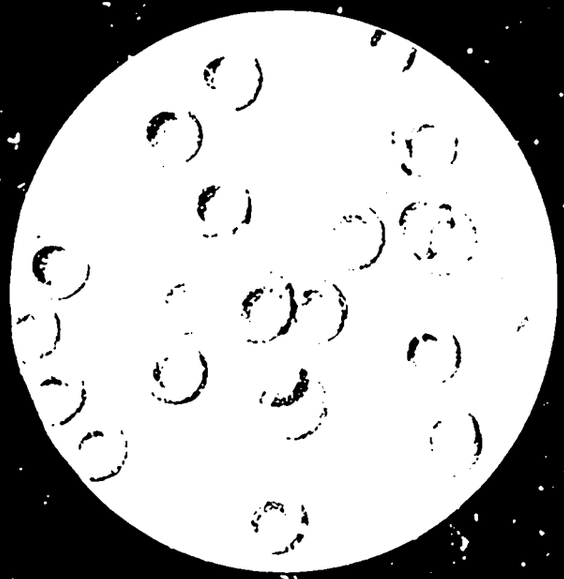
IRON DEFICIENCY IN INFANCY AND CHILDHOOD



The purpose of the International Nutritional Anemia Consultative Group (INACG) is to guide international activities aimed at reducing nutritional anemia in the world. The group offers consultation and guidance to various operating and donor agencies who are seeking to reduce iron deficiency and other nutritionally preventable anemias. As part of this service, INACG has prepared guidelines and recommendations for:

— Assessing the regional distribution and magnitude of nutritional anemia.

— Developing intervention strategies and methodologies to combat iron deficiency anemia.

— Evaluating effectiveness of implemented programs on a continuing basis so this evaluation of intervention techniques is a continuing and dynamic procedure.

— Research needed to support the assessment, intervention and evaluation of programs.

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IRON DEFICIENCY IN INFANCY AND CHILDHOOD

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the International Nutritional Anemia Consultative Group (INACG)
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I.
Introduction

Iron deficiency is the most commonly recognized form of nutritional deficiency in developing countries as well as in affluent societies. It is particularly prevalent among infants and young children because rapid growth imposes large iron needs and because most infant diets contain a marginal supply of iron. In recent years, iron requirements during early development have become better defined and greater attention is being paid not only to preventing deficiency, but also to avoiding unnecessary supplementation.* Studies of iron absorption have shown that the *form* of iron in the diet is at least as important as the amount, and that the age at which iron is administered influences its effectiveness. Much of this information is already being applied in new recommendations for infant feeding, in iron-fortification* of infant foods, and in planning school lunches, all of which are effective means of preventing iron deficiency in infants and children. Despite the rapid accumulation of new information, the diversity of dietary practices and economic circumstances in various parts of the world pose special problems that call for a variety of solutions. In this report we will review some of the characteristics of iron metabolism and nutrition in infants and children and discuss the application of this information to the prevention of iron deficiency.

*In accord with recent INACG and WHO publications, the term "fortification" will be applied to the addition of a nutrient (iron) to a food, whereas "supplementation" will refer to use of that nutrient in liquid or tablet form.

II.
Salient Features of Iron Metabolism

A. *Iron Compounds in the Body and Their Function*: Iron-containing compounds in the body may be grouped conveniently into two categories: (a) those that serve metabolic or enzymatic functions; and (b) those associated with iron storage. The first category includes hemoglobin, myoglobin, the cytochromes, and several other proteins that function in the transport, storage, and utilization of oxygen. Depending on the stage of development, these compounds account for 25 to 55 mg/kg body weight, more than 80% of which is in hemoglobin.[7,8]

The storage compounds account for 5 to 25 mg iron/kg body weight, primarily as ferritin and hemosiderin. These compounds are involved in the maintenance of iron homeostasis. When the supply of dietary iron becomes inadequate, iron is mobilized from ferritin and hemosiderin and serves to maintain the production of hemoglobin and other iron compounds with known metabolic functions,[8,9] not until this production becomes restricted is there likely to be any impairment of body function.

B. *Iron Balance*: A distinctive feature of iron metabolism is the remarkable extent to which the body conserves and re-utilizes iron once it has been absorbed. The quantity of body iron is normally maintained within narrow limits at each stage of growth and development. This is accomplished primarily through the regulation of intake: at the placental interface in the fetus, and post-natally by the intestinal mucosa. The amount assimilated each day is a small fraction of the total in the diet, but its regulation is the means by which iron homeostasis is maintained. Iron excretion occurs primarily through desquamation of the cells of the intestinal mucosa. The amount of iron lost in this manner is relatively fixed and is thought to vary over no more than a four-fold range in response to iron deficiency or overload.[8,9] This relatively restricted range of iron loss indicates the importance of absorption as the determining factor in iron homeostasis.

The degree of iron absorption may vary considerably depending on the abundance of body iron stores, the form and amount of iron in foods, and on the combination of foods in the diet. The major difference in iron balance between infants or children and adults is in the degree of their dependence on dietary iron. In adult men, about 95% of the iron required for the production of red blood cells is recycled from the breakdown of senescent red cells and only 5% comes from dietary sources.[8] In contrast, the one-year old infant, due to his rapid growth, is estimated to derive less than 70% of red cell iron from senescent red cells and requires about 30% from the diet (Table I).

