Chapter 8
NUTRITIONAL STATUS

NUTRIENTS

Nutrition refers to the intake, digestion, and absorption of the nutrients needed to maintain the body in good health and, in children and adolescents, to allow for growth. The major classes of nutrients are proteins, carbohydrates, lipids, the major minerals, the trace minerals, and vitamins. Proteins, carbohydrates, lipids, and the major minerals are referred to as macronutrients because of the relatively large quantities of these nutrients ingested daily in a typical Western diet. The trace minerals and vitamins are called micronutrients. The trace minerals, vitamins, the major minerals, and some fatty acids and amino acids (Table 8.1) are essential nutrients in that they cannot be synthesized at all or they cannot be produced in an adequate amount under normal conditions so they must be derived from the diet (Linder 1991). Calciferols, the dietary forms of the prohormone cholecalciferol, are a conditional essential nutrient. In tropical and southern temperate latitudes, adequate amounts of cholecalciferol can be synthesized from 7-dihydrocholesterol by ultraviolet photolysis in skin cells. However, at northern latitudes, the exposure to sunlight is less and the synthesis of cholecalciferol cannot meet the needs of growing children. Hence, for children living in northern lands, the endogenous production of cholecalciferol must be supplemented by the intake of dietary calciferols.

The diets of peoples living in affluent countries, such as the United States, almost invariably completely satisfy the dietary needs for the macronutrients and the widespread practice of fortifying foods with micronutrients assures that even individuals consuming unbalanced diets will still obtain a

Table 8.1
Essential Nutrients

amino acids
- isoleucine
- leucine
- lysine
- methionine
- phenylalanine
- threonine
- tryptophan
- valine

fatty acids
- linoleic acid (n-6 fatty acids)
- linolenic acid (n-3 fatty acids)

water and major minerals
- water
- potassium
- sodium
- calcium
- magnesium
- chloride
- phosphate

trace minerals
- chromium
- cobalt
- copper
- iodine
- iron
- manganese
- molybdenum
- selenium
- zinc

fat-soluble vitamins
- calciferols (vitamin D)
- menaquinones and phylloquinones (vitamin K)
- retinoids (vitamin A)
- tocopherols (vitamin E)

water-soluble vitamins
- ascorbic acid (vitamin C)
- biotin
- cobalamin (vitamin B_{12})
- folate
- niacin (vitamin B_3)
- pantothenic acid (vitamin B_5)
- pyridoxine (vitamin B_6)
- riboflavin (vitamin B_2)
- thiamin (vitamin B_1)

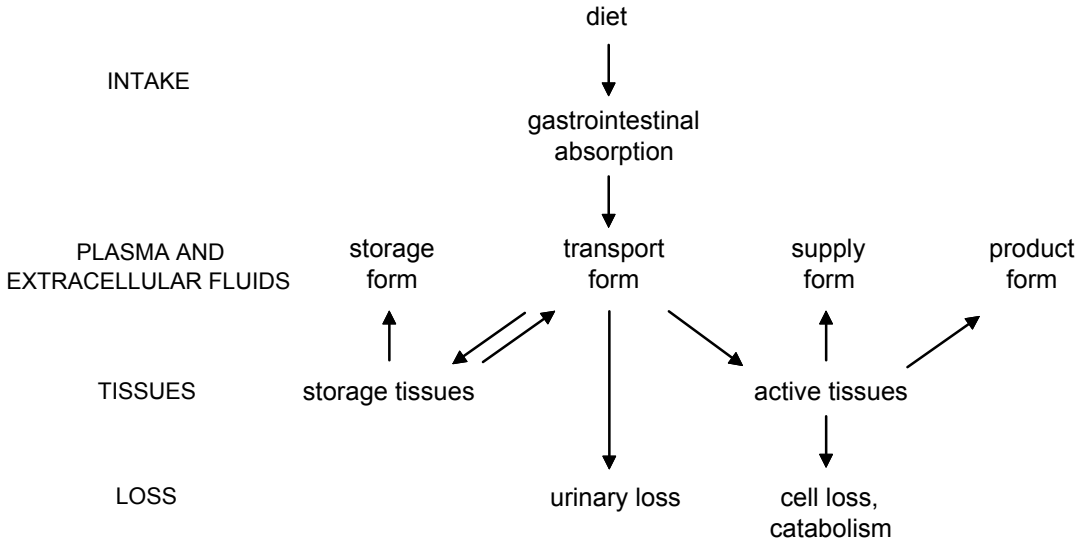

Figure 8.1 A model of the disposition of trace minerals and vitamins.

