated reticulocyte count, and normocytic or macrocytic red cell indices. Nutritional deficiency states often result in combinations of decreased hemoglobin synthesis, abnormal red cell maturation, increased red cell destruction, or decreased RBC production, making straightforward classification as shown in Figure 1 difficult.

The most common nutritional deficiency responsible for anemia is iron deficiency. Nutritional deficiencies of folic acid or vitamin B_{12} are also occasionally encountered in clinical practice and patients with chronic illness may have other vitamin, mineral, and protein deficiencies contributing to anemia. In this chapter, we will review relationships between nutritional deficiencies and anemia and discuss responsible mechanisms. The etiology of the vitamin and mineral deficiencies will not be discussed in detail as this information is provided elsewhere in this book.

MEGALOBLASTIC ANEMIAS: FOLATE AND VITAMIN B_{12} DEFICIENCIES

Nutrient deficiency of either folate or vitamin B_{12} results in a megaloblastic anemia. The fundamental biochemical defect related to vitamin B_{12} or folate deficiencies is decreased synthesis of deoxyribonucleoprotein (DNA). This appears to be the result of the inadequate conversion of deoxyuridylate to thymidylate related to inadequate quantities of 5,10-methylene tetrahydrofolate for the single carbon transfer reaction.[4] Vitamin B_{12} is required for the release of folate from its methyl form so that it can return to the tetrahydrofolate pool for conversion to 5,10-methylene tetrahydrofolate. In vitamin B_{12} deficiency, folate is trapped as methylfolate which is metabolically inactive. This abnormality of folate metabolism is known as the "folate trap" hypothesis.[5–7]

The morphologic representation of this decreased DNA synthesis is the megaloblast. Megaloblasts are nucleated red blood cells that display lacy nuclear chromatin and prominent parachromatin pattern, and an apparent dyssynchrony of maturation between the nucleus and the cytoplasm. This dyssynchrony is produced by the slow DNA synthesis in the nucleus in relation to the near-normal synthesis of RNA in the cytoplasm. Although these morphologic abnormalities in the maturing erythroid precursors are regarded as the hallmark of folic acid or vitamin B_{12} deficiency, these same abnormalities are also evident in the myeloid and megakaryocytic cell lines.

In an established case of megaloblastic anemia, the circulating red cells are macrocytic, with an MCV increased to a range of 105 to 160 femtoliters (fl). The presence of simultaneous iron deficiency may obscure the macrocytosis resulting in a normocytic anemia. Polychromia and fine

Table 1 Red Blood Cell Values at Various Ages: Mean and Lower Limit of Normal (–2 SD)

	Hemoglobin (g/dL)		Hematocrit (%)		MCV (fl)	
	Mean	Lower Limit	Mean	Lower Limit	Mean	Lower Limit
Age						
Birth (cord blood)	16.5	13.5	51	42	108	98
1–3 d (capillary)	18.5	14.5	56	45	108	95
1 wk	17.5	13.5	54	42	107	88
2 wk	16.5	12.5	51	39	105	86
1 mo	14.0	10.0	43	31	104	85
3–6 mo	11.5	9.0	35	28	96	77
0.5–1.9 yr	11.5	9.5	35	29	91	74
2–4 yr	12.0	11.0	37	33	77	70
5–7 yr	12.5	11.0	38	34	79	73
8–11 yr	13.0	11.5	39	35	81	75
12–14 yr	13.5	12.0	40	36	83	76
Female	13.5	12.0	41	36	85	78
Male						
15–17 yr	14.0	12.5	43	37	84	77
Female	14.0	12.0	41	36	87	79
Male						
19–49 yr	15.0	13.0	46	38	86	78
Female	14.0	12.0	42	37	90	80
Male	16.0	14.0	47	40	90	80

Adapted from references 1 and 2.
MCV = mean corpuscular volume.

With permission from Duggan C, et al. *Nutrition in Pediatrics.* 4th ed. Hamilton, Ontario, Canada: BC Decker Inc; 2008.

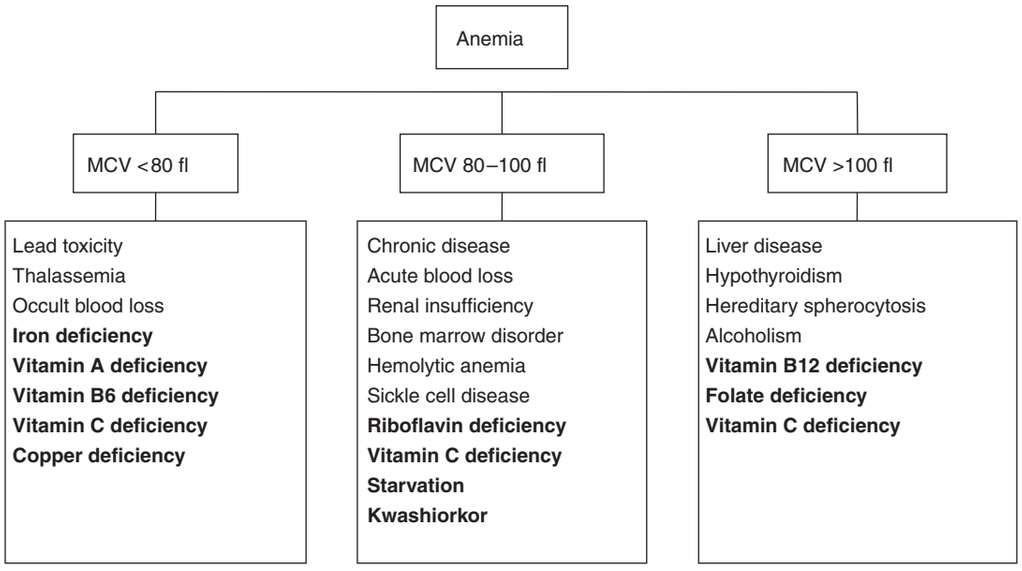

Figure 1 Initial laboratory evaluation of anemia. (Adapted from reference 3.)

basophilic stippling of the erythrocytes are observed and Howell–Jolly bodies, nuclear remnants normally extruded from the erythrocyte during cell maturation, may be present. The concentration of serum iron and nontransferrin bound iron is increased due to impaired erythrocytosis.[8] The red cell life span is modestly reduced, perhaps due to an abnormality in membrane properties related to the dyserythropoietic state. A significant degree of ineffective erythropoiesis is present,[9] which may be reflected by increases in serum bilirubin, serum LDH,[10] and nontransferrin bound iron.[8]

The earliest morphologic change in the megaloblastic anemias is alteration in the circulating granulocytes, which become hypersegmented. In the normal child, the average number of nuclear segments is 2.6. In megaloblastic anemias, the average number of segments generally exceeds a mean value of 3.0 and is often six or greater.[10] In severe forms of deficiency, both neutropenia and thrombocytopenia may be present.

Deficiencies of either folate or B_{12}, from any mechanism, are uncommon in the pediatric population in the United States. However, when they do occur, it may be as a result of inadequate ingestion, absorption, or utilization due to congenital or acquired defects. Not all megaloblastic anemias are due to a lack of these vitamins. Megaloblastosis can also be seen in thiamine deficiency, Lesch–Nyhan syndrome, congenital dyserythropoietic anemias, or as a side effect of chemotherapeutic agents (methotrexate and cytosine arabinoside), antimalarials, and antibacterials (trimethoprim). These antifolates have an affinity for dehydrofolate reductase (DHFR). When intracellular folate concentrations are low, they may inhibit DHFR activity and cause folate deficiency.

FOLIC ACID DEFICIENCY

The clinical manifestations of folate deficiency include megaloblastic changes (not necessarily anemia or pancytopenia), gastrointestinal symptoms such as glossitis, anorexia, gastrointestinal discomfort or occasional diarrhea, humoral and cellular immune defects, and neurologic abnormalities including depression, poor judgment, and some affective disorders.[11]

Folate is present in a wide variety of foods. Cow's milk, human milk, and proprietary infant formulas normally provide approximately 50 μg/L. In contrast, goat's milk contains only 2 to 11 μg/L, and the feeding of goat's milk to infants as the sole source of nutrition will result in the appearance of a megaloblastic anemia.[13] Although dietary folic acid deficiency appears to be unusual in the United States, it is a common cause of megaloblastic anemia in developing countries. Folate deficiency may accompany kwashiorkor, and its incidence has been found to range from 10 to 70%. This variation presumably reflects regional dietary practices such as overcooking of foods or boiling of milk can reduce the folate content by approximately 50%.[14] Both malabsorption and chronic infection may also contribute to this folic acid deficiency.

Table 2 Etiologic and Pathophsiologic Classification of Folate Deficiency

I. Nutritional causes
  A. Decreased dietary intake
    1. Poverty and famine (associated with kwashiorkor, marasmus)
    2. Institutionalized individuals (psychiatric and nursing homes)
    3. Chronic debilitating disease
    4. Goat's milk, special diets (phenylketonuria, maple syrup urine disease, slimming)
    5. Cultural/ethnic cooking techniques (food folate destroyed) or habits (folate-rich foods not consumed)
  B. Decreased diet and increased requirements
    1. Physiologic
      (a) Pregnancy
      (b) Lactation
      (c) Prematurity, infancy
    2. Pathologic
      (a) Intrinsic hematologic disease [autoimmune hemolytic anemia, drugs; malaria, hemoglobinopathy (ie, sickle cell disease, thalassemia); membrane defects; hereditary spherocytosis, paroxysmal nocturnal hemoglobinuria]
      (b) Abnormal hematopoiesis (leukemia, lymphoma, myelodysplastic syndrome, agnogenic myeloid metaplasia with myelofibrosis)
      (c) Infiltration with malignant disease
      (d) Dermatologic (psoriasis, methotrexate dermatopathies)

II. Folate malabsorption
  A. With normal intestinal mucosa
    1. Some drugs (controversial)
    2. Congenital folate malabsorption
  B. With mucosal abnormalities
    1. Tropical sprue
    2. Nontropical sprue
    3. Regional enteritis

III. Defective cellular folate uptake
  A. Familial aplastic anemia

IV. Inadequate cellular utilization
  A. Folate antagonists (methotrexate)
  B. Hereditary enzyme deficiencies involving folate

V. Drugs (multiple effects on folate metabolism)
  A. Alcohol
  B. Sulfasalazine
  C. Triamterene
  D. Pyrimethamine
  E. Trimethopirm-sulfamethoxazole
  F. Anticonvulsants (diphenylhydantoin, barbiturates)

VI. Acute folate deficiency

Adapted from reference 12.

Developmental changes in folate balance are seen during infancy. Serum and red cell folate concentrations are higher in both preterm and term infants than in normal adults.[15] After birth, serum values decline rapidly, but the decline is most severe in infants weighing less than 1,700 g at birth. Approximately two-thirds of low-birth-weight infants may display subnormal serum folate concentrations between 1 and 3 months of age, but they rarely have a megaloblastic anemia. Administration of supplemental folic acid, in the absence of megaloblastic anemia, has not been demonstrated to produce any increase in the hemoglobin concentration. The normal preterm infant absorbs folic acid without difficulty and the dietary provision of 20 to 50 μg/d appears adequate to prevent the development of a deficiency. The presence of chronic infection or diarrhea may impair absorption or increase needs in these small infants.