C. *Iron Absorption*.[11,12] 1. *Heme vs. non-heme (inorganic) iron*. Food iron exists primarily in the non-heme form of inorganic iron III (ferric) complexes, with a smaller amount present in the heme proteins, hemoglobin, and myoglobin, which are present in meat. Inorganic iron III complexes in food are broken down during digestion, and the iron is partly reduced to the more readily absorbed iron II (ferrous) form. This conversion is facilitated by endogenous factors such as hydrochloric acid in the gastric secretions and by dietary constituents such as ascorbic acid. Ascorbic acid also enhances assimilation of ionic iron by another mechanism, the formation of readily absorbed iron-ascorbate complexes. Absorption of non-heme iron can be decreased by a number of dietary constituents such as phosphate and oxalate, which form insoluble iron compounds, a process that is favored by the alkaline

TABLE I.
GREATER DEPENDENCE ON DIETARY IRON IN THE INFANT COMPARED TO THE ADULT MALE

	Infant. Age 1 Year (Wt: 10 Kg)	*Adult Man (Wt: 70 Kg)*
Hemoglobin Iron		
In Circulation	270.0 mg	2,200 mg
Turnover day*	2.3 mg	18 mg
Dietary Iron		
Assimilated day*	0.7 mg	0.9 mg
% of Hgb Iron Turnover	30%	5%

*based on a red cell litespan of 120 days
*from references 3 and 10 for infants and 9 for the adult

environment of the small intestine. Absorption of inorganic iron is also decreased by the presence of food fiber and by certain metal chelators.

The smaller portion of dietary iron that is present in the form of heme is handled differently from non-heme iron. Heme is split from the globin portions of hemoglobin and myoglobin in the intestinal lumen. The heme is then assimilated intact by the intestinal mucosa, and ionic iron is released by a heme-splitting enzyme within the mucosal cell. Dietary constituents that decrease the absorption of non-heme iron appear to have significantly less effect on heme iron; a larger percentage of the dietary heme iron is therefore assimilated. In one study, for example, 37% of the heme iron was absorbed from a test meal in contrast to only 5% of the non-heme iron.[14] Thus, the quantitative relationship between heme and non-heme iron in the diet strongly influences the percentage of food iron that will be absorbed.

In infancy, there is almost no heme iron in the diet during the period when milk is the major source of calories. The relatively small amount of iron in milk is primarily in the non-heme form. After infancy, the amount of heme iron in the diet will increase at varying rates and to varying degrees according to eating patterns in different parts of the world. For example, the per capita intake of heme iron among adults in Australia, the United States and Sweden is estimated to be greater than 2.5 mg/day. In Tanzania and India it is less than 1.0 mg/day.

2. *Quantitation of iron absorption.* A reliable method of quantitating iron absorption is essential for determining which diets and methods of food fortification are likely to be successful in preventing iron deficiency. During the last decade, the techniques for measuring iron absorption have become more refined as radioisotopic techniques have supplanted the more tedious and imprecise balance methods. Many recent studies have employed the whole body counter to measure the amount of isotope retained after oral administration. Iron absorption can also be estimated from the amount of isotope incorporated into hemoglobin, measured in a sample of peripheral blood.

The most physiologic method of studying iron absorption involves feeding biosynthetically labelled plant and animal foods. Iron absorption from many foods has been studied by this laborious method. Fortunately, subsequent studies established that dietary iron absorption takes place from two pools, a heme iron and a non-heme iron pool, each of which can be labelled with a different radioactive isotope of iron.[14-16] Under the proper conditions[14,17,18] the labelled heme and non-heme iron added to food will exchange in the intestinal lumen with the corresponding forms of intrinsic dietary iron in the food or meal. In diets that contain little heme iron, total iron absorption can be measured with considerable accuracy by using a single non-heme iron isotope. These and other studies which were recently reviewed[19] have advanced the methodology and make it more practical than previously to measure iron absorption from foods or meals.

Despite standardization of experimental conditions, individual values for iron absorption fluctuate considerably among individuals with comparable iron stores and even in repeated studies on the same individual. However, relatively consistent results are obtained for specific foods or standard meals when absorption of iron is measured in sufficiently large groups of individuals.

3. *Results of iron absorption studies.*

a. *Standard doses of non-heme iron under fasting conditions*

When a standard dose of labelled iron (0.56 mg ^{59}Fe(II) with 17.6 mg ascorbate, a 1:10 molar ratio) is administered under fasting conditions, an inverse relationship between stainable bone marrow iron and iron absorption is evident both in children[12,20] and adults[21] (Fig. 1). However, there is a tendency to higher absorption values in children that can be largely attributed to their normal tendency to have scant iron stores. Thus, extrapolating absorption data obtained from adults will generally result in an underestimation of the percentage of iron absorbed by infants and children. Studies in both children and adults show that the intestinal mucosa plays a homeostatic role in decreasing iron absorption when stores are abundant and in increasing absorption when stores are diminished. This adaptive response favors the self-correction of mild iron depletion in infants and children.