sufficient supply of trace minerals and vitamins. These salubrious circumstances have made nutritional diseases uncommon and, consequently, make laboratory evaluation of nutritional status an infrequent undertaking. A notable exception to this generalization is the laboratory evaluation of the nutritional status of the micronutrients involved in red cell production: anemia due to impaired absorption of folate and cobalamin are often seen in patients with intestinal malabsorption; anemia caused by deficient absorption of cobalamin as a result of intrinsic factor deficiency can be seen in the elderly; and anemia due to iron deficiency is not rare in infants and young children, in menstruating and pregnant women, and in individuals with gastrointestinal bleeding.

TRACE MINERALS AND VITAMINS

A simple model of the disposition of trace minerals and vitamins is shown in Figure 8.1. The tissues that utilize a trace substance receive their supply of the substance from the circulation, where it is available in its transport form, and from local intracellular supplies of the substance. The transport form arises from intestinal absorption of the substance present in the diet and, if the trace substance is stored, from the release of substance from the storage form. The water-soluble vitamins other than cobalamin are transported in the plasma in their free form or loosely bound to albumin. Vitamins in the free form are freely filtered at the glomerulus and lost in the urine. For some vitamins, notably pantothenic acid and biotin, this can be the primary route of loss of the vitamin. Most of the trace metals are transported in the plasma bound to albumin. Iron, however, is bound to an iron-specific binding protein, apotransferrin, which releases the iron only after the iron-apotransferrin complex (called transferrin) is bound to cell surface transferrin receptors and taken up into the acidic environment of early endosomes. This facilitates the differential delivery of iron to tissues expressing the transferrin receptor, such as red cell precursors, placenta, and the liver, the storage organ for iron. Cholecalciferol, cobalamin, and retinol are also transported bound to specific binding proteins: vitamin-D binding globulin, transcobalamin II, and retinol-binding globulin, respectively. The cobalamin-transcobalamin II complex (called holotranscobalamin II) enters cells by holotranscobalamin-specific receptor-mediated endocytosis. Thus, in a way similar to that of protein binding of iron, the protein binding of cobalamin directs the delivery of cobalamin to cells requiring the micronutrient. The protein binding of cholecalciferol and retinol allows both of these vitamins, which are otherwise sparingly soluble in water, to enter the circulation for delivery to tissues. Because the dynamic equilibrium between the free and protein-bound forms of these vitamins is so much in favor of the bound form, very little of the free form of either vitamin is available for diffusion into most tissues. Tissues that possess high

concentrations of intracellular binding protein shift the local equilibrium so that the vitamins are unloaded from the transport protein and onloaded to the intracellular binding protein. In this way, protein binding also results in directed delivery of these vitamins. The other fat-soluble vitamins are transported in lipoproteins and are widely distributed in cell membranes and body fat.

When the dietary intake of a trace mineral or vitamin exceeds the body needs for the micronutrient, the excess may be accumulated in the active tissues as reserve supplies or it may be distributed to storage sites. Iron is accumulated both in marrow cells as a supply reserve and in hepatocytes as a store. Additionally, the iron from senescent red cells is recovered by and stored in fixed tissue macrophages of the marrow and spleen. At all these sites, the surplus iron complexes with the intracellular binding protein apoferritin. The iron-apoferritin complex is referred to as ferritin. Iodine is the only other trace mineral that the body accumulates. It is taken up into thyroid follicles where it exists as a supply reserve covalently bound to thyroglobulin. For both iron and iodine it is important for the cell to maintain the mineral complexed or bound to protein to protect against cellular toxicity from the free ionic forms. Cobalamin and folate accumulate in the marrow and in hepatocytes primarily bound to cytosolic and mitochondrial enzymes. In addition, folate is retained in the cell as free folylpolyglumates which, unlike folylmonoglumate, do not diffuse out of the cell, owing to the highly charged polyglumate tail. Retinol is stored in the liver in specialized accessory cells called stellate cells (also called Ito cells). The other fat-soluble vitamins are also stored in the liver but in the hepatocytes. Tocopherol, menaquinone and phylloquinone are present in adipose tissue but this is a site of slow turnover which may not function as an available store.