Folate requirements appear greater in the preterm infant, in pregnant women, in whom folate is shunted to the developing fetus and urinary folate loss is increased, and in lactating women, who secrete 50 μg or more into each liter of milk.[16] Other groups of patients with higher than normal folate requirements include those with celiac disease and other diseases of the small intestine, those taking antiepileptic medication or birth control pills, and persons with hemolytic anemia, including those with sickle cell anemia and thalassemia.[11]

Laboratory Diagnosis of Folate Deficiency

In addition to the characteristic laboratory findings of megaloblastic anemia, such as macrocytosis, multilobulated polymorphonuclear cells, increased LDH and bilirubin, bone marrow showing megaloblastic changes, increased plasma iron and transferrin saturation, and decreased serum cholesterol and lipids, specific measurements provide direct evidence of deficiency of folate. These include decreased serum folate, decreased erythrocyte folate, homocysteinemia, and sometimes homocystinuria.[11] RBC folate is considered a better indicator of folate status than serum folate. RBC levels are significantly higher than normal serum levels and thus less subject to processing artifacts. Red cell folate also has been reported to parallel tissue stores and is, therefore, more useful than serum folate for documenting the presence of a long-standing deficiency.[17] The normal range of serum folate is 3 to 15 ng/mL. Serum folate values in the 1.5 to 3.0 range are regarded as "indeterminate." The red cell folate normally ranges from 150 to 600 ng/mL, and values of less than 150 ng/mL are diagnostic of deficiency.

VITAMIN B_{12} DEFICIENCY

Vitamin B_{12}, or cobalamin, is an organometallic complex consisting of two major moieties: a corrin nucleus containing a covalently bound cobalt atom, and a nucleotide base lying at right angles to the corrin nucleus. Hydroxycobalamin is the predominant dietary form of the vitamin. Vitamin B_{12} is synthesized by bacteria, is present in animal foods, and is not present in plants; therefore it does not occur in vegetables or fruit.

Only two metabolic functions for the vitamin B_{12} coenzymes, 5-adenosylcobalamin and methylcobalamin, have been identified in humans. The first of these is the conversion of L-methylmalonyl-CoA to succinyl-CoA, a reaction catalyzed by methylmalonyl-CoA mutase and requiring 5-adenosylcobalamin. Defects in the conversion of L-methylmalonyl-CoA to succinyl-CoA result in methylmalonic acidemia (MMA) and methylmalonic aciduria. The second reaction in which vitamin B_{12} is a cofactor is the homocysteine 5-methyl-tetrahydrofolate methyltransferase reaction. This reaction generates methionine from homocysteine using methionine synthase and produces tetrahydrofolic acid from 5-methyl-tetrahydrofolic acid.[18]

Dietary vitamin B_{12}, primarily in the form of hydroxycobalamin, is absorbed in the terminal ileum and requires the presence of intrinsic factor that has been secreted in the parietal cells of the stomach. Nutritional deficiency of vitamin B_{12} is extremely rare in infants and children.

It is estimated that the older child and adult require 1.0 μg of vitamin B_{12}/d, and the infant 0.1 μg/d, to maintain normal erythropoiesis. The normal diet usually contains far more B_{12} than this minimal requirement. Western diets usually contain 5 to 10 μg daily. The highest concentrations are found in liver, kidney, meat muscle, fowl, shellfish, and dairy products. There is probably no vitamin B_{12} in fruits, vegetables, nuts, or cereal unless they are contaminated with vitamin B_{12}-producing bacteria. Vitamin B_{12} is usually not destroyed by cooking. Under alkaline conditions and the presence of ascorbic acid, some vitamin B_{12} may be lost when milk is boiled or when meat is overcooked.

The average daily vitamin B_{12} output in breast milk is approximately 0.3 μg and closely parallels the serum vitamin B_{12} concentration of the mother.[19] The term infant receives approximately 30 μg of vitamin B_{12} from the mother during the course of gestation. Liver stores are about 26 μg in term infants but only 10 μg in premature infants.[20] Serum vitamin B_{12} values in the newborn are normally much higher than those of the mother. Because of this adequate endowment, vitamin B_{12} deficiency due to inadequate ingestion in the first year or two of life is seen only when an infant is born to a severely vitamin B_{12}-deficient mother and is exclusively breastfed by her or placed on a strict vegetarian diet. Megaloblastic anemia in the infants of strict vegetarian mothers has been reported from India.[21] Megaloblastic anemia in breastfed infants of mothers who either have untreated pernicious anemia[22] or are on vegetarian diets has also been observed in 5- to 6-month-old infants in the United States.[23] In many of these cases, the infant presents with evidence of neurologic involvement as well as a macrocytic anemia. Both resolve following treatment with vitamin B_{12}.

The juvenile form of pernicious anemia resembles that seen in adults. These children have gastric atrophy, gastric achlorhydria, absent intrinsic factor, and a high incidence of antibody against intrinsic factor. Some children with pernicious anemia, like adults, have associated endocrinopathies such as hypoparathyroidism, hypothyroidism, and Addison's disease. An association of selective IgA deficiency with juvenile pernicious anemia has also been observed.

The biochemical changes associated with vitamin B_{12} deficiency include MMA and aciduria. The secondary effects of MMA accumulation including acidosis, hyperglycinemia, and possibly inhibition of other enzymes and of bone marrow stem cells by accumulated MMA,[24–27] homocystinemia, and homocystinuria, can be found. Inadequate levels of THF in cells, producing defective rates of polyglutamyl folate synthesis and deficiency of folates required for some intracellular reactions, are observed. Defective thymidylate synthesis, defective synthesis of endogenous purines, defective detoxification of formate, and accumulation of formiminoglutamate, 5(4)-amidoimidazole-4(5) carboxyribonucleotide and other intermediates of folate-dependent reactions are present. Biochemical central nervous system (CNS) changes, which might relate to defective methylation of myelin basic protein, have been reported.[28–30] Finally, intracellular folate levels are lower than expected for the concentration of folate in plasma.[11]

The onset of symptoms is often insidious and may include pallor, apathy, fatigability, and anorexia. These symptoms are not specific for vitamin B_{12} deficiency. A beefy red and sore tongue with papillary atrophy may be observed. Paresthesias may be reported in the older child. Signs of subacute dorsolateral degeneration are uncommon in children, but loss of vibration and position sense may be seen. When neurologic disease is present, the lower limbs are more severely affected. The neurologic changes may precede the appearance of anemia. A number of gastrointestinal symptoms have been reported. The peripheral blood findings and bone marrow findings in vitamin B_{12} deficiency resemble those observed with folic acid deficiency.

Laboratory Diagnosis of B_{12} Deficiency

The general findings secondary to megaloblastic anemia are similar to those indicated above for deficiency of folic acid. Specific measurements for vitamin B_{12} deficiency include decreased serum cobalamin, excess methylmalonic acid in the urine or serum, homocystinemia and sometimes homocystinuria, and abnormal deoxyuridine suppression test in bone marrow, corrected in vitro by addition of MeCbl or 5-formyl-THF but not by methyl-THF.

The serum vitamin B_{12} level is deficient when it is less than 100 pg/mL (normal 200 to 800 pg/mL). If the dietary history indicates a

normal vitamin B_{12} intake, absorption of cobalt-labeled B_{12} should be performed by the Schilling test. For this purpose, a standard dose of the labeled vitamin (0.5 μg) is given orally after an overnight fast, and then a "flushing dose" of 1,000 μg of vitamin B_{12} is given parenterally 2 hours after the oral dose. Less than 7% of the labeled dose will appear in the urine during the 24-hour collection period if there is a lack of intrinsic factor or malabsorption of vitamin B_{12} for any other reason. If absorption is impaired, the Schilling test is repeated with the simultaneous administration of both intrinsic factor and labeled vitamin B_{12}. Improvement in the urinary excretion of the labeled B_{12} confirms the diagnosis of intrinsic factor deficiency. Gastric biopsy, the measurement of gastric acid secretion, and assay of intrinsic factor in gastric juice all help to categorize the nature of the underlying disturbance.

A therapeutic trial may be employed for the diagnosis of vitamin B_{12} deficiency. A dose of 0.5 μg of cyanocobalamin or hydroxycobalamin is given parenterally for 7 to 10 days. A reticulocytosis and increase in hemoglobin concentration should be observed. If the diagnosis has been firmly established, a therapeutic trial may be omitted and the patient may be treated with daily oral doses of 25 to 100 μg. If the patient has any form of B_{12} malabsorption, rather than a primary nutritional deficiency, maintenance therapy should consist of monthly injections of 50 to 1,000 μg, depending on the patient's age and weight. Recently, a nasal gel form of vitamin B_{12} (Questcor Pharmaceuticals, Union City, CA) was approved and has been shown to be safe and effective in treating pernicious anemia.

IRON-DEFICIENCY ANEMIA

Iron deficiency continues to be the most common nutritional cause of anemia worldwide, affecting as many as 4 to 5 billion people.[31] The prevalence of iron deficiency has been declining in industrialized countries over the past few decades, in part, due to an increase in breastfeeding and fortification of infant formulas and cereals.[32] Unfortunately, most of the rest of the world has not experienced such a decline; an estimated 90% of cases occur in developing countries, impacting significantly on morbidity, mortality, and national development.[31]

Iron is a vitally important nutrient that serves multiple functions in the body. It is the functional group in hemoglobin for oxygen transport in the red blood cell and it helps with storage of oxygen in myoglobin in muscle. Iron is also present in peroxidase, catalase, and the cytochromes. Anemia, a treatable condition, is the most common clinical problem associated with iron deficiency. However, the real public health concerns are the cognitive and behavioral impairments seen in infants and children,[33–37] the fatigue and decreased work capability in older children and adults,[33] and the association of severe iron-deficiency anemia in pregnant women with prematurity, perinatal mortality, and low-birth-weight infants.[37–40]

Iron is found in many different food sources; however, the bioavailability is highly variable. Heme iron is more readily absorbed than nonheme iron, as it is absorbed in the intestinal mucosa in the intact protoporphyrin ring. Nonheme iron must be reduced from the ferric state to the ferrous state, which requires a low gastric pH, and its absorption is influenced by many other constituents in the concurrent meal.[41,42] Orange juice facilitates absorption whereas tea hinders it. Other enhancers of iron absorption include fructose, certain amino acids, meat, fish, and poultry. Inhibitors include phosphates, phytates (common in vegetarian diets), tannins, and oxalates.[43,44]

The body, based on its needs, attempts to maintain a balance between iron stores, recycled iron, and dietary iron intake. One-third of an infant's iron needs come from dietary sources; the remainder is recycled from red blood cell breakdown. Over time this changes, so that by adulthood an even greater proportion of iron needs come from recycled iron such that there is less reliance on the diet for iron.[45]

FETAL–MATERNAL RELATIONSHIPS

The iron content of the normal newborn infant is approximately 75 mg/kg as determined by carcass analysis of stillbirths.[46] Studies performed during various stages of pregnancy indicate that the iron content of the fetus and the weight of the fetus increase proportionately with age. Under usual circumstances, 66 to 75% of the infant's iron is present in the red cell mass. Storage iron in the liver and spleen, although subject to great variability, makes up approximately 6% of the total iron while nonhemoglobin iron in the form of cytochromes, myglobin, and other iron-containing enzymes account for approximately 24%.[47]

The bulk of the infant's iron endowment at birth is represented by the red cell mass. As a result, the amount of iron in the body at the time of birth depends on the blood volume and the hemoglobin concentration. Any complications during pregnancy or the perinatal period that result in fetal blood loss will compromise the infant's iron endowment. Unless extreme, the presence of maternal iron deficiency does not appear to compromise the iron endowment of the fetus. The hemoglobin concentration in the cord blood of infants born to anemic iron-deficient mothers does not differ from that of infants born to iron-sufficient mothers until maternal hemoglobin values fall below 6.0 g/dL.[48] With extreme maternal iron-deficiency anemia, the placenta is small and the cord blood hemoglobin concentration is reduced. Some studies have observed that women with low serum iron values tend to have infants with lower than normal serum iron levels[49]; other studies have found no difference in the state of the infant's iron nutrition at 6, 12, and 18 months of age, regardless of whether their mothers had received iron supplementation during pregnancy.[50,51] Employing plasma ferritin concentrations as an index of iron sufficiency, Rios and coworkers were unable to document any correlation between plasma ferritin concentrations in mothers with high and low values and the plasma ferritin concentrations of their infants at birth or at 1.5 months of age.[52] It may be concluded from these studies that, except in the most unusual circumstances, maternal iron deficiency by itself does not result in iron-deficiency anemia in the infant, either at birth or later in the first year of life. Factors such as rate of growth relative to birthweight, initial iron endowment that has been compromised by blood loss, and infant nutrition seem to be far more important in determining the later appearance of anemia.