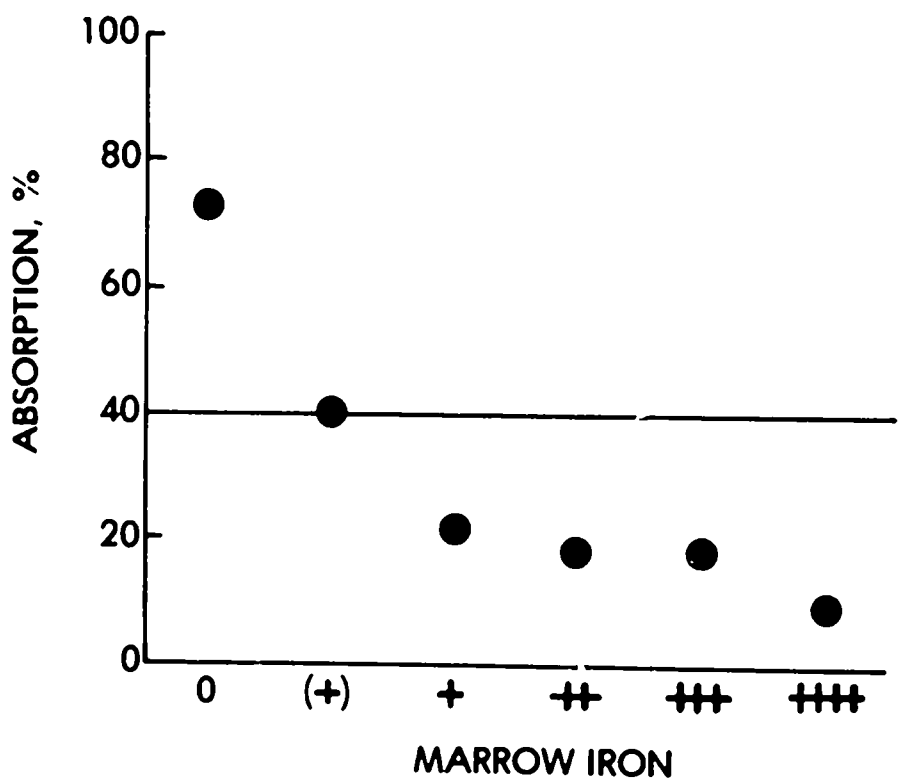

FIGURE 1—MARROW IRON

IRON ABSORPTION IN CHILDREN. Iron absorption is increased when stainable bone marrow iron is decreased. Studies were done by whole body counter after administration of 0.56 mg ^{59}Fe(II) under fasting conditions. The percent of iron absorbed is plotted against stainable iron in the bone marrow. No stainable marrow iron is designated as 0, traces of stainable iron as (+) and increasing amounts of stainable iron from + to + + + +. The horizontal line at 40% indicates the upper limit of iron absorption in adults with normal marrow iron stores (between + and + + + +). The figure is drawn from Bender-Götze et al.[12]

within the constraints imposed by the diet. Also, a dose of fortification or supplemental iron will be better utilized when it is given for an established deficiency than when it is administered in anticipation of future need. This consideration applies particularly to the first few months of life, when infants have abundant iron stores and iron is less well absorbed (Fig. 2).[22] A higher percentage of iron is absorbed during later infancy when iron stores are marginal.

b. *Iron absorption from single foods.* The percentage of iron absorbed by adults from individual, biosynthetically labelled foods averages from a mean of less than 1% to over 20%.[13] Iron is absorbed least well from foods of vegetable origin and best from meat.

Iron absorption from infant foods has been less extensively studied and almost entirely with exogenous label. In interpreting the results, both the percent and the amount absorbed from an average daily intake should be considered (Fig. 3). Milk, for example, is the dominant source of calories during the first year of life and the major determinant of infant nutrition; other foods are eaten in much smaller quantities. Both breast milk and cow's milk are unusually iron-poor foods.[23] The iron content of *breast milk* gradually falls from about 0.5 mg/liter during the first month after birth to about 0.3 mg/liter between 4 and 6 months of age.[24] The iron content of cow's milk or unfortified cow's milk formula may be influenced by introduction of contaminating exogenous iron in the course of its handling;