Vitamins are lost by urinary excretion and by catabolism within the active tissues. Trace minerals are lost by urinary excretion, by desquamation of skin and intestinal cells, and by insensible fluid losses. Only the unbound plasma species are available for urinary excretion.

Circulating forms of micronutrients

Trace minerals and vitamins circulate not only in their transport forms but may also be present in their supply and storage forms which enter the plasma as a result of cellular turnover in the active tissues and in the storage tissues, respectively. Trace minerals may also be incorporated into plasma constituents (product forms) secreted into the plasma from their sites of synthesis.

The plasma concentration of the storage form of a trace substance can be used as a marker of the body stores of the substance. The plasma concentration of the storage form depends upon the rate at which the storage form enters the plasma. That entry rate is the product of the rate of turnover of storage cells and the amount of substance stored in each cell. In the absence of other perturbing influences, the rate of cell turnover among storage cells is constant. The plasma entry rate then depends only upon the amount of the storage form liberated per dead storage cell which is, in turn, determined by the tissue stores of the substance. Thus, the plasma concentration of the storage form is proportional to the body stores of the substance. When the rate of cell turnover in the storage tissue is increased, as occurs with injury or in neoplastic tissue, the release of the storage form is increased and its plasma concentration will be elevated, thereby compromising the relationship between the concentration and the size of the body stores.

In a similar fashion, the plasma concentration of the supply form of a micronutrient can be used as a marker of the supply of the micronutrient in the active tissues. Additionally, a plasma product form of a micronutrient substance can be used as a marker of the supply of the trace substance in the tissue secreting the product. A reduced supply of micronutrient results in diminution in the rate of synthesis and secretion of the product form. Because the plasma concentration of the product form depends upon its rate of secretion, the magnitude of the deficiency in the active tissue supply will be reflected in the product form concentration. Of course, other influences upon the rate of secretion of the product form can cause alterations in its plasma concentration that can be confused with or can obscure changes due to decreased micronutrient supply.

The plasma concentration of the transport form of a trace substance depends upon the rate of entry of the form into the plasma from the diet and from body stores and its rate of egress from the plasma into sites of utilization and back into body stores. If the major source of the transport form is the body stores, the concentration of the transport form will generally parallel those stores. If the transport form

Table 8.2
Circulating Forms of Micronutrients Essential for Red Cell Production (TC, transcobalamin)

Micronutrient	Transport Form	Storage Form	Supply Form	Product Form
Iron	transferrin	ferritin	red cell ferritin	red cell hemoglobin
Cobalamin	holo-TC II	holo-haptocorrin	red cell cobalamin	
Folate	free folate folate-albumin		red cell folate	

arises predominantly from dietary intake, its plasma concentration will merely reflect recent dietary intake of the substance, regardless of the state of the body stores.

Table 8.2 lists the circulating forms for the micronutrients involved in red cell production. Iron circulates in four forms. The iron present in red cell hemoglobin is a product form. The ferritin contained within red cells represents erythroblast iron left over from the process of hemoglobinization. As such, it is a supply form of iron. Most of the iron in the plasma is present as transferrin, the transport form of iron. Plasma iron is also present as ferritin, the storage form of iron. Normally the ferritin in plasma arises from hepatic iron stores. However, in chronic anemias treated by repeated red cell transfusions, large amounts of ferritin are deposited in the marrow and spleen fixed tissue macrophages. Then this source of ferritin contributes significantly to the circulating ferritin.

Cobalamin is present in the circulation in three forms. The cobalamin in red cells is a supply form. Plasma cobalamin is present as holo-transcobalamin II, the transport form of cobalamin, and the more abundant holo-haptocorrin which is a complex of cobalamin and haptocorrin. In cobalamin-replete individuals, holo-haptocorrin typically constitutes about 0.85 of the circulating cobalamin (Wickramasinghe and Fida 1993). Among other physiologic functions, haptocorrin is probably a minor intracellular cobalamin-binding protein (Jacobs *et al*. 1980, Fernandes-Costa and Metz 1982), so holo-haptocorrin represents a storage form of cobalamin. The usual sites of origin of plasma holo-haptocorrin are not known. An isoform of haptocorrin with low affinity for cobalamin is present in the specific granules of granulocytes. In the myeloproliferative disorders, increased turnover of immature granulocytic cells can result in elevated plasma holo-haptocorrin concentrations due to holo-haptocorrin of granulocyte origin.