At birth the newborn has a large number of reticulocytes and a relatively high hemoglobin concentration with a mean of 16.6 g/dL.[53] The hemoglobin increases over the first few days and then, as erythropoiesis slows in the marrow and extramedullary spaces, the hemoglobin falls, reaching a nadir in 6 to 8 weeks. Erythropoiesis is then stimulated and the hemoglobin rises to 12.5 g/dL, which is the mean throughout infancy.[53] Preterm infants have a more dramatic fall in hemoglobin to a mean of about 9 g/dL at the nadir, though the marrow recovery is good, and at 6 months of age these infants have the same mean hemoglobin concentration as a term infant.[54]

Iron Requirements

Iron requirements are age dependent. It is rare for a term infant to become iron deficient before 4 months of age, but a preterm infant can become deficient by 2 to 3 months because of relatively faster growth and smaller iron stores at birth.[53] Children, from birth through adolescence, need iron for growth and increasing blood volume, as well as to replace losses. Although the amounts are small in an infant, averaging 20 μg/kg/d, iron is lost in sweat, urine, bile, desquamation of skin and intestinal cells, and through hair and nail loss.[32] The Committee on Nutrition of the American Academy of Pediatrics made the following recommendation for daily iron requirements for premature infants: (1) breastfed infants, 2 mg/kg/d from 1 month to 12 months of age; (2) formula-fed infants, 1 mg/kg/d through the first year of life.[16] Adequate intake (AI) for infants 0 to 6 months is 0.27 mg/d and the dietary reference intake (DRI) is: 7 to 12 months, 11 mg/d; 1 to 2 years, 7 mg/d; 3 to 8 years, 10 mg/d; 9 to 13 years, 8 mg/d; 14 to 18 years, boys 11 mg/d, girls 15 mg/d; pregnancy 27 mg/d.[55]

Manifestations

With the decline in iron-deficiency anemia in industrialized countries, children with the manifestations of severe longstanding iron-deficiency anemia are rare. Sadly, they are still

seen elsewhere. Mild to moderate iron-deficiency anemia is most common. Frequently the first signs and symptoms of iron-deficiency anemia are pallor, fatigue, exercise intolerance, and occasionally palpitations. The pallor is most notable in the nail beds, conjunctiva, and palms, especially if the individual has dark skin. Fatigue and exercise intolerance have been best documented in studies of adults employed at physical labor revealing that even mild anemia can affect exercise tolerance in settings involving strenuous energy expenditure, and that correction of the anemia increased exercise tolerance.[56–58] The phenomenom of pica or compulsive consumption of nonnutritional items such as dirt, clay, cornstarch, laundry detergent, or ice is a characteristic finding of iron-deficiency anemia. Ingestion of these items often exacerbates the underlying iron deficiency by further impairing absorption.[59]

The recognition that iron deficiency produces a broad array of systemic effects has attracted increasing attention.[45,60] A partial list of nonhematologic manifestations of iron deficiency are provided in Table 3.[61] Over time, glossitis, stomatitis, and angular cheilosis may develop. These symptoms may be the result of tissue iron deficiency rather than the anemia itself.[62] Koilonychia, or spooning of the fingernails, is caused by abnormal proliferation of the cells of the nail bed. Other features of severe longstanding iron-deficiency anemia can actually make treatment with oral iron supplementation difficult. The presence of esophageal webs, or Plummer–Vinson syndrome, impairs swallowing, atrophic gastritis, and abnormal duodenal mucosa can each decrease absorption.

There have been numerous studies investigating immune function associated with iron-deficiency anemia.[33,63–72] Paradoxically, iron overload has also been shown to impair normal immune function, decreasing lymphocyte proliferation and the ability of macrophages to kill intracellular pathogens.[73] Similarly, both iron deficiency and iron overload have been associated with increased infection. In one study, anemia was associated with malaria, acute respiratory infection, and diarrhea.[74] and in other studies, individuals with malaria,[75] hepatitis C virus infection[76] and human immunodeficiency virus infection[77] have worse outcomes if they have excess iron. This suggests that there is a level of optimal body iron with deficiency or overload leading to abnormal immune function.

Iron plays an important role in brain development and many studies have indicated that iron deficiency is associated with cognitive impairment, poor motor development, and behavioral problems.[33–36,78–80] This is particularly disturbing as these problems may continue long term, even after correction of the anemia. However, it should be noted that a causal relationship has not been established as important potential confounding variables have not been fully accounted for. It is also not clear that CNS effects can be remedied by iron therapy, as many of the clinical trials have failed to demonstrate benefit from therapy.[81,82]

Iron is essential to the metabolism of certain amine neurotransmitters.[33] Children with iron-deficiency anemia have increased catecholamines in their urine which return to normal levels after iron therapy.[83] There have been animal studies suggesting abnormal dopamine receptor function in association with iron-deficiency anemia.[84] The exact meaning of these findings and how they relate to cognitive and behavioral development remains unclear.

Table 3 Nonhematologic Manifestations of Iron Deficiency

1. Impaired growth
2. Skin and mucous membranes
   (a) Koilonychia
   (b) Angular stomatitis
   (c) Glossitis
3. Gastrointestinal tract
   (a) Anorexia
   (b) Dysphagia with postcricoid webs
   (c) Gastric achlorhydria
   (d) Malabsorption
   (e) Beeturia
   (f) Exudative enteropathy and occult bleeding
4. Central nervous system
   (a) Irritability
   (b) Decreased attention span
   (c) Poor performance in standardized developmental testing
   (d) Breath-holding spells
5. Impaired exercise tolerance
6. Immunologic response
   (a) Impaired lymphocyte mitogen response
   (b) Decreased leukocyte killing

Laboratory Findings

The diagnosis of iron-deficiency anemia is rarely made based on a single laboratory test, but rather on a combination of several tests and the comparison to age-specific norms reflecting the changes that occur as a child grows. Commonly used tests assess the iron storage, erythropoiesis, and severity of the anemia (Table 4). Together these tests can help to rule out other causes of anemia.

Iron deficiency occurs in various stages. First, there is depletion of iron stores in the body. This stage is unlikely to be diagnosed by any routine screening laboratory tests. Next, there is iron deficiency with anemia, initially with a normal MCV. As the deficiency continues, erythropoiesis is significantly impaired, and the diagnosis becomes more evident with obvious hypochromia and microcytosis on peripheral blood smear, in conjunction with some of the nonhematologic manifestations of this condition.

Peripheral Smear. Often microscopic examination of the blood smear can be normal early in iron deficiency, but as the anemia progresses hypochromia, microcytosis, anisocytosis, and occasional target cells and elliptocytes are seen.

Hemoglobin. Anemia starts when this value is greater than 2 standard deviations below the normal mean for age. Thus it is essential to use age-based mean hemoglobin values for accurate interpretation of these results.

Table 4 Laboratory Measurements of Iron Status

1. Storage iron depletion
   (a) Bone marrow iron stain (decreased)
   (b) Serum ferritin (decreased)
   (c) Serum transferrin (increased)
   (d) Transferrin receptor (increased)
   (e) Serum iron (decreased)*
2. Iron deficiency erythropoiesis
   (a) Transferrin saturation (decreased)
   (b) Total iron-binding capacity (increased)
   (c) Free erythrocyte protoporphyrin (increased)
   (d) Zinc protoporphyrin (increased)
   (e) Red cell mean corpuscular volume (decreased)
   (f) Red cell mean corpuscular hemoglobin (decreased)
   (g) Red cell distribution width (increased)
   (h) Reticulocyte heme (decreased)
3. Iron deficiency anemia
   (a) Hemoglobin (decreased)
   (b) Therapeutic trial with increase in hemoglobin concentration

*See text.

Red Cell Distribution Width. This rarely used index may provide early evidence for iron-deficiency anemia.[85] It represents the variation in size of the red blood cells. In thalassemia, infection, and inflammation, it is usually normal, but in iron-deficiency anemia this value is elevated and can be >20%.[45]

MCV. This value represents the average size of the red blood cell and it varies with age. When anemia becomes significant enough to interfere with hemoglobin synthesis microcytosis occurs (low MCV). This is one of the red cell indices readily available in the routine complete blood count (CBC) from an electronic counter in the laboratory.

MCH. As with the MCV, this index is available from an electronic counter. Initially with iron deficiency the MCH is normal, but with advancing anemia the red blood cell becomes more hypochromic and this change is evident by a low MCH.

Serum Ferritin. Ferritin, the primary intracellular iron storage protein, is also found in small amounts in the circulation. Serum ferritin generally correlates with total body iron although, as an acute phase reactant, ferritin may be increased by inflammatory, infectious, and malignant processes, and this correlation becomes less reliable. In contrast, a few conditions, including vitamin C deficiency, reduced serum ferritin, and scurvy, or vitamin C deficiency, should be evaluated as a potential cause of low ferritin. In general, values less than 10 μg/L are indicative of iron deficiency.

Serum Iron. Serum iron has not been a reliable test for iron-deficiency anemia because of its diurnal pattern with a peak in the morning and a trough in the evening. As with serum ferritin it can be elevated by many other conditions. The concentration is usually low in iron-deficiency anemia. However, even a single dose of iron

within the 24 hours prior to the test can cause a transient elevation in the serum iron level, thus possibly masking an underlying iron-deficiency anemia.[62]

Total Iron-Binding Capacity (TIBC) and Transferrin Saturation (TS). Most circulating iron is bound to a plasma protein called transferrin. TIBC is a measure of the total transferrin. As the serum iron decreases, the TIBC increases, and the ratio of these variables in iron-deficiency anemia is less than 1:6.[85] TS is the ratio of serum iron to TIBC multiplied by 100 to yield a percentage. It represents the percent of transferrin that is saturated with iron. A low percentage is suggestive of iron deficiency.

Soluble Transferrin Receptor (sTfR). This receptor can be measured in the circulation and is indicative of the concentration of cellular transferrin receptor. Since receptor synthesis increases with iron deficiency but not with anemia of chronic disease, it is a sensitive indicator of iron deficiency.[86] Because sTfR is also elevated in the presence of ineffective erythropoiesis and sideroblastic anemias, this must be taken into account and evaluated appropriately.