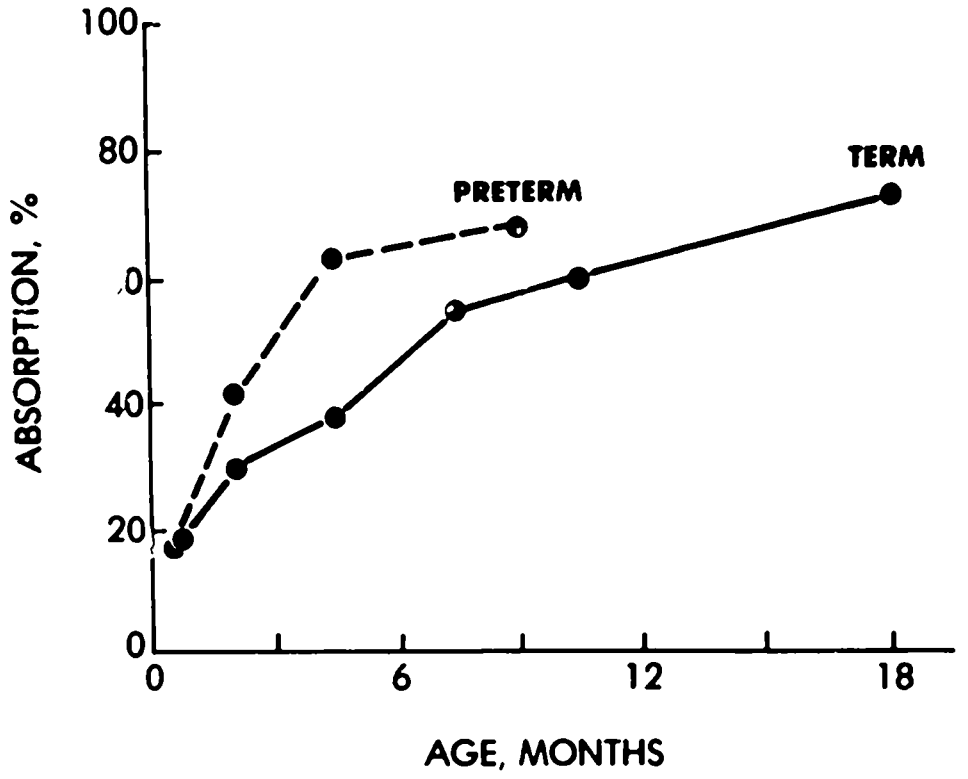

FIGURE 2—AGE, MONTHS

DEVELOPMENTAL CHANGES IN ABSORPTION OF IRON IN INFANCY. Studies were done by whole body counter after administration of 0.5o mg ^{59}Fe(II) under fasting conditions. Iron absorption increases with age as iron stores diminish. These changes occur earlier in the preterm than in the term infant. The data are from Götze et al[22] and additional unpublished results from the same group.

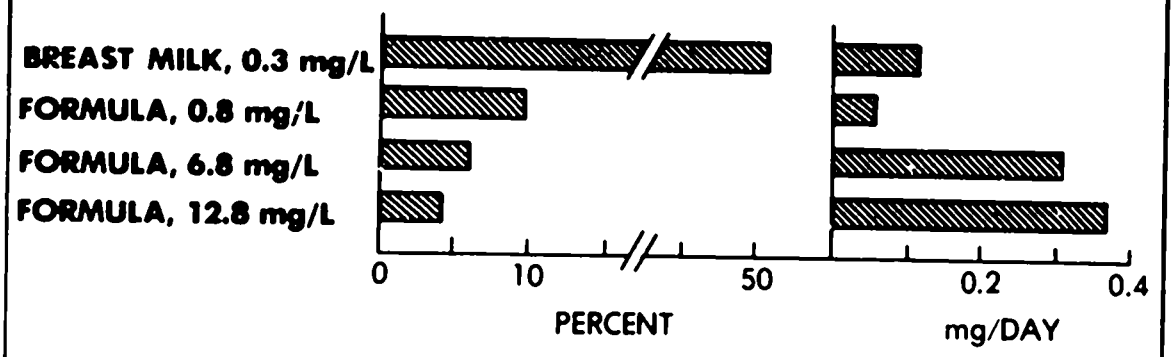

FIGURE 3

IRON ABSORPTION FROM BREAST MILK AND INFANT FORMULA. The percentage of iron absorbed is shown on the left. The total amount of iron assimilation that would be predicted from a usual daily intake of 750 ml of breast milk or formula is shown on the right. Calculations are based on absorption data in references 26 and 27. The iron content of breast milk or formula is indicated at the far left in mg per liter.

values range between about 0.5mg/liter and 1.0 mg/liter.[26] Expressed in terms of kilocalories, both human milk and cow's milk average less than 1 mg of iron/1000 kCal in contrast to the 6 mg/1000 kCal in the average mixed diet of developed countries.[27] The concentration of iron is decreased further when cow's milk is diluted with water for young infants.

Despite the small amount of iron in milk, differences in the percentage absorbed can have an important impact because milk accounts for most or all of the infant's diet (Fig. 3). An average of 49% of the iron in breast milk is absorbed, based on studies with an exogenous non-heme iron label;[28] this is an extraordinarily high figure compared to the value of 10 to 12% from unfortified cow's milk or cow's milk formula.[29] Analogous results were obtained when exogenous isotope was added to breast milk or cow's milk fed to adult subjects.[30] These findings help to explain the observation that prolonged breast feeding confers partial protection against the development of iron deficiency.[31,32] The basis for the excellent absorption of iron from breast milk is not known. The lower calcium and protein content of breast milk compared to cow's milk, and the high concentration of the iron binding protein, lactoferrin, have been postulated to play a role but cannot explain this phenomenon entirely.[33] There is some evidence that ingestion of breast milk, *per se* may condition the intestinal mucosa in a manner that facilitates iron absorption even when it is not administered with the breast milk.[34] This possibility warrants further study.

Most *iron-fortified cow's milk formulas* contain between 11 and 12 mg iron per liter as iron(II) sulfate or iron(II) gluconate; this represents about 15 to 20 times the iron in unfortified milk. The mean absorption of iron from fortified formulas is about 4%.[35,36] Although the percentage absorbed is less than half that of iron in unfortified formula, the absolute amount of iron retained is more than six times greater (Fig. 3). Since the percentage of iron absorbed is inversely proportional to the amount administered, it is reasonable to anticipate that lower concentrations of fortification iron would be almost as effective. Indeed, the amount of iron retained from formula containing 6 mg of added iron per liter is almost equivalent to that with 12 mg per liter[37] (Fig. 3). In evaluating these studies, it is important to bear in mind that the results were based on proprietary formulas containing other added vitamins and minerals. Actually, it is likely that the added ascorbic acid in proprietary formulas is a major factor in facilitating iron absorption (See Case Study).