Circulating folate is present as transport forms in the plasma and as a supply form in red cells.

The transport markers of these micronutrients that are most often used clinically are plasma iron concentration, plasma holo-transcobalamin II concentration, and plasma folate concentration. Because transferrin contains most of the iron in the plasma in normal individuals and in iron deficiency, total plasma iron concentration is a very satisfactory way to measure this transport species. Plasma cobalamin concentration serves as the storage marker for cobalamin. Total plasma cobalamin concentration is a satisfactory measure of plasma holo-haptocorrin concentration because the majority of the plasma cobalamin is in this form. The plasma concentration of ferritin-bound iron could be used as a storage marker for iron but the separation of ferritin-bound iron from transferrin-bound iron is technically difficult. Instead, as discussed in a subsequent section, the plasma concentration of ferritin measured as a protein is used as the storage marker for iron. There is no marker of folate stores. Red cell folate concentration is used as the marker of the marrow supply of folate. Red cell ferritin and cobalamin have not proven to be very reliable markers of micronutrient supply in the marrow so they are not employed clinically. Instead, measures of a product form are used to gauge the state of marrow supplies. For iron, red cell hemoglobinization is usually evaluated. It can be assessed using either measures of individual red cell hemoglobin (mean red cell volume or mean red cell hemoglobin) or measures of total red cell hemoglobin (hematocrit and blood hemoglobin concentration). For cobalamin, the *in vivo* activity of an intracellular enzyme that uses cobalamin as a cofactor is evaluated.

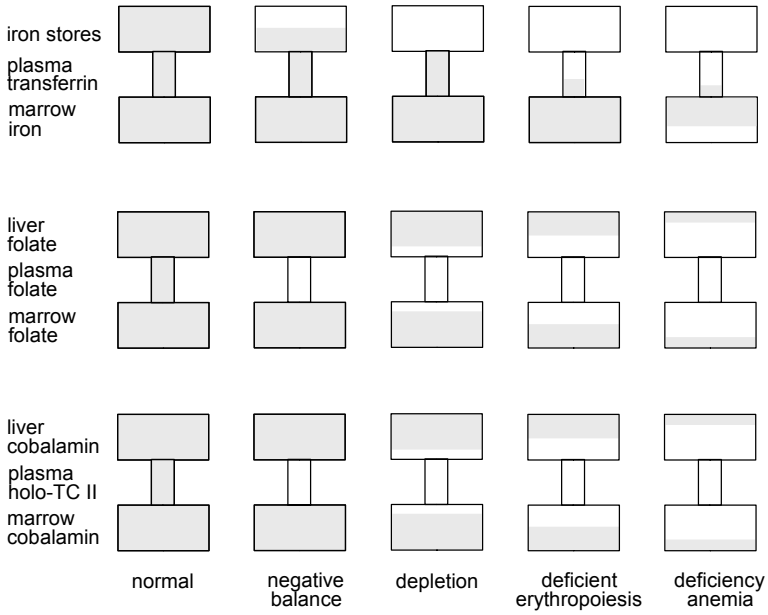

Figure 8.2 Pathobiologic stages in the development of micronutrient deficiency. Adapted from figures in Herbert (1987). holo-TC II, holo-transcobalamin II.