Erythrocyte Protoporphyrin (EP). Under normal circumstances iron combines with protoporphryin in the red blood cell to form heme, a reaction catalyzed by the enzyme ferrochelatase. In iron-deficiency anemia there is an elevation in EP, usually >35 μg/dL.[87] Lead poisoning, which often causes a higher level of EP than iron deficiency, does so by inhibiting ferrochelatase. Inflammatory, infectious, and/or malignant processes also can raise the EP level.[62]

Zinc Protoporphyrin (ZPP). In iron deficiency and lead poisoning, ZPP is formed instead of EP because zinc fills the iron pocket in the protoporphyrin molecule. This is a sensitive, inexpensive test that will show an elevation in iron deficiency before anemia is present.[88] When iron stores are sufficient, the ratio of iron to zinc incorporation into protoporphyrin is 30,000 to 1.[89] Iron deficiency produces an increase in the normal ratio of $\leq$40 umol ZPP/mol heme.[90] This test alone does not differentiate between iron deficiency and lead poisoning, and so further tests must be done. The issue is complicated because iron deficiency increases the absorption of lead so that in many situations the two disturbances exist together. A lead level and a therapeutic trial of oral iron therapy may be necessary to clarify the diagnosis.

Reticulocyte Heme. Reticulocyte hemoglobin content (CHr) measurement by automated flow cytometry assesses the iron status of red blood cells when they are released from the bone marrow as reticulocytes.[91] It can be used to detect early iron deficiency and has been found to be the strongest predictor of iron deficiency when compared to other commonly used laboratory parameters.[92]

Bone Marrow Biopsy. If the usual tests are equivocal or there are confounding factors making the diagnosis of iron deficiency difficult, such as infection or inflammation, the bone marrow iron stores can be assessed. Staining the specimen with Prussian blue and counterstaining with safranin O helps to show the characteristic paucity of ferritin and hemosiderin, the final breakdown product of iron and ferritin.[54] Normally 10 to 20% of red cell precursors in the marrow contain iron granules; however, in iron deficiency few if any will be present.[93]

Therapy

If iron deficiency is diagnosed or suspected based on some or all of the laboratory tests discussed above, treatment with iron should begin immediately. In most circumstances, oral iron supplementation is used since it is inexpensive and easily absorbed. The dose of iron for infants and children is 4 to 6 mg/kg of elemental iron, depending on the severity of the anemia, divided into two to three doses per day. For adolescents the dose is 100 to 300 mg of elemental iron per day.[94] Traditionally, 3-times-daily dosing has been used but recent research has demonstrated that once-daily dosing results in similar treatment of anemia,[94] and once- or twice-weekly dosing results in similar improvement in hemoglobin but not ferritin.[96] Further work in this area is ongoing, in an attempt to reduce side effects, simplify treatment, and improve compliance.

Ferrous iron is more easily absorbed than ferric iron, and thus the usual treatment for infants and children is ferrous sulfate. Premature infants are frequently vitamin E deficient due to decreased intake, decreased stores, and poor absorption of vitamin E. Since iron therapy inhibits absorption of vitamin E, this deficiency must be corrected before iron therapy is started.[97] The absorption of iron on an empty stomach is about twice that of a full stomach; therefore it is recommended that the dose be given about an hour prior to a meal.

The duration of treatment is 2 to 4 months after the hemoglobin has returned to a level normal for age, in order to replenish the iron stores.[45] If these stores are not replenished because iron therapy is discontinued too soon, a rapid recurrence of iron-deficiency anemia may result. Response to treatment is initially evident by a reticulocytosis peaking in 5 to 10 days from the onset of treatment. During the first week of therapy, the hemoglobin rises about 0.25 to 4 g/dL/d and then slows to about 0.1 g/dL/d.[94] It is important to follow up no more than a month later to see if there is improvement, because if there is no change in that period of time on an adequate dose, and compliance is not an issue, further studies must be done to determine the underlying problem.[45] Failure of oral iron therapy can be the result of impaired absorption, incorrect diagnosis, ongoing blood loss greater than hemoglobin generation, inadequate dose, ineffective iron preparation, superimposed malignancy or inflammatory disease, or, most commonly, simple noncompliance. Compliance can be an issue because of the taste of iron, gastrointestinal distress, or concern of parents that the drops will stain the infant's teeth. These problems can be dealt with by giving the iron with a small amount of food or liquid, preferably something that will enhance the absorption, and by giving the drops in the back of the mouth. If noncompliance is suspected but denied, the stool can be examined. In the presence of iron supplements, it should be black. The stool can also be tested specifically for iron if necessary.

Parenteral iron therapy has greater side effects and is therefore reserved for only specific circumstances. These include true intolerance to oral iron (extremely rare), severe gastrointestinal disease that prevents absorption and may be exacerbated by oral iron therapy, chronic bleeding, or repeated noncompliance.[98,99] There are three parenteral preparations available in the United States: iron dextran, iron sucrose, and iron gluconate, and the maximum dose that can be given at once varies among them.[100] The dose is calculated to correct the anemia and replenish the iron stores. The formula is:

$$\text{Normal Hgb} - \text{Initial Hgb} \times \text{blood volume} \times 3.4 \times 1.5 = \text{mg iron } 100$$

The normal hemoglobin should be based on the age. Blood volume is approximately 80 mL/kg of body weight. The value 3.4 converts the grams of hemoglobin to milligrams of iron, and the factor 1.5 provides a little extra iron to supply the iron stores.[98,99]

A small portion of patients have an anaphylactic reaction to dextran-containing iron preparations, and this should be considered when choosing which preparation to use. Whenever iron dextran treatment is used, a test dose must first be given, and if there is no sign of reaction 5 minutes after the test dose, the therapeutic dose may be started. Epinephrine should be kept at the bedside, as well as resuscitation equipment. Less severe side effects are hypotension and flushing (2%), phlebitis (10%), and a delayed rash with or without arthritis (20%).[98,99]

In extreme iron-deficiency anemia with a very low hemoglobin and/or cardiovascular instability, a packed red blood cell transfusion is given first to stabilize the patient. This is usually followed by oral iron therapy. In this situation the transfusion is given slowly, at 5 cc/kg over 4 hours to start. Depending on how much more blood is necessary to increase the hemoglobin to the desired level, the rest of the transfusion can be broken into aliquots and given slowly or at the usual rate for a transfusion.[94]

OTHER VITAMIN AND METAL DEFICIENCY-RELATED ANEMIAS

Vitamin A Deficiency

Chronic vitamin A deficiency results in an anemia with certain characteristics similar to those observed in iron deficiency. Mean red cell volume and MCHC are reduced, anisocytosis and poikilocytosis may be present, and serum iron values are low.[101–104] Unlike iron deficiency, however, liver and marrow iron stores are increased, serum ferritin values are normal, serum transferrin

concentration is usually normal or decreased, and the administration of therapeutic doses of iron may not correct the anemia. These findings suggest that this hypochromic, microcytic anemia may be related to a failure of iron transport from storage sites to active metabolic pools.

Several large pediatric studies conducted in developing countries support a relationship between serum concentration of vitamin A and blood hemoglobin concentration.[104–108] This relationship was confirmed in experimental studies showing a correlation between plasma retinol and hemoglobin in adult men with induced vitamin A deficiency.[102] Finally, studies in underdeveloped countries have shown that treating anemia with vitamin A in combination with iron is more effective than iron alone, again supporting a causal relationship.[102,109–112]

Pyridoxine Deficiency

The vitamin B_6 group includes pyridoxal, pyridoxine, and pyridoxamine. These substances are all phosphorylated to pyridoxal-5-phosphate (PLP), which is an essential cofactor in heme biosynthesis mediated by D-aminolevulinic acid synthetase.[113,114] Limited animal and human studies support a relationship between pyridoxine deficiency and anemia. In animals with experimentally induced pyridoxine deficiency, Bottomly and colleagues observed anemia and ringed sideroblasts.[115] Experimental induction of vitamin B_6 deficiency in two infants was associated with a hypochromic, microcytic anemia,[116] and a malnourished patient has been described whose hypochromic anemia failed to respond to iron therapy but subsequently responded to the administration of vitamin B_6.[117]

Nutritional deficiencies of pyridoxine are extremely rare, although patients receiving therapy with antituberculosis drugs such as isoniazid, which interferes with the transport of pyridoxine to cells,[115,118,119] can develop a microcytic anemia. This anemia was corrected with large doses of pyridoxine.[120,121] A small percentage of patients with congenital or acquired sideroblastic anemia improve on treatment with pharmacologic doses of pyridoxine.[122,123] There are also reports of sideroblastic anemia due to vitamin B_6 deficiency occurring in patients on hemodialysis.[113] Administration of pyridoxine with isoniazid or during dialysis may decrease the risk of these complications.

Riboflavin Deficiency

While anemia secondary to riboflavin deficiency has been demonstrated in several animal models, it has been difficult to establish a direct causal role in humans.[124] The best evidence has been provided by experimental studies. Human volunteers with malignancies maintained on a riboflavin-deficient diet and fed the riboflavin antagonist, galactoflavin, developed pure red cell aplasia.[125] Vacuolated erythroid precursors were evident prior to the development of aplasia. Red cell indices were normal and the reticulocyte count was low. This anemia was corrected by the administration of riboflavin. Confirmation of this response to riboflavin supplementation has been seen in both human adult and pediatric populations with anemia.[126,127]

Riboflavin-deficient children given riboflavin demonstrated a decrease in serum iron and TS accompanied by an increase in hemoglobin if the initial hemoglobin was below 13.5 g/mL, supporting a relationship between riboflavin and iron utilization.[127] Abnormal iron utilization as a possible mechanism for riboflavin-induced anemia was explored in rodent studies that have shown that riboflavin deficiency was associated with reduced iron absorption and increased iron loss.[128,129] In addition, ferrokinetic studies in humans receiving the riboflavin-deficient diet and galactoflavin demonstrated distribution of iron primarily to liver and spleen in contrast to bone marrow in normals. Clearly, riboflavin has an important role in erythropoiesis, probably by altering iron metabolism.

Riboflavin deficiency has been shown to produce a decrease in red cell glutathione reductase,[130,131] which is necessary for glutathione peroxidase to effectively regulate cellular redox potential.[132,133] Under normal conditions, the decrease in glutathione reductase activity is not associated with an increased sensitivity of the red cell to oxidant-induced injury.[134] It is theoretically possible that riboflavin deficiency in conjunction with increased oxidant stress leaves the red cell vulnerable to oxidation injury, causing hemolytic anemia; however, this has not been described in humans.