Transitional or weaning foods become important sources of iron when the diet becomes more diversified late in infancy. Pertinent studies of single grain products or

legumes that might serve as weaning foods have been done primarily in adults. For example, adults were found to absorb 7% of the iron in soy beans, which is a relatively high value compared to other grains such as rice and maize, from which about 1 and 3.5% respectively were absorbed.[13] The method of commercial processing and/or home preparation may strongly influence the amount of iron absorbed. For example, it was found that children absorbed far more iron from a baked soy bean preparation than from one that was boiled.[32] Soy-based infant formulas have been extensively studied and are generally well-tolerated.[33] However, raw soy flour contains a trypsin inhibitor and possibly other heat-labile, physiologically active substances that presumably cause adverse intestinal reactions.

In the United States, proprietary dry cereal for infants is fortified with electrolytic iron of small particle size at a level of 45 mg/100 g dry cereal. This amount is about ten times greater than the iron present in whole grain products. Electrolytic iron is one type of elemental iron powder. Elemental iron powders are identified according to the process by which they are produced; other types include carbonyl iron, hydrogen-reduced iron and carbon monoxide-reduced iron. Factors that are associated with a high bioavailability of iron powders include small particle size, high solubility in hydrochloric acid, and a large reactive surface area.[34] The electrolytic iron currently used in dry infant cereals has a relatively small particle size and a large reactive surface area that would favor a high bioavailability. However, the absorption of electrolytic iron has not been studied in infants; the most pertinent studies in infants were performed with hydrogen-reduced iron of small particle size and showed absorption of about 4% of this iron from a mixture of wheat, barley, and maize.

Physiologic amounts of ascorbic acid in adults can result in a 2 to 5-fold enhancement of absorption of the intrinsic iron and fortification iron in cereal products.[35,36] It has been suggested that a ratio by weight of at least 1.5 iron to ascorbic acid be used in infant cereal and milk products that are fortified with iron.[11] Another option particularly for developing countries is to fortify purchasable food products, such as salt[37] or sugar[40] that are customarily added to cereals and other foods. Thus, iron absorption could be improved even with foods that would ordinarily not be ideal vehicles for fortification iron. However, salt and sugar should not be recommended in more generous amounts than is customary. Excessive salt is suspected of predisposing to later hypertension and frequent sugar use is associated with dental caries.

c. *Absorption from meals.* Iron absorption in adults is strongly influenced by the interaction of various foods consumed in the same meal and by the amounts of non-heme and heme iron that they contain.[7,14] These interactions also appear to apply to the infant's diet after solid foods have been introduced and breast milk or formula is no longer the exclusive source of nourishment. Evidence for such effects can be derived from estimates of the rate of increase in total body iron during the four-month period spanning the introduction of solid foods.[41] When total body iron was calculated from hemoglobin concentration, serum ferritin and body weight, it was found that the rate of iron accumulation changed at the age when unfortified solid foods (primarily fruits and vegetables) were introduced (Fig. 4). However, whether the rate of iron accumulation increased or decreased depended on the type of milk feeding. In breast-fed infants who were calculated to have a higher baseline body iron, the estimated rate of iron accumulation decreased after introduction of solid foods. In contrast, infants fed a home-prepared cow's milk formula who had a lower calculated baseline body iron were estimated to have an increased rate of iron accumulation after initiation of solid foods. More studies are clearly needed, not only to confirm these results, but also to determine the overall influence on iron absorption of various types of fortified and unfortified transitional infant foods.

Iron absorption from composite meals has been studied primarily in adults, but the general conclusions should also apply to children; absolute values for iron absorption are likely to be higher in children because of their normally lower iron stores. Meat and foods rich in ascorbic acid effect a substantial increase in iron absorption from a meal, whereas milk or tea will have the reverse effect. For example, the presence of meat,

poultry, or fish resulted in the absorption of about four times as much iron from a standard meal as when these were replaced by equivalent portions of cheese, milk, or eggs.[42] Other studies have shown the importance of the type of beverage that accompanies the food. The use of orange juice, for example, more than doubled the absorption of iron from the entire meal.[43] Milk was found to decrease the absorption of ferrous iron in infants by about 75% compared to the iron given alone.[44] Tea had an effect of similar magnitude on iron alone or iron in various foods.[45] On the basis of these dietary interactions, one would anticipate a high prevalence of iron deficiency when the diet consists almost entirely of cereals or when there is a high intake of milk or tea. Conversely, diets rich in meat and or ascorbic acid would be less likely to be associated with dietary iron deficiency.

d. *Absorption of iron used in food fortification.*[46] In addition to being well assimilated, fortification iron must also be stable during storage and should not result in undesirable changes in the color or appearance of the food to which it is added. Iron (II) sulfate, for example, is well absorbed when freshly added to foods but is readily oxidized during storage, causing discoloration and alteration in flavors if the container is not airtight. It has therefore been most successfully used in fortification of foods in airtight containers, such as canned infant formulas and certain infant cereals in jars, and in flour products, to which it can be added just prior to baking.