Circulating forms of micronutrients in deficiency states

Micronutrient deficiency does not occur all of a sudden; it is the result of a long-term inequality between the dietary intake of a micronutrient and its loss from the body. During the period that the deficiency is developing, the body passes through a characteristic series of pathobiologic stages each of which is associated with a predictable pattern of findings among the circulating forms of the micronutrient (Hastka *et al.* 1996, Herbert 1994, Herbert 1987). The stages as they apply to iron, cobalamin, and folate deficiency are illustrated in Figure 8.2. At first, the body merely experiences a negative balance in the micronutrient; the shortfall in dietary intake is compensated for by tapping the body stores and supply reserves of the micronutrient. For cobalamin and folate, the transport form of the micronutrient is decreased in this stage due to the decreased contribution of the dietary component of the form. For iron, the plasma transferrin concentration remains essentially normal because of ready access to the hepatic iron stores. As the negative balance persists, the stores and supply reserves are progressively depleted. During this stage, the concentration of the circulating storage form of the micronutrient decreases. At some point, the stores and supply reserves of the micronutrient become depleted to the point that there is insufficient delivery of the micronutrient to the active tissues. Then the circulating supply and product forms of the micronutrient begin to decline. During the stage of deficient erythropoiesis, only the marrow and the most recently manufactured red cells show the effects of insufficient micronutrient supply. As the normal red cells (those that were produced prior to the onset of deficient erythropoiesis) senesce, the reduced rate of effective red cell production leads to anemia. With continued micronutrient deficiency, individual red cell abnormalities become more pronounced and the rate of effective red cell production falls ever lower resulting in progressively more severe anemia.

Other markers of micronutrient status in deficiency states

In addition to direct measurement of circulating forms of trace substances, there are a number of other laboratory approaches for the evaluation of micronutrient status (Gibson 1990, Taylor 1996). One such approach, the measurement of the *in vivo* activity of an enzyme that depends upon a trace substance, was mentioned in a preceding section as the preferred means of assessing the supply status of cobalamin. The enzyme, methylmalonyl CoA mutase, catalyzes the conversion of methylmalonyl CoA to succinyl CoA. When tissue concentrations of the mutase are depressed due to cobalamin

deficiency, the substrate accumulates intracellularly, undergoes spontaneous hydrolysis, and diffuses out of the cells, thereby raising the plasma concentration of methylmalonate. Plasma methylmalonate concentration can therefore be used as a marker of mutase activity and, in turn, of tissue cobalamin supply (Nexř *et al.* 1994, Chanarin and Metz 1997). As a rule, enzyme substrates increase in concentration in plasma and are excreted in the urine at greater than normal rates as a consequence of micronutrient deficiency. The *in vivo* activity of micronutrient-dependent enzymes can also be assessed by measurement of the usual products of the metabolic transformation that is catalyzed or by measurement of substances or processes that are metabolically downstream of the catalytic step. Trace substance deficiency leads to a reduction in the plasma concentration of the catalysis products and a reduced rate of urinary excretion of the products. Lastly, direct, *in vitro* assay of the activity of micronutrient-dependent enzymes can be undertaken when the enzymes are present in marrow cells. Enzyme activities measured *in vitro* decrease in deficiency states. Nonenzymatic *in vitro* assay of micronutrients in marrow cells is also possible; the most important example being the semi-quantitation of erythroblast iron by light microscopy using iron-specific histologic stains.

Another laboratory approach that can be used to evaluate trace substance supply is measurement of a precursor substance in the metabolic pathway of the trace substance. When a trace substance is not freely available for incorporation into a product because of deficiency of the substance, precursors accumulate intracellularly and, if able to diffuse out of the cell, they accumulate in the plasma and are excreted at increased rates in the urine. In the case of iron, in the later stages of deficiency, when there is iron-deficient erythropoiesis, protoporphyrin, the immediate precursor of heme, accumulates in red cells. Red cell protoporphyrin concentration then correlates with the severity of the iron deficiency (Hastka *et al.* 1996).

In deficiency states, there is typically a decrease in the synthesis of the intracellular micronutrient-binding protein with an associated decline in the plasma concentration of the protein. In iron deficiency, decreased synthesis of apoferritin leads to a fall in the intracellular and plasma concentrations of ferritin. Because it is the protein concentration of ferritin rather than the ferritin-bound iron concentration that is measured when ferritin is assayed in the laboratory, it is the decreased synthesis of apoferritin that underlies the inverse relationship between plasma ferritin concentration and the status of the iron stores.