Vitamin C (Ascorbic Acid) Deficiency

It is unclear whether ascorbic acid has a direct role in hematopoiesis or whether the anemia observed in subjects with ascorbic acid deficiency (scurvy) is a result of the interactions of ascorbic acid with folic acid and iron metabolism. Although approximately 80% of individuals with scurvy are anemic,[135] attempts to induce anemia in human volunteers by severe restriction of dietary ascorbic acid have been unsuccessful.[136,137] Confusion arises because the anemia observed in subjects with scurvy may be hypochromic, normocytic, or macrocytic; and the marrow may be hypocellular, normocellular, or hypercellular. In about 10% of patients the marrow is megaloblastic.[135]

Ascorbic acid is required to maintain folic acid reductase in its reduced, or active form. Impaired folic acid reductase activity results in an inability to form tetrahydrofolic acid, the metabolically active form of folic acid. It has been demonstrated that patients with scurvy and megaloblastic anemia excrete 10-formylfolic acid as the major urinary folate metabolite. Following ascorbic acid therapy, 5-methyltetrahydrofolic acid becomes the major urinary folate metabolite in these patients. This observation has led to the suggestion that ascorbic acid prevents the irreversible oxidation of methyltetrahydrofolic acid to formylfolic acid.[138] Failure to synthesize tetrahydrofolic acid or to protect it from oxidation in scurvy ultimately results in the appearance of a megaloblastic anemia. Under these circumstances, ascorbic acid therapy will produce a hematologic response only if sufficient folic acid is present to interact with the ascorbic acid.[139]

Iron deficiency in children often occurs in association with ascorbic acid deficiency. Scurvy may cause iron deficiency as a consequence of external bleeding. Iron balance may be further compromised by ascorbic acid deficiency because this vitamin facilitates intestinal iron absorption.[140] Ascorbic acid increases the bioavailability of iron through two mechanisms. First, it can reduce Fe^{3+} to Fe^{2+}, which is more soluble and less likely to form insoluble hydroxides at low pH than Fe^{3+}. Second, it forms a stable complex with iron, preventing iron from complexing with dietary constituents, such as tannins and phytates, which block transport into gastrointestinal mucosal cells.[141] Patients with scurvy, particularly children, often require both iron and ascorbic acid to correct a hypochromic, microcytic anemia.[142] Rarely, a combination of iron, ascorbic acid, and folate may be required.[143]

Despite the evidence linking ascorbic acid deficiency to alterations in either folic acid or iron metabolism, many patients with scurvy have a normocytic, normochromic anemia accompanied by a persistent reticulocytosis of 5 to 10%. Ascorbic acid has antioxidant properties, and it is possible that a deficiency of this vitamin renders the cell susceptible to oxidant injury. This is of particular interest in regard to the possible reducing effects of ascorbic acid on oxidized vitamin E, much like those described above for folic acid.[144] Administration of ascorbic acid to patients with scurvy who have hemolysis produces an initial increase in reticulocyte count, followed by a rise in hemoglobin concentration and an ultimate correction of hematologic abnormalities.[145]

Vitamin E Deficiency

The human fetus at 5 months gestation has a total body vitamin E content of only 1 mg.[146] A term newborn has a body vitamin E content of 20 mg. The normal adult male averages 50 mg/kg, and the female averages 160 mg/kg. This difference in ratio of body vitamin E to body weight presumably reflects differences in body fat composition. The ratio of vitamin E to fat remains relatively constant throughout gestation and averages 0.27 mg of vitamin E per gram of lipid; ratios of 0.24:1.00 have been described for adult males and 0.46:1.00 for adult females.[147,148]

Plasma vitamin E levels average close to 0.9 mg per 100 mL in the mother at term, with a corresponding value of only 0.2 mg per 100 mL in the infant.[149] Although there is a direct relationship between the plasma level of vitamin E in the infant at birth and that of the mother, infants are not born with values above 0.6 mg per 100 mL, the value regarded as the lower limit of normal for older children and adults. When

vitamin E concentrations are expressed as vitamin E-lipid ratios, perhaps a more nutritionally valid means of determining vitamin E deficiency, differences between mothers and their infants disappear. The vitamin E-total lipid ratio of mothers at term has been found to average 1.57:1.00, with a ratio of 2.40:1.00 in cord blood.[150]

Infants, both term and preterm, who are fed colostrum and human milk achieve normal plasma vitamin E concentrations by 1 week of age.[3,151] Most of the simulated human milk formulas presently available will produce vitamin E concentrations within the normal adult range when fed to infants during the first 2 weeks of life. The relationship between iron content and vitamin E in the diet may determine the turnover of vitamin E as iron-mediated reactions stimulate lipid peroxidation and increase the requirements for vitamin E.

Hemolytic Anemia and the Premature Infant

Prior to 1967, despite the fact that it was recognized that most proprietary formulas fed to low-birth-weight infants resulted in a prolonged period of vitamin E deficiency, no hematologic abnormalities could be demonstrated.[152,153] In 1967, the presence of a hemolytic anemia that could be corrected by vitamin E or prevented by maintaining vitamin E sufficiency from early infancy was described.[154]

This syndrome was seen almost exclusively in infants with birth weights of less than 1,500 g and was most pronounced during the period of 6 to 10 weeks of life. The hematologic findings consisted of anemia with hemoglobin of 6 to 8 g/dL, reticulocytosis of 4 to 5% or greater, and thrombocytosis. Red cell morphologic changes included the presence of anisocytosis, poikilocytosis, red cell fragments, and irregularly contracted erythrocytes and spherocytes. The hydrogen peroxide hemolysis test was abnormal, and the red cell life span was reduced.[154,155] These findings were all consistent with alterations in the membrane due to oxidant injury. Treatment with vitamin E, in total doses ranging from 200 to 1,000 mg, produced a prompt rise in hemoglobin, a reduction in the reticulocyte count, and a gradual decline in the platelet count to the normal range. In addition to the hematologic abnormalities, many of the small infants also displayed edema of the lower legs and scrotum, watery nasal discharge, and, on occasion, tachypnea. The mechanisms responsible for these changes are not known.

Melhorn and Gross studied infants of less than 36 weeks gestation receiving a commercial formula and found that, at 6 to 10 weeks of age, infants who had been supplemented with 25 IU of vitamin E per day from Day 8 of life had significantly higher hemoglobin concentrations and lower reticulocyte counts and hydrogen peroxide tests than the unsupplemented infants.[156] It was also found that the daily administration of ferrous sulfate, 10 to 12 mg/kg/d from Day 8, exaggerated the hemolytic anemia in the infants not receiving the vitamin E supplement. Williams and coworkers demonstrated that hemolytic anemia due to vitamin E deficiency occurred in infants receiving iron-fortified formulas only if the formula was unusually rich in polyunsaturated fats.[157]

It has been hypothesized that hemolysis occurs in vitamin E deficiency as a consequence of peroxidation of lipid components of the red cell membrane. The peroxidation is initiated by the generation of a free radical (an unpaired electron) and proceeds autocatalytically in the absence of an antioxidant. Iron, in the reduced state, particularly in the presence of ascorbic acid, is recognized to generate free radicals. The requirements for lipid peroxidation include the presence of a suitable substrate (eg, a long-chain unsaturated fatty acid), a lack of antioxidant (eg, vitamin E), and a source of a free radical generating system (eg, heavy metals, molecular oxygen). Most proprietary formulas have now reduced their polyunsaturated fatty acid (PUFA) content and increased their vitamin E concentration, resulting in vitamin E-PUFA ratios in excess of 0.6:1.00, a value generally regarded as sufficient to prevent the development of vitamin E deficiency.[158] The potential for the development of vitamin E deficiency anemia is present when infants receive intravenous lipid preparations without adequate vitamin E supplementation.

The diagnosis should be suspected in an infant who displays anemia in the presence of persistent reticulocytosis, nonspecific red cell morphologic abnormalities, and thrombocytosis. Confirmation of the diagnosis requires: (1) evidence of a reduced plasma vitamin E concentration ($<$0.5 mg/dL) or a reduced vitamin E to a total lipid ratio ($<$0.6); (2) an abnormal hydrogen peroxide hemolysis test (usually $>$30% hemolysis with a normal laboratory value of $<$10%); (3) an increase in hemoglobin and a fall in reticulocyte count after vitamin E therapy. Response should be apparent within 10 days, provided correction of criteria 1 and 2 has occurred.

Miscellaneous Conditions

Patients with various forms of malabsorption will become vitamin E deficient. These include patients with exocrine pancreatic insufficiency (cystic fibrosis), congential biliary atresia, abetalipoproteinemia, and extensive small bowel resections. In patients with reduced concentrations of plasma vitamin E, the red cell half-life is usually shortened,[159,160] although anemia is usually not evident or, when present, is usually not correctable by the administration of vitamin E alone. The relative anemia observed in most patients with chronic lung disease with cystic fibrosis will not respond to iron alone but may improve when iron and vitamin E are given concurrently.[161]

Copper Deficiency

Copper is present in a number of metalloproteins. Among the cuproenzymes are cytochrome c oxidase, dopamine-β-hydroxylase, urate oxidase, tyrosine and lysyl oxidase, ascorbic acid oxidase, and superoxide dismutase (erythrocuprein). More than 90% of the copper in the blood is carried bound to ceruloplasmin, an α_2-globulin with ferroxidase activity.

Copper appears to be required for the absorption and utilization of iron. It has been proposed that copper, in the form of ferroxidases, converts and maintains iron in the Fe^{3+} state for its transfer by transferrin.[162] Decreased absorption of iron and anemia have been demonstrated in rats with induced dietary copper deficiency.[107]

Copper deficiency has been described in malnourished children[163] and in both infants[164] and adults[165] receiving parenteral alimentation. Copper deficiency in humans is characterized by (1) a microcytic anemia that is unresponsive to iron therapy, (2) hypoferremia, (3) neutropenia, and usually (4) the presence of vacuolated erythroid precursors in the marrow. In infants and young children with copper deficiency, radiologic abnormalities are generally present. These abnormalities include osteoporosis, flaring of the anterior ribs with spontaneous rib fractures, cupping and flaring of long bone metaphyses with spur formation and submetaphyseal fractures, and epiphyseal separation. These radiologic changes have frequently been misinterpreted as signs of scurvy.

The diagnosis of copper deficiency can be established by the demonstration of a low serum ceruloplasm or serum copper level. Adequate normal values for the first 2 to 3 months have not been well defined and are normally lower than those observed later in life. Despite these limitations, a serum copper level of less than 40 μg/dL or a ceruloplasmin value of less than 15 mg/dL after 1 or 2 months of age can be regarded as evidence of copper deficiency. In later infancy, childhood, and adulthood, serum copper values should normally exceed 70 μg/dL.

Preterm infants are at risk due to their lower hepatic copper stores which largely accumulate in the third trimester.[166] Low serum copper values may be observed in hypoproteinemic states, such as exudative enteropathies and nephrosis, as well as in Wilson's disease. In these circumstances, a diagnosis of copper deficiency cannot be established by serum measurements alone, but instead requires analysis of liver copper content or clinical response after a therapeutic trial of copper supplementation. Dietary zinc decreases copper absorption and may lead to copper deficiency and anemia.[167,168]

The anemia does not respond to iron but is quickly corrected by administration of copper. Therapy in a dose of 0.2 mg/kg of body weight will cause a prompt reticulocytosis and rise in the leukocyte count. This can be given as a 10% solution of copper sulfate ($CuSO_4 \cdot 5H_2O$), which contains about 25 mg of copper per milliliter.

The daily requirement of copper is 0.1 to 1 mg for infants and 1.0 to 5.0 mg for children and adolescents. Copper intake in infants is usually low since breast milk contains only 0.2 to 0.4 mg

copper per liter and infant formulas are generally fortified to 0.4 to 0.6 mg/L; however, copper deficiency is rarely seen in healthy term infants. Older infants and children tend to have increased copper intake as cereals and other foods provide more copper than milk.[16]

NUTRITIONAL STATES CAUSING ANEMIAS

Anemia of Starvation

Studies conducted during World War II with conscientious objectors demonstrate that semistarvation for 24 weeks produces a mild to moderate normocytic, normochromic anemia.[169] Marrow cellularity was usually reduced and was accompanied by a decrease in the erythroid-myeloid ratio. Measurements of red cell mass and plasma volume suggested that dilution was a major factor responsible for the reduction in hemoglobin concentration. In persons subjected to complete starvation, either for experimental purposes or to treat severe obesity, anemia was not observed during the first 2 to 9 weeks of fasting.[170] Starvation for 9 to 17 weeks produced a fall in hemoglobin and marrow hypocellularity.[171] Resumption of a normal diet was accompanied by a reticulocytosis and disappearance of anemia. It has been suggested that the anemia of starvation is a response to a hypometabolic state with its attendant decrease in oxygen requirements.