The less reactive iron compounds such as iron (III) pyrophosphate, iron (III) orthophosphate, and metallic, reduced iron of large particle size came into use to fortify infant cereal and flour

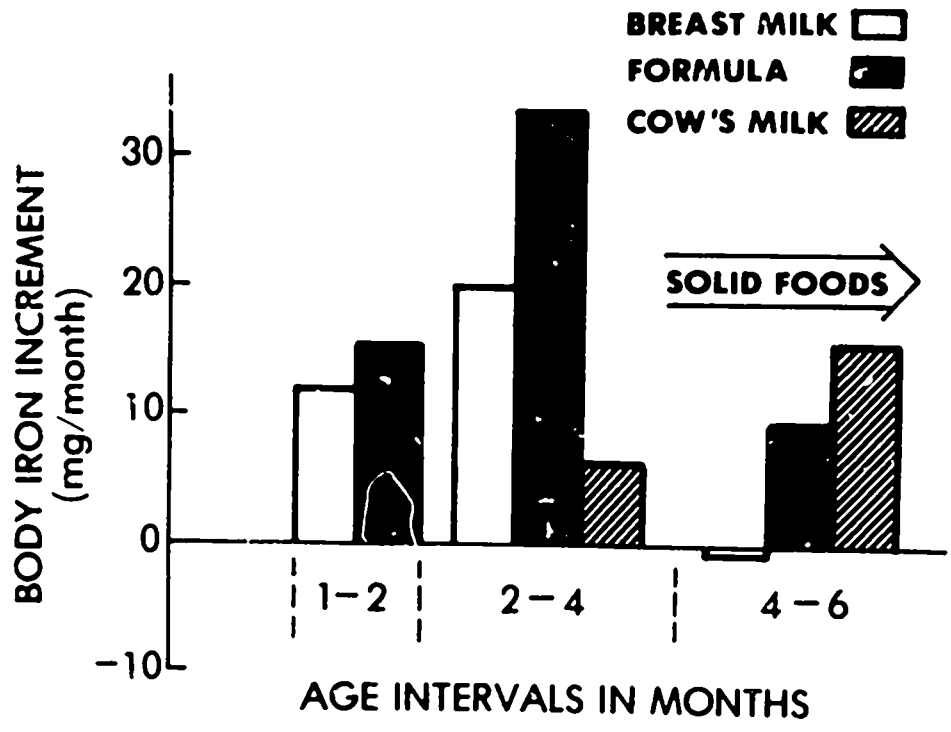

FIGURE 4 – AGE INTERVALS IN MONTHS

Monthly increments of estimated total body iron in the three study groups during the first six months of life (Saarinen and Siimes[47]). The rate of accumulation of body iron appears to decrease in breast-fed infants after solid foods are introduced.

products because they did not result in undesirable changes in the color or flavor of foods in packages that are permeable to air. However, these compounds have been found to be so poorly absorbed that they have probably had little impact on iron nutrition. This realization has led to the testing of compounds that meet the criteria of being both reasonably well assimilated (at least 50% as well as iron (II) sulfate) as well as allowing storage of foods for prolonged periods. Among the compounds that appear to fulfill these criteria is electrolytic iron powder of small particle size (in which most of the iron by weight is in particles <10 μm in diameter) which is used to fortify infant cereal and bread. Alternatively, a compound such as iron (III) pyrophosphate, which is poorly absorbed when used alone, may become effective when combined with ascorbic acid. This combination has been successfully used to fortify salt without causing discoloration during storage. This topic is discussed more fully under heading V.

e. *Conclusions.* From this brief review of the bioavailability of iron from various foods and iron compounds, it can be appreciated that strategies for preventing iron deficiency in infants and children must be considered primarily in terms of dietary composition and food habits and must be tested under pilot conditions before implementation in large populations (see Case Study). Because iron absorption varies so markedly according to the diet, recommended dietary allowances for iron merely provide rough guidelines. It is possible on the one hand to develop iron deficiency while ingesting large amounts of iron-rich foods in which the iron is poorly absorbed, or conversely, to escape deficiency despite ingesting far less than the recommended amount but in forms that are particularly well absorbed. It is also important to consider any dietary recommendations aimed at the improvement of iron nutrition from a broader economic and nutritional perspective. For example, overemphasis of meat and avoidance of cereal in the diet might be effective in preventing iron deficiency, but could be economically impractical or actually defeat other nutritional objectives.

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III.
Changing Iron Needs During Development

A. *Maternal-Fetal Relationships* Pregnancy is a period of increased need for dietary iron However, the consequences of iron deficiency affect the mother far more than the fetus In the fetus, the ratio of iron to body weight remains relatively constant throughout pregnancy at an average level of 75 mg/kg (Fig. 5).[1] The iron requirements of pregnancy during the latter half of pregnancy are high, and iron deficiency anemia is common in the mother, particularly during the latter half of pregnancy. However, with few exceptions,[2] comparisons of iron-deficient and iron-sufficient mothers not only show that the hemoglobin concentrations of their offspring are indistinguishable at birth,[1,4] but that they remain so during the remainder of the first year of life.[5,6] Similarly, estimates of neonatal iron stores based on the serum ferritin concentration show little[7] or no[8] difference between groups of infants whose mothers had iron deficiency anemia and those who were non-anemic The high priority of the infant for available iron continues into lactation. The iron status of the mother has little influence on the concentration of iron in breast milk.[9,10] These findings should not be construed as minimizing the importance of iron nutrition in pregnant and lactating women. However it should be realized that treating the mother with iron is of more demonstrable benefit to her than to the fetus or to the nursing infant

B. *Postnatal Development*[11,12] The newborn infant is well supplied with iron, not only in terms of substantial concentrations of storage iron, but also in the form of a high concentration of hemoglobin. However, at birth the reliable trans-placental delivery of iron is replaced by a smaller and less consistent dietary supply. Nevertheless, within the first year of life, the average term infant must almost double his body iron as he triples his body weight. At birth, the *term infant* has a total body iron averaging about 75 mg/kg.[1] Iron stores contribute approximately 25% of this amount. After birth, there are profound changes in iron metabolism and in rate of erythropoiesis that can be conveniently divided into three stages (Fig. 6.)