Deficiency states can also lead to a number of physiologic alterations that serve to improve tissue access to a deficient micronutrient. There may be an increase in the absorptive capacity of the intestine due to increased expression of enterocyte receptors for the micronutrient; there may be an increased rate of hepatic secretion of the plasma transport protein, and there may be an increase in the tissue expression of cellular receptors for the micronutrient or the transport complex. Many of these alterations occur in response to cobalamin deficiency (Herbert 1994) and all of them are seen in iron deficiency (Melefors and Henze 1993). Changes in enterocyte iron receptors can be assessed by performance of a radio-iron absorption study but absorption studies are less reliable than the much more easily performed blood studies. Changes in tissue transferrin receptor expression are reflected in the concentration of the receptor in plasma (Skikne *et al.* 1990). Because transferrin receptors enter the plasma as a result of cell turnover, the increased turnover of red cells in the marrow and in the circulation that is present in iron-deficiency leads to a diagnostically useful exaggeration of the plasma receptor concentration in that state. Alterations in the rate of secretion of apotransferrin are quantified by measurement of the plasma concentration of the protein, usually as total iron binding capacity. It is common practice to measure the concentration of the plasma iron as well as total iron binding capacity and to calculate the ratio of the two, which is expressed as percent transferrin saturation. In iron deficiency, once iron stores are depleted and plasma iron concentration begins to fall, transferrin saturation is directly related to micronutrient stores and in a fashion that is much more steep, and therefore much more sensitive and precise, than that of either of the two measures separately.

Deficiency of a trace substance leads to a decreased cellular concentration of the substance and thereby directly increases tissue access for the substance if the substance readily diffuses into cells. This phenomenon underlies the use of load tests for the evaluation of deficiency states of the water-soluble vitamins and some of the trace minerals. In a load test, the urinary excretion of a test dose of

Table 8.3
Laboratory Studies for the Assessment of Iron, Cobalamin, and Folate Deficiency
(TC, transcobalamin; dU, deoxyuridine)

Pathophysiology	Iron	Cobalamin	Folate
↓ Transport form	plasma iron	plasma holo-TC II	plasma folate
↑ Transport protein	plasma iron binding capacity	plasma TC II (as protein)	
↓ Tissue stores	plasma ferritin (as protein)	plasma cobalamin	
↑ Tissue receptors	plasma transferrin receptor		
↓ Tissue supply	red cell ferritin marrow erythroblast iron	red cell cobalamin	red cell folate
↑ Tissue precursor	red cell protoporphyrin		
↓ Tissue product	individual red cell hemoglobin mean red cell volume mean red cell hemoglobin		
	total red cell hemoglobin blood hematocrit blood hemoglobin		
↑ Tissue enzyme substrate		plasma methylmalonate	
↓ Tissue enzyme product		anemia blood hematocrit blood hemoglobin blood cell megaloblastosis marrow megaloblastosis dU suppression test	anemia blood hematocrit blood hemoglobin blood cell megaloblastosis marrow megaloblastosis dU suppression test
+ Therapeutic response	reticulocyte count blood hemoglobin	reticulocyte count blood hemoglobin	reticulocyte count blood hemoglobin

trace substance is used to measure the extent of uptake of the substance into the tissues. Increased tissue uptake due to cellular deficiency results in decreased excretion of the load.

Tissue deposits that do not serve as stores or supply reserves have been investigated as possible markers of the long-term trend in the concentration of transport forms of trace minerals. Scalp hair is by far the best studied of the sites. Trace minerals are more concentrated in hair, making the elements easier to assay, and hair specimens can be obtained without trauma, require no special preservatives, and can be stored without deterioration (Gibson 1990). However, the concentrations of trace minerals in hair are influenced by hair color and the rate of hair growth and by contamination from water supplies, shampoos, hair treatments, and hair grooming agents (Taylor 1986). Consequently, hair analysis does not have a place in the clinical evaluation of nutritional status. Fingernails and toenails have been proposed as potential tissues for the analysis of trace mineral deposition but they are also highly susceptible to environmental contamination. It is unlikely that nail analysis will ever be used clinically.