Anemia of Protein Deficiency (Kwashiorkor)

Most cases of Kwashiorkor occur in developing countries as a result of malnutrition and poverty. In the United States, though rare, Kwashiorkor may be observed in individuals consuming restricted diets.[172] The mechanisms for anemia in Kwashiorkor have been examined in several laboratories. Protein deficiency leads to a reduction in oxygen consumption and erythropoietin production, with a subsequent drop in erythropoiesis and reticulocyte count.[173] Red cell maturation is blocked at the erythroblast level and the erythropoietin-sensitive stem cell pool is slightly decreased.[174]

In infants and children with protein-calorie malnutrition, the hemoglobin concentration may fall to 8 g/dL of blood,[175,176] but some children with kwashiorkor are admitted to the hospital with normal hemoglobin levels, probably due to a decreased plasma volume. The anemia is normocytic and normochromic, but there is a considerable variation in size and shape of the red cells on the blood film. The white blood cells and the platelets are usually normal. The marrow is most often normally cellular or slightly hypocellular, with a reduced erythroid-myeloid ratio. Erythroblastopenia, reticulocytopenia, and a marrow containing a few giant pronormoblasts may be found, particularly if these children have an infection. With treatment of the infection, erythroid precursors may appear in the marrow, and the reticulocyte count may rise. When nutrition is improved by feeding high-protein diets (powdered milk or essential amino acids), there is reticulocytosis, a slight fall in hematocrit due to hemodilution, and then a rise in hemoglobin, hematocrit, and red blood cell count. Improvement is slow, however, and during the third or fourth week, when the children are clinically improved and the serum proteins are approaching normal, another episode of erythroid marrow aplasia devoid of giant pronormoblasts may develop. This relapse is not associated with infection, does not respond to antibiotics, and does not remit spontaneously. It does respond to either riboflavin or prednisone, and unless treated with these agents, children who develop this complication may die suddenly. It has been suggested that the erythroblastic aplasia is a manifestation of riboflavin deficiency.[177]

Although the plasma volume is reduced to a variable degree in children with kwashiorkor, the total circulating red cell mass decreases in proportion to the decrease in lean body mass as protein deprivation reduces metabolic demands.[176] During repletion, an increase in plasma volume may occur before an increase in red cell mass, and the anemia may seem to become more severe, despite reticulocytosis. The erythropoietin level increases as the hemoglobin concentration falls[178] and, more important, as oxygen demand increases. The increased oxygen demand may in part account for the reticulocytosis. Also, during the repletion period, occult deficiencies of iron, folic acid, and occasionally of riboflavin, vitamin E, and vitamin B_{12} may become manifest unless these essential nutrients are supplied in adequate amounts.

Anemia and Obesity

The prevalence of obesity has been increasing dramatically in developed countries, particularly over the past two decades.[179] Children who are obese or overweight may not consume more calories than nonobese children,[180,181] and yet they may have inadequate micronutrient intake despite adequate or excessive energy intake.[182] One study of an overweight population found that intake of saturated fat, calcium, fruits, and vegetables was below recommended amounts.[183] Overweight or obese children have been shown to have lower levels of vitamin B_{12},[184] an increased prevalence of iron deficiency,[185] and a greater likelihood of iron-deficiency anemia than children of normal weight.[186] These findings suggest that children who are overweight or obese may be at risk for multiple micronutrient deficiencies that may lead to anemia.

REFERENCES

1. Oski, Brugnara, Nathan. A diagnostic approach to the anemic patient In: Nathan, Orkin, Ginsburg, Look, editors. Nathan and Oski's Hematology of Infancy and Childhood. Philadelphia, PA: Saunders; 2003.
2. Oski Pallor. In: Kay, Oski, Barness, editors. Core Textbook of Pediatrics. Philadelphia, PA: Lippincott; 1989.
3. Karnath BM. Anemia in the adult patient. Hosp Physician. 2004;32–6.
4. Herbert V. Folic acid. In: Shils ME, Olson JA, Shike M, et al., editors. Modern Nutrition in Health and Disease, 9th edition. Philadelphia, PA: Lippincott Williams & Wilkins; 1999.p. 433–46.
5. Herbert V, Zalusky R. Interrelations of vitamin B_{12} and folic acid metabolism: Folic acid clearance studies. J Clin Invest 1962;41:1263.
6. Baumgartner ER, Stokstad ELR, Wick H, et al. Comparison of folic acid coenzyme distribution patterns in patients with mehtylenetetrahydrofolate reductase and methionine synthetase deficiencies. Pediatr Res 1985;19:1288–92.
7. Hoffbrand AV, Jackson BFA. Correction of the DNA synthesis defect in vitamin B_{12} deficiency by tetrahydrofolate: Evidence in favour of the methyl-folate trap hypothesis as the cause of megaloblastic anaemia in vitamin B_{12} deficiency. Br J Haematol 1993;83:642–47.
8. Gafter-Gvili A, Prokocimer M, Breuer W, et al. Non-transferrin-bound serum iron (NTBI) in megaloblastic anemia: Effect of vitamin B_{12} treatment. Hematol J 2004;5:32–34.
9. Finch CA. Erythrokinetics in pernicious anemia. Blood 1956;9:807.
10. Herbert V. Megaloblastic anemia: Mechanism and management. Disease-A-Month. Chicago, IL: Year Book; 1965.
11. Cooper BA, Rosenblatt DS. Disorders of cobalamin and folic acid metabolism. In: Handin RJ, Lux SE, Stossel TP, editors. Blood: Principles and Practice of Hematology. Philadelphia, PA: J.B. Lippincott; 1995.
12. Antony A. Megaloblastic anemias In: Hoffman R, et al., editors. Hematology: Basic Principles and Practice. New York: Churchill Livingstone; 1991.
13. Lanzkowsky P. Megaloblastic anemias and other nutritional anemias. In: Miller DR, et al., editors.Blood Diseases of Infancy and Childhood. St. Louis, MO: Mosby; 1978.
14. Streiff RR. Disorders of folate metabolism. Dis Mon 1972; 1–32.
15. Baker H, Frank O, Thomson AD, et al. Vitamin profile of 174 mothers and newborns at parturition. Am J Clin Nutr 1975;28:59–65.
16. American Academy of Pediatrics. Pediatric Nutrition Handbook, 5th edition. American Academy of Pediatrics, 2004.
17. Hoffbrand AV, Newcomb B, Mollin D. Method of assay of red cell folate activity and the value of the assay as a test for folate deficiency. J Clin Pathol 1966;19:17–28.
18. Rosenblatt DS, Fenton WA. Inherited disorders of folate and cobalamin transport and metabolism. In: Scriver CR, Beaudet AL, Sly WS, et al., editors. The Metabolic and Molecular Bases of Inherited Disease, 8th edition. New York: McGraw-Hill; 2001.
19. Baker SJ, et al. Vitamin B_{12} deficiency in pregnancy and the puerperium. Br Med J 1962;1:1658.
20. Rappazzo ME, Salmi HA, Hall CA. The content of vitamin B_{12} in adult and foetal tissue: A comparative study. Br J Haematol 1970;18:425–33.
21. Jadhav M, et al. Vitamin B_{12} deficiency in Indian infants. Lancet 1962;2:903.
22. Lampkin BC, Shore NA, Chadwick D. Megaloblastic anemia of infancy secondary to maternal pernicious anemia. N Engl J Med 1966;274:1168–71.
23. Higginbottom MC, Sweetman L, Nyhan WL. A syndrome of methylmalonic aciduria, homocystinuria, megaloblastic anemia and neurologic abnormalities in a vitamin B_{12}-deficient breast-fed infant of a strict vegetarian. N Engl J Med 1978;299:317–23.
24. Coulombe JT, Shih VE, Levy HL. Massachusetts metabolic disorders screening program. II. Methylmalonic aciduria. Pediatrics 1981;67:26–31.
25. Halperin ML, Schiller CM, Fritz IB. The inhibition by methylmalonic acid of malate transport by the dicarboxylate carrier in rat liver mitochondria. A possible explantation for hypoglycemia in methylmalonic aciduria. J Clin Invest 1971;50:2276–82.
26. Montgomery JA, Mamer OA, Scriver CR. Metabolism of methylmalonic acid in rats. Is methylmalonyl-coenzyme a racemase deficiency symptomatic in man? J Clin Invest 1983;72:1937–47.
27. Rosenberg LE. The inherited methylmalonic acidemias. Prog Clin Biol Res 1982;103:187–209.
28. Deacon R, Purkiss P, Green R, et al. Vitamin B_{12} neuropathy is not due to failure to methylate myelin basic protein. J Neurol Sci 1986;72:113–17.
29. van Tonder SV, Ruck A, van der WJ, et al. Dissociation of methionine synthetase (EC 2.1.1.13) activity and impairment of DNA synthesis in fruit bats (Rousettus aegyptiacus) with nitrous oxide-induced vitamin B_{12} deficiency. Br J Nutr 1986;55:187–92.
30. Young PR, Waickus CM. Time dependence of the methylation of myelin basic protein from bovine brain; Evidence for protein-methylarginine demethylation. Biochem Biophys Res Commun 1987;142:200–4.
31. World Health Organization. Micronutrient deficiencies: Battling iron deficiency anaemia. http://www.who.int/nut/ida.htm. 3-13-2002.