The *first stage*, encompassing the first six to eight weeks of life, is marked by a decline in the concentration of hemoglobin from the highest to the lowest levels observed during any period of development. This decline is attributable primarily to an abrupt decrease in erythropoiesis, in response to increased postnatal delivery of oxygen to the tissues. The percentage of erythroid precursor cells in the bone marrow declines from a mean of about 30% at birth[13] to 10% between one week[14] and a month of age.[10] Extra-medullary hematopoiesis comes to a halt during the same period. In the peripheral blood, these changes are reflected in the disappearance of nucleated red blood cells and the precipitous fall in the reticulocyte count.[15] During this marked depression of erythropoiesis, the concentration of hemoglobin decreases at a rate that is determined primarily by the lifespan of red blood cells that were produced before birth. Because a relatively small proportion of the iron from senescent red cells is reutilized for erythropoiesis, the remaining iron temporarily augments iron stores.[16,21,22] The percentage of dietary iron absorbed during this period is lower than in later infancy, probably due to the inhibitory effect of large iron stores on absorption.[24]

During the *second stage* the normal decline in hemoglobin concentration is reversed. Erythropoiesis becomes more active, as indicated by an increase in the percentage of erythroid precursors in the bone marrow[25] and by an elevation of the reticulocyte count[26] to far above the normal adult level (Fig. 6). The concentration of hemoglobin rises from its

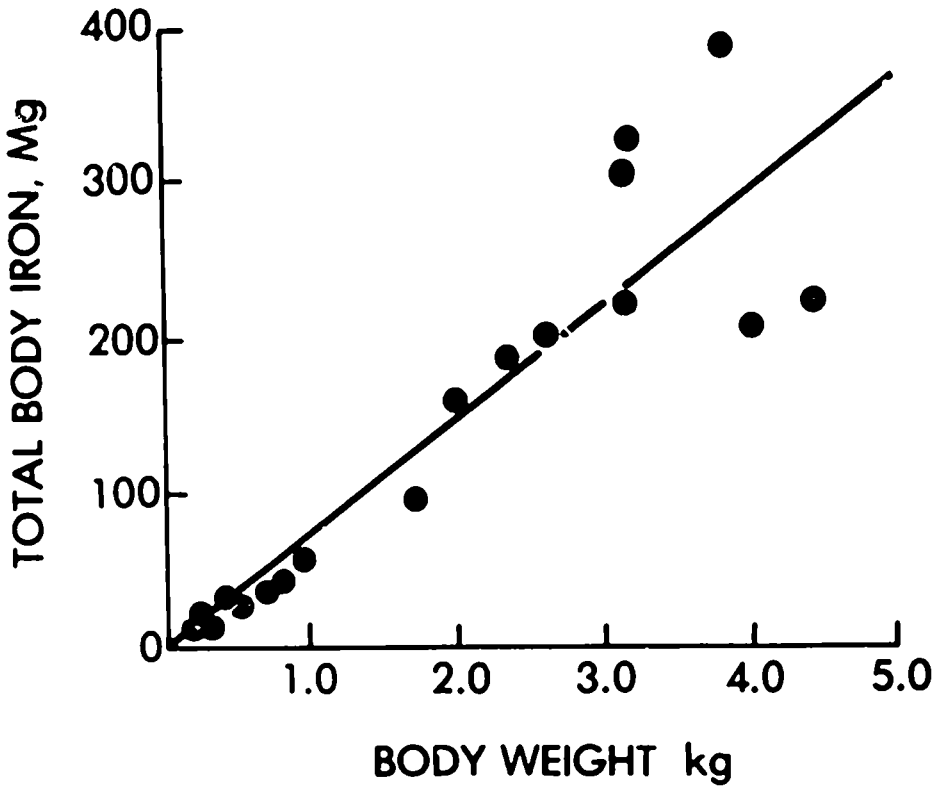

FIGURE 5

IRON CONTENT AND BODY WEIGHT IN THE FETUS AND NEWBORN CHILD. Total body iron increases in a linear fashion as body weight increases. From Widdowson and Spray.[1]

lowest mean value of about 11 g/dl and is subsequently maintained at an average value of about 12.5 g/dl during the remainder of the first year of life.[illegible]

A *third stage* is characterized by an increased dependence on dietary iron. A rapid rate of growth and the low iron content of either breast or unsupplemented cow's milk products predispose to the depletion of iron stores. If iron stores are exhausted, then the rise in concentration of hemoglobin that started during the second state can be either curtailed or reversed. Not until the third stage is additional dietary iron likely to be effective in augmenting the concentration of hemoglobin.[illegible]

In *infants of low birth weight* the total body iron at birth is less than in the full-term infant; however, the ratio of iron to body weight is the same.[illegible] Infants of low birth weight have a more rapid rate of postnatal growth and exhaust their iron stores at an earlier age. Consequently, the requirements for exogenous iron are greater than in the term infant[illegible] and the third stage of iron nutrition begins earlier.[illegible] As in the term infant, the postnatal fall in hemoglobin concentration to a low value between six weeks and two months of age occurs before iron stores have become depleted and is not influenced by iron supplementation. However, in the infant of low birth weight, the nadir in concentration of hemoglobin is much lower than in the term infant (Fig. 6) and is followed by a rise in reticulocyte count that is higher than in term infants. If the supply of dietary iron is adequate, the concentration of hemoglobin in preterm infants gradually catches up with that of term infants until mean values are equal by nine months of age.[illegible]

The highest prevalence of iron deficiency anemia is likely to occur between about six months and two to

three years of age when iron stores are likely to become depleted.[11] Subsequently, the demand for iron decreases as the rate of growth slows.