As a summary of the many different laboratory approaches that can be used in the assessment of nutrition status of a trace substance, the laboratory studies pertinent to the evaluation of iron, cobalamin, and folate deficiency are listed in Table 8.3. Not all of the studies are clinically available: those

that have been shown to be inferior diagnostic tests, such as plasma folate, are generally not offered by clinical laboratories, and some of the studies, such as holo-transcobalamin II, are performed only in a few research laboratories. Among the studies that are available, there are a number of clearly preferred studies. For iron deficiency, the storage marker, plasma ferritin, is by far the best diagnostic marker (Guyatt *et al*. 1992), although it must be remembered that the plasma ferritin concentration tends to be increased somewhat (on average 20 μg/L based on the data reported by Guyatt *et al*.) by the presence of an inflammatory condition such as an infection, a rheumatologic disorder, or a malignancy. The storage marker, (total) plasma cobalamin, is the best diagnostic marker of cobalamin deficiency (Chanarin and Metz 1997). Folate does not have a circulating storage form to utilize but the circulating supply marker, red cell folate, has been found to be a reliable marker of folate deficiency. The hematologic marker, mean red cell volume, is useful in patients in late stages of micronutrient deficiency as it indicates whether a microcytic disease (such as iron deficiency) or a macrocytic disorder (such as cobalamin or folate deficiency) is present. Similarly, megaloblastic features in the circulating blood cells of patients with late stage cobalamin and folate deficiency strongly suggest the deficiency of one of these micronutrients as the etiology of the anemia. A positive response to micronutrient administration is an important test of the validity of the diagnosis. The absence of a response or a partial response following full replenishment of the micronutrient can be taken either as evidence of an error in diagnosis or of the existence of a concurrent disorder, especially of a concurrent micronutrient deficiency as it is not uncommon for patients to present with multiple micronutrient deficiencies.

Micronutrient excess

A trace substance is present in excess, also called overload, when the storage form of the substance accumulates to the point of causing damage to the storage tissue or when, because of increased body stores, there is sufficient deposition of the substance in nonstorage tissues to cause injury to them. The clinical findings are attributable to the tissue injury and its sequelae. Excessive dietary intake of micronutrients is very unusual but in individuals with hereditary hemochromatosis, a not uncommon autosomal recessive disorder of iron metabolism in which there is excessive intestinal absorption of iron (Adams and Valberg 1994), iron overload can develop despite a normal dietary intake of iron.

Table 8.4
Laboratory Studies for the Assessment of Iron Overload

Pathophysiology	Study
↑ Transport form	plasma iron
↓ Transport protein	plasma iron binding capacity
↑ Tissue stores	plasma ferritin (as protein) hepatic iron index

The increased hepatic iron stores in hereditary hemochromatosis prompt an increased synthesis of ferritin and a decreased synthesis and secretion of apotransferrin (Table 8.4). Because of the increased intrahepatic concentrations of ferritin, the plasma ferritin concentration (measured as protein) increases and it does so in rough proportion to the magnitude of the hepatic ferritin stores. The presence of increased hepatocyte turnover and decreased hepatic ferritin clearance further increases plasma ferritin concentrations in advanced hemochromatosis. The decreased hepatic secretion of apotransferrin results in a decreased plasma concentration of the protein which is manifest as a decreased (total) iron binding capacity. As there is abundant iron available for transport to the marrow; plasma transferrin iron concentrations are normal. However, because plasma transferrin concentrations are measured as (total) plasma iron, the contribution of ferritin-bound iron, which is present in elevated concentrations, results in an increased total iron concentration. As a consequence of the opposite direction of change for plasma iron concentration and plasma iron binding capacity, transferrin saturation is markedly increased in hemochromatosis. (Note that the ratio of the two measures is not the true transferrin saturation because the numerator includes nontransferrin iron and the denominator includes nontransferrin iron binding capacity). Most experts recommend using transferrin saturation as the screening study for hereditary hemochromatosis (Adams and Valberg 1994, Phatak and Cappuccio 1994) but the diagnostic performance of plasma ferritin concentration is much better than that of transferrin saturation in early disease (Bassett *et al*. 1984) making it the more appropriate screening study.

If a patient does not have a family history of hemochromatosis, the diagnosis is confirmed by chemical demonstration of increased iron stores in liver tissue obtained by percutaneous liver biopsy. The hepatic iron index, which is calculated as the ratio of hepatic iron concentration (in μmol/g dry tissue) to age (in years), very accurately identifies patients with homozygous disease; they have an index greater than 2. Patients with heterozygous disease or with hepatic siderosis due to alcoholism and individuals with normal hepatic iron stores have an hepatic iron index less than 2.

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