32. Oski FA. Iron deficiency in infancy and childhood. N Engl J Med 1993;329:190–93.
33. Cook JD, Lynch SR. The liabilities of iron deficiency. Blood 1986;68:803–9.
34. Lozoff B, Brittenham GM, Wolf AW, et al. Iron deficiency anemia and iron therapy effects on infant developmental test performance. Pediatrics 1987;79:981–95.
35. Oski FA, Honig AS. The effects of therapy on the developmental scores of iron-deficient infants. J Pediatr 1978;92: 21–5.
36. Walter T, De AI, Chadud P, et al. Iron deficiency anemia: Adverse effects on infant psychomotor development. Pediatrics 1989;84:7–17.
37. Walter T, Kovalsky J, Stekel A. Effect of mild iron deficiency on infant mental development scores. J Pediatr 1953;102:519.
38. Cook JD, Skikne BS, Baynes RD. Iron deficiency: The global perspective. Adv Exp Med Biol 1994;356:219–28.
39. Lops VR, Hunter LP, Dixon LR. Anemia in pregnancy. Am Fam Physician 1995;51:1189–97.
40. Swain S, Singh S, Bhatia BD, et al. Maternal hemoglobin and serum albumin and fetal growth. Indian Pediatr 1994;31: 777–82.
41. Beard JL, Dawson H, Pinero DJ. Iron metabolism: A comprehensive review. Nutr Rev 1996;54:295–317.
42. Bothwell T. Overview and mechanisms of iron regulation. Nutr Rev 1995;53:237–45.
43. Cook JD, Monsen ER. Food iron absorption in human subjects. III. Comparison of the effect of animal proteins on nonheme iron absorption. Am J Clin Nutr 1976;29:859–67.
44. van Dokkum W. Significance of iron bioavailability for iron recommendations. Biol Trace Elem Res 1992;35:1–11.
45. Dallman PR, Yip R, Oski FA. Iron deficiency and related nutritional anemias. In: Nathan DG, Oski FA, editors. Hematology of Infancy and Childhood. Philadelphia, PA: W.B. Saunders; 1993.
46. Iob V, Swanson WW. Mineral growth of the human fetus. Am J Dis Child 1934;47:302.
47. Dallman PR. Nutritional anemias. In: Rudolph AM, Hoffman JIE, Rudolph CD, editors. Rudolph's Pediatrics, 20th edition. Stamford, CT: Appleton & Lange; 1996.
48. Singla PN, Chand S, Khanna, S et al. Effect of maternal anaemia on the placenta and the newborn infant. Acta Paediatr Scand 1978;67:645–48.
49. Shott RJ, Andrews BF, Thomas PT. Maternal and infant serum iron and total iron binding capacity at birth in a high risk population. Clin Res 1972;20:100.
50. DeLeeuw NKM, Lowenstein L, Hsieh Y-S. Iron deficiency and hydremia in normal pregnancy. Medicine 1966;45:291.
51. Lanzkowsky P. The influence of maternal iron deficiency on the hemoglobin of the infant. Arch Dis Child 1961;36:205.
52. Rios E, Lipschitz DA, Cook JD, et al. Relationship of maternal and infant iron stores as assessed by determination of plasma ferritin. Pediatrics 1975;55:694–99.
53. Brown MS. Physiologic anemia of infancy: Normal red cell values and physiology of neonatal erythropoiesis. In: Stockman JA, Pochedly C, editors. Developmental and Neonatal Hematology. New York: Raven Press; 1988.
54. Bridges KR, Seligman PA. Disorders of iron metabolism. In: Handin RJ, Lux SE, Stossel TP, editors. Blood: Principles and Practice of Hematology. Philadelphia, PA: J.B. Lippincott; 1995.
55. Russell RM. New micronutrient dietary reference intakes from the National Academy of Sciences. Nutr Today 2001;36:163–71.
56. Edgerton VR, Gardner GW, Ohira Y, et al. Iron–deficiency anaemia and its effect on worker productivity and activity patterns. Br Med J 1979;2:1546–49.
57. Gardner GW, Edgerton VR, Senewiratne B, et al. Physical work capacity and metabolic stress in subjects with iron deficiency anemia. Am J Clin Nutr 1977;30:910–17.
58. Viteri RE, Torun B. Anemia and physical work capacity. Clin Hematol 1974;3:609.
59. Moore DF, Jr, Sears DA. Pica, iron deficiency, and the medical history. Am J Med 1994;97:390–93.
60. Oski FA. The nonhematologic manifestations of iron deficiency. Am J Dis Child 1979;133:315–22.
61. Lanzkowsky P. Iron metabolism and iron deficiency anemia. In: Miller DR, et al., editors. Blood Diseases of Infancy and Childhood St. Louis: Mosby, 1978.
62. Fairbanks VF, Beutler E. Iron deficiency. In: Beutler E, editor. Williams Hematology. New York: McGraw–Hill; 1995.
63. Chandra RK, Saraya AK. Impaired immunocompetence associated with iron deficiency. J Pediatr 1975;86:[V20] 899–902.
64. Dallman PR. Iron deficiency and the immune response. Am J Clin Nutr 1987;46:329–34.
65. Feng XB, Yang XQ, Shen J. Influence of iron deficiency on serum IgG subclass and pneumococcal polysaccharides specific IgG subclass antibodies. Chin Med J (Engl) 1994; 107:813–16.
66. Hershko C, Peto TE, Weatherall DJ. Iron and infection. Br Med J (Clin Res Ed) 1988;296:660–4.
67. Joynson DH, Walker DM, Jacobs A, et al. Defect of cell-mediated immunity in patients with iron-deficiency anaemia. Lancet 1972;2:1058–9.
68. Krantman HJ, Young SR, Ank BJ, et al. Immune function in pure iron deficiency. Am J Dis Child 1982;136:840–4.
69. Kulapongs P, Vithayasai V, Suskind R, et al. Cell-mediated immunity and phagocytosis and killing function in children with severe iron-deficiency anaemia. Lancet 1974;2:689–91.
70. Macdougall LG, Anderson R, McNab GM, et al. The immune response in iron-deficient children: Impaired cellular defense mechanisms with altered humoral components. J Pediatr 1975;86:833–43.
71. Srikantia SG, Prasad JS, Bhaskaram C, et al. Anaemia and immune response. Lancet 1976;1:1307–9.
72. Thibault H, Galan P, Selz F, et al. The immune response in iron-deficient young children: Effect of iron supplementation on cell-mediated immunity. Eur J Pediatr 1993;152:120–4.
73. Weiss G. Iron and immunity: A double-edged sword. Eur J Clin Invest 2002;32:70–8.
74. Gebreselassie H. Iron supplementation and malaria infection: Results of a randomized controlled field trial. Montreal, Canada: McGill University;1996.
75. Gordeuk VR, Thuma PE, Mclaren CE, et al. Transferrin saturation and recovery from coma in cerebral malaria. Blood 1995;85:3297–301.
76. Barton AL, Banner BF, Cable EE, et al. Distribution of iron in th eliver predicts the response of chronic hepatitis C infection to interferon therapy. Am J Clin Pathol 1995;103:419–24.
77. de Monye C, Karcher DS, Boelaert JR, et al. Bone marrow macrophage iron grade and survival of HIV-seropositive patients. AIDS 1999;13:375–80.
78. Hurtado EK, Claussen AH, Scott KG. Early childhood anemia and mild or moderate mental retardation. Am J Clin Nutr 1999;69:115–9.
79. Lozoff B, Jiminez E, Hagen J, et al. Poorer behavioral and developmental outcome more than 10 years after treatment for iron deficiency in infancy. Pediatrics 2000;105:E51.
80. Wasserman G, Graziano JH, Factor-Litvak P, et al. Independent effects of lead exposure and iron deficiency anemia on developmental outcome at age 2 years. J Pediatr 1992;121:695–703.
81. Iannotti LL, Tielsch JM, Black MM, et al. Iron supplementation in early childhood: Health benefits and risks. Am J Clin Nutr 2006;84:1261–76.
82. Lozoff B, Georgieff MK. Iron deficiency and brain development. Semin Pediatr Neurol 2006;13:158–65.
83. Voorhess ML, Stuart MJ, Stockman JA, et al. Iron deficiency anemia and increased urinary norepinephrine excretion. J Pediatr 1975;86:542–47.
84. Youdin MBH, Yehuda S, Ben-Shachar D, et al. Behavioral and brain changes in iron-deficient rats: The involvement of iron in dopamine receptor function. In: Politt E, Leibel RL, editors. Iron Deficiency: Brain Biochemistry and Behavior. New York: Raven Press; 1982.
85. Thompson WG, Meola T, Lipkin M, Jr, et al. Red cell distribution width, mean corpuscular volume, and transferrin saturation in the diagnosis of iron deficiency. Arch Intern Med 1988;148:2128–30.
86. Whelby MS, Jones-Garrison S. Clinical use of transferrin receptor assay. Blood 1992;80:278a.
87. Yip R, Schwartz S, Deinard AS. Screening for iron deficiency with the erythrocyte protoporphyrin test. Pediatrics 1983;72:214–9.
88. Siegel RM, LaGrone DH. The use of zinc protoporphyrin in screening young children for iron deficiency. Clin Pediatr (Phila) 1994;33:473–79.
89. Labbe RF, Rettmer RL. Zinc protoporphyrin: A product of iron-deficient erythropoiesis. Semin Hematol 1989;26:40–6.
90. Hastka J, Lasserre J, Schwarzbeck A, et al. Zinc protoporphyrin in anemia of chronic disorders. Blood 1993; 81:1200–4.
91. Brugnara C, Laufer MR, Friedman AJ, et al. Reticulocyte hemoglobin content (CHr): Early indicator of iron deficiency and resonse to therapy. Blood 1994;83:3100–1.
92. Brugnara C, Zurakowski D, DiCanzio J, et al. Reticulocyte hemoglobin content to diagnose iron deficiency in children. J Am Med Assoc 1999;281:2225–30.
93. Ellis LD, Jensen WN, Westerman MP. Marrow iron: An evaluation of depleted stores in a series of 1,322 needle biopsies. Ann Intern Med 1964;61:44.
94. Lanzkowsky P. Iron-deficiency anemia. Manual of Pediatric Hematology and Oncology. New York: Churchill Livingstone; 1995.
95. Zlotkin S, Arthur P, Antwi KT, et al. Randomized, controlled trial of single versus 3-times-daily ferrous sulfate drops for treatment of anemia. Pediatrics 2001;108:613–6.
96. Kianfar H, Kimiagar M, Ghaffarpour M. Effect of daily and intermittent iron supplementation on iron status of high school girls. Int J Vitam Nutr Res 2000;70:172–7.
97. Brown MS. Physiologic anemia of infancy: Nutritional factors and abnormal states. In: Stockman JA, Pochedly C, editors. Developmental and Neonatal Hematology. New York: Raven Press; 1988.
98. Hamstra RD, Block MH, Schocket AL. Intravenous iron dextran in clinical medicine. JAMA 1980;243:1726–31.
99. Wallerstein RO. Intravenous iron-dextran complex. Blood 1968;32:690–5.
100. Macdougall IC. Intravenous administration of iron in epoetin-treated haemodialysis patients-which drugs, which regimen? Nephrol DialTransplant 2000;15:1743–45.
101. Blackfan KD. Vitamin A deficiency in infants, a clinical and pathological study. J Pediatr 1933;3:679.
102. Hodges RE, Sauberlich HE, Canham JE, et al. Hematopoietic studies in vitamin A deficiency. Am J Clin Nutr 1978;31: 876–85.
103. Majia LA, Hodges RE, Arroyave G, et al. Vitamin A deficiency and anemia in Central American children. Am J Clin Nutr 1977;30:1175–84.
104. Mohanram M, Kulkarni KA, Reddy V. Hematological studies in vitamin A deficient children. Int J Vitam Nutr Res 1977;47:389–93.
105. Nutrition Survey of Paraguay, May-August. 1965. Washington, D.C., U.S. Printing Office.
106. Ahmed F, Rahman A, Noor A, et al. Anaemia and vitamin A status among adolescent schoolboys in Dhaka City, Bangladesh. Public Health Nutrit 2006;9:345–50.
107. Hashizume M, Chiba M, Shinohara A, et al. Anaemia, iron deficiency and vitamin A status among school-aged children in rural Kazakhstan. Public Health Nutr 2005;8:564–71.
108. Molla A, Khurshid M, Molla AM, et al. Is anemia an accurate predictor of vitamin A status in Pakistani children? Am J Trop Med Hyg 1993;49:276–9.
109. Ahmed F, Khan MR, Jackson AA. Concomitant supplemental vitamin A enhances the repsonse to weekly supplemental iron and folic acid in anemic teenagers in urban Bangladesh. Am J Clin Nutr 2001;74:108–15.
110. Muslimatun S, Schmidt MK, Schultink W, et al. Weekly supplementation with iron and vitamin A during pregnancy increases hemoglobin concentration but decreases serum ferritin concentration in Indonesian pregnant women. J Nutr 2001;131:85–90.
111. Mwanri L, Worsley A, Ryan P, et al. Supplemental vitamin A improves anemia and growth in anemic school children in Tanzania. J Nutr 2000;130:2691–6.
112. Suharno D, West CE, Muhilal, et al. Supplementation with vitamin A and iron for nutritional anaemia in pregnant women in West Java, Indonesia. Lancet 1993;342:1325–8.
113. Nankivell BJ. Vitamin B_6 deficiency on hemodialysis causing sideroblastic anemia. Nephron 1991;59:674–5.
114. Weissgarten J, Modai D, Oz D, et al. Vitamin B_6 therapy does not improve hematocrit in hemodialysis patients supplemented with iron and erythropoietin. Nephron 2001;87: 328–32.
115. Bottomley SS. Porphyrin and iron metabolism in sideroblastic anemia. Semin Hematol 1977;14:169–85.
116. Snyderman SE, Holt LE, Carretero R, et al. Pyridoxine deficiency in the human infant. J Clin Nutr 1953;1:200.
117. Foy H, Kondi A. Hypochromic anemias of the tropics associated with pyridoxine and nicotine acid deficiencies. Blood 1958;13:1054.
118. Bottomley SS. Sideroblastic anaemia. Clin Haematol 1982;11:389–409.
119. Verwilghen R, Reybrouck G, Callens L, et al. Antituberculous drugsand sideroblastic anemia. Br J Haematol 1965;II:92.
120. Frimpter GW. Pyridoxine (B_6) dependency syndromes. Ann Intern Med 1968;68:1131–2.
121. McCurdy PR, Donohoe RF, Magovern M. Reversible sideroblastic anemia caused by pyrazinoic acid (Pyrazinamide). Ann Intern Med 1966;64:1280–4.
122. Horrigan DL, Harris JW. Pyridoxine-responsive anemia: Analysis of 62 cases. Adv Intern Med 1989;12:103.
123. Raab SO, Haut A, Cartwright GE, et al. Pyridoxine-responsive anemia. Blood 1961;18:285.
124. Alfrey CP, Lane M. The effect of riboflavin deficiency on erythropoiesis. Semin Hematol 1970;7:49–90.
125. Lane J, Alfrey CP. The anemia of human riboflavin deficiency. Blood 1963;22:811.
126. Ajayi OA, Okike OC, Yusuf Y. Haematological response to supplements of riboflavin and ascorbic acid in Nigerian young adults. Eur J Haematol 1990;44:209–12.
127. Buzina R, Jusic M, Milanovic N, et al. The effects of riboflavin administration on iron metabolism parameters in a school-going population. Int J Vitam Nutr Res 1979;49:136–43.
128. Powers HJ, Wright AJ, Fairweather-Tait SJ. The effect of riboflavin deficiency in rats on the absorption and distribution of iron. Br J Nutr 1988;59:381–7.

129. Powers HJ, Weaver LT, Austin S, et al. Riboflavin deficiency in the rat: Effects on iron utilization and loss. Br J Nutr 1991;65:487–96.
130. Beutler E. Effect of flavin compounds on glutathione reductase activity: In vivo and in vitro studies. J Clin Invest 1969;48:1957–66.
131. Beutler E. Glutathione reductase: Stimulation in normal subjects by riboflavin supplementation. Science 1969;165:613–5.
132. Staal FJT, Ela SW, Roederer M, et al. Glutathione deficiency and human immunodeficiency virus infection. Lancet 1992;339:909–12.
133. Delmas-Beauvieux M, Peuchant E, Couchouron A, et al. The enzymatic antioxidant system in blood and glutathione status in human immunodeficiency virus (HIV)-infected patients; effects of supplementation with selenium or B-carotene. Am J Clin Nutr 1996;64:101–7.
134. Beutler E, Srivastava SK. Relationship between glutathione reductase activity and drug-induced haemolytic anaemia. Nature 1970;226:759–60.
135. Vilter RW, Woolford RMSTL. Severe scurvy, a clinical and hemaotlogical study. J Lab Clin Med 1946;31:609.
136. Crandon JH, Lund CC, Dill DB. Experimental human scurvy. N Engl J Med 1940;223:353.
137. Hodges RE, Baker EM, Hood J, et al. Experimental scurvy in man. Am J Clin Nutr 1969;22:535–48.
138. Stokes PL, Melikian V, Leeming RL, et al. Folate metabolism in scurvy. Am J Clin Nutr 1975;28:126–9.
138. Cox EV, Meynell MJ, Northan BE, et al. The anemia of scurvy. Am J Med 1966;42:220.
140. Moore CV, Dubach R. Observations on the absorption of iron from foods tagged with radioiron. Trans Assoc Am Physicians 1951;64:245.
141. Fairweather-Tait S, Fox T, Wharf SG, et al. The bioavailability of iron in different weaning foods and the enhancing effect of a fruit drink containing ascorbic acid. Pediatr Res 1995;37:389–94.
142. Zucker W, Hutnoff L, Apt L. Relationship of anemia and scurvy. Am J Dis Child 1949;77:128.
143. Clark N, Sheard NF, Kelleher JF. Treatment of irondeificiency anemia complicated by scurvy and folic acid deficiency. Nutr Rev 1992;50:134–7.
144. van den Berg JJ, Kuypers FA, Roelofsen B, et al. The cooperative action of vitamins E and C in the protection against peroxidation of parinaric acid in human erythrocyte membranes. Chem Phys Lipid 1990; 53:309–20.
145. Cox EV. The anemia of scurvy. In: Harris RS, Wool IG, Loraine JA, editors. International Symposium on Vitamin-Related Anemias. Vitamins and Hormones. Advances in Research and Applications. New York: Academic; 1968.
146. Dju MV, Mason KE, Filer LJ. Vitamin E in human fetuses and placentae. Etud Neo-Natales 1952;1:49.
147. Durnin JV, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: Measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 1974;32:77–97.
148. Quaife ML, Dju MY. Chemical estimation of vitamin E in tissue and the tocopherol content of some normal human tissues. J Biol Chem 1949;180:263.
149. Leonard PJ, Doyle E, Harrington W. Levels of vitamin E in the plasma of newborn infants and of the mothers. Am J Clin Nutr 1972;25:480–4.
150. Martinez FE, Goncalves AL, Jorge SM, et al. Brief clinical and laboratory observations. Vitamin E in placental blood and its interrelationship to maternal and newborn levels of vitamin E. J Pediatr 1981;99:298–300.
151. Wright SW, Filer LJ, Mason RE. Vitamin E blood levels in premature and full term infants. Pediatrics 1951;7:386.
152. Goolbloom RB, Cameron D. Studies of tocopherol requirements in health and disease. Pediatrics 1963;32:36.
153. Hassan H, Hashim SA, Van Itallie TB, et al. Syndrome in premature infants associated with low plasma vitamin E levels and high polyunsaturated fatty acid diet. Am J Clin Nutr 1966;19:147–57.
154. Oski FA, Barness LA. Vitamin E deficiency: A previously unrecognized cause of hemolytic anemia in the premature infant. J Pediatr 1967;70:211–20.
155. Ritchie JH, Fish MB, McMasters V, et al. Edema and hemolytic anemia in premature infants. A vitamin E deficiency syndrome. N Engl J Med 1968;279:1185–90.
156. Melhorn DK, Gross S. Vitamin E-dependent anemia in the premature infant. I. Effects of large doses of medicinal iron. J Pediatr 1971;79:569–80.
157. Williams ML, Shoot RJ, O'Neal PL, et al. Role of dietary iron and fat on vitamin E deficiency anemia of infancy. N Engl J Med 1975;292:887–90.
158. Bell EF, Filer LJ, Jr. The role of vitamin E in the nutrition of premature infants. Am J Clin Nutr 1981;34:414–22.
159. Bieri JG, Corash L, Hubbard VS. Medical uses of vitamin E. N Engl J Med 1983;308:1063–71.
160. Farrell PM, Bieri JG, Fratantoni JF, et al. The occurrence and effects of human vitamin E deficiency. A study in patients with cystic fibrosis. J Clin Invest 1977;60:233–41.
161. Ater JL, Herbst JJ, Landaw SA, et al. Relative anemia and iron deficiency in cystic fibrosis. Pediatrics 1983;71:810–4.
162. Frieden E. The ferrous to ferric cycles in iron metabolism. Nutr Rev 1973;31:41–4.
163. Graham GG, Cordano A. Copper depletion and deficiency in the malnourished infant. Johns Hopkins Med J 1969;124:139–50.
164. Joffe G, Etzioni A, Levy J, et al. A patient with copper deficiency anemia while on prolonged intravenous feeding. Clin Pediatr (Phila) 1981;20:226–8.
165. Dunlap WM, James GW, III, Hume DM. Anemia and neutropenia caused by copper deficiency. Ann Intern Med 1974;80:470–6.
166. Committee on Nutrition AAoP. Pediatric Nutrition Handbook, 4th edition. Elk Grove Village, IL: American Academy of Pediatrics; 1998.
167. Fischer P, Giroux A, L'Abbe M. The effect of dietary zinc on intestinal copper absorption. Am J Clin Nutr 1981;34:1670–5.
168. Forman W, Sheehan D, Cappelli S, et al. Zinc abuse–An unsuspected cause of sideroblastic anemia. West J Med 1990;152:190–2.
169. Keys A, et al. The Biology of Semistarvation Minneapolis: University of Minnesota Press, 1950.
170. Thomson TJ. Treatment of obesity by total fasting for up to 249 days. Lancet 1966;2:992.
171. Drenick EJ, Swendseid ME, Blahd WH, et al. Prolonged starvation as a treatment for severe obesity. J Am Med Assoc 1964;187:100.
172. Fischer P. Commentary. Clin Pediatr 2004;43:306.
173. Delmonte L, Aschenasy A, Eyquen A. Studies on the hemolytic nature of protein deficiency anemia in the rat. Blood 1964;24:49.
174. Naets JP, Wittek M. Effect of starvation on the response to erythropoietin in the rat. Acta Haematol 1974;52:141–50.
175. Adams EB. Observations on the etiology and treatment of anemia in kwashiorkor. Br Med J 1967;3:451.
176. Viteri RE, Alvaredo J, Luthringer DG, et al. Hematological changes in protein-calorie malnutrition. In: Harris RS, Wool IG, Loraine JA, editors. International Symposium on Vitamin-Related Anemias. Vitamins and Hormones. Advances in Research and Applications. New York: Academic; 1968.
177. Foy H, Kondi A. Comparison between erythroid aplasia in marasmus and kwashiorkor and the experimentally induced erythroid aplasia in baboons by riboflavin deficiency. In: Harris RS, Wool IG, Loraine JA, editors. International Symposium on Vitamin-Related Anemias. Vitamins and Hormones. Advances in Research and Applications. New York: Academic; 1968.
178. McKenzie D, Friedman R, Katz S, et al. Erythropoietin levels in anaemia in kwashiorkor. S Afr Med J 1967;41:1044–6.
179. Eisenmann J. Secular trends in variables associated with the metabolic syndrmoe of North American children and adolescents: A review and synthesis. Am J Hum Biol 2003;15:786–94.
180. Rocandio A, Ansotegui L, Arroyo M. Comparison of dietary intake among overwieght and non-overweight schoolchildren. Int J Obes Relat Metab Disord 2001;25:1651–55.
181. Rodriguez G, Moreno L. Is dietary intake able to explain differences in body fatness in children and adolescents? Nutr Metab Cardiovasc Dis 2006;16:294–301.
182. Gillis L, Gillis A. Nutrient inadequacy in obese and non-obese youth. Can J Diet Pract Res 2005;66:237–42.
183. Davy B, Harrell K, Stewart J, et al. Body weight status, dietary habits, and physical activity levels of middle school-aged children in rural Mississippi. South Med J 2004;97:571–7.
184. Pinhas-Hamiel O, Doron-Panush N, Reichman B, et al. Obese children and adolescents: A risk group for low vitamin B12 concentration. Arc Pediatr Adolesc Med 2006;160:933–6.
185. Nead K, Halterman J, Kaczorowski J, et al. Overweight children and adolescents: A risk group for iron deficiency. Pediatrics 2004;114:104–8.
186. Pinhas-Hamiel O, Newfield R, Koren I, et al. Greater prevalence of iron deficiency in overweight and obese children and adolescents. Int J Obes Relat Metab Disord 2003;27:416–18.