C. *Childhood* Although more investigation has been focussed on infancy, iron deficiency is also a common problem throughout childhood. During the preschool period, between one and five years of age, the composition of the diet is often unfavorable for iron absorption. This is particularly true for developing countries, in which cereals are usually the major constituent of the diet. Economic factors often limit the intake of meat, poultry, or fish, foods that would enhance the absorption of iron from cereal. Cultural patterns also frequently favor giving the father and other adults first access to these more expensive foods of animal origin. If unrefined cereal products are used, their high fiber content may also restrict the absorption of iron as well as other trace minerals, such as zinc.

In developed countries, the availability of dietary iron is often restricted by an excessive use of milk among pre-school children. Milk not only displaces other more iron-rich foods from the diet, but is rich in calcium and phosphorus which form insoluble

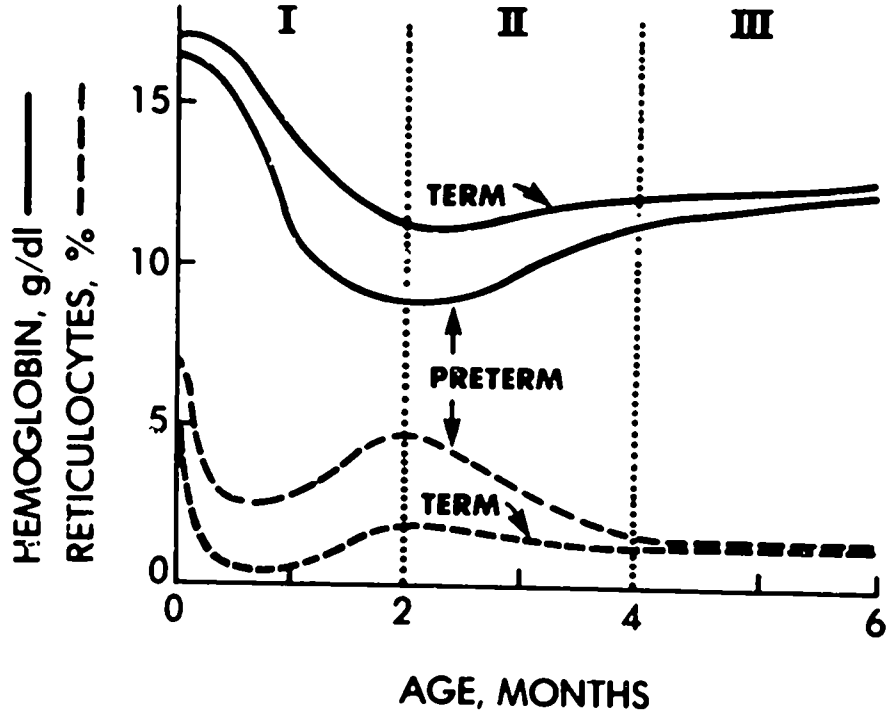

FIGURE 6

THREE POSTNATAL STAGES OF IRON BALANCE AND ERYTHROPOIESIS. Mean values for concentration of hemoglobin and reticulocyte count are shown for term infants and preterm infants. The duration of the stages is shown for term infants. I Immediately after birth, the rate of erythropoiesis (reticulocyte count) and concentration of hemoglobin decrease in response to increased availability of oxygen. Storage iron is augmented. II At about 2 months of age, the concentration of hemoglobin has fallen to its lowest point and the rate of erythropoiesis is increased. Storage iron gradually decreases. III Iron stores become depleted by about 3 months of age in preterm infants and 4 months in term infants unless replenished by an adequate exogenous supply of iron. During this stage anemia is likely to respond to iron administration. (Data for newborns from Oski and Naiman[4] and other hemoglobin values are from Lundstrom et al.[7] and Saarinen and Siimes;[11] reticulocyte values are from unpublished data on the same infants described in the preceeding two studies).

complexes with iron.

In tropical and semi-tropical parts of the world where children are often barefoot, hookworm infestation is a common problem. Intestinal blood loss can then become a major factor contributing to iron deficiency.[12,13] Pinworm infestation may also result in substantial blood loss[14] and is also common in temperate climates. In order to prevent iron deficiency in hookworm areas more dietary iron is generally required than elsewhere.

D *Adolescence* Acceleration of growth, particularly during the years of sexual maturation, imposes increased requirements of iron primarily for the production of hemoglobin. For example, during the peak year of their adolescent growth spurt, boys gain an average of 10 kg (Fig. 7).[15] This can be calculated to require a net increase of about 300 mg of iron merely to maintain a constant concentration of hemoglobin in an expanding blood volume. However, the concentration of hemoglobin also increases between 0.5 and 1.0 g/dl/year at this age.[16,17] An increase in hemoglobin concentration of 0.5 g/dl in a representative 55 kg adolescent boy requires more than 50 mg iron. Consequently, the average adolescent boy requires a net increase of about 350 mg of iron during his year of peak growth. This peak rate of growth, and presumably the concurrent peak increase in concentration of hemoglobin, are far sharper than is apparent from growth and hemoglobin percentile curves; this is because there are individual variations in age of peak growth which become obscured when average values for each age are calculated.[15]

In adolescent girls, iron needs are also large, but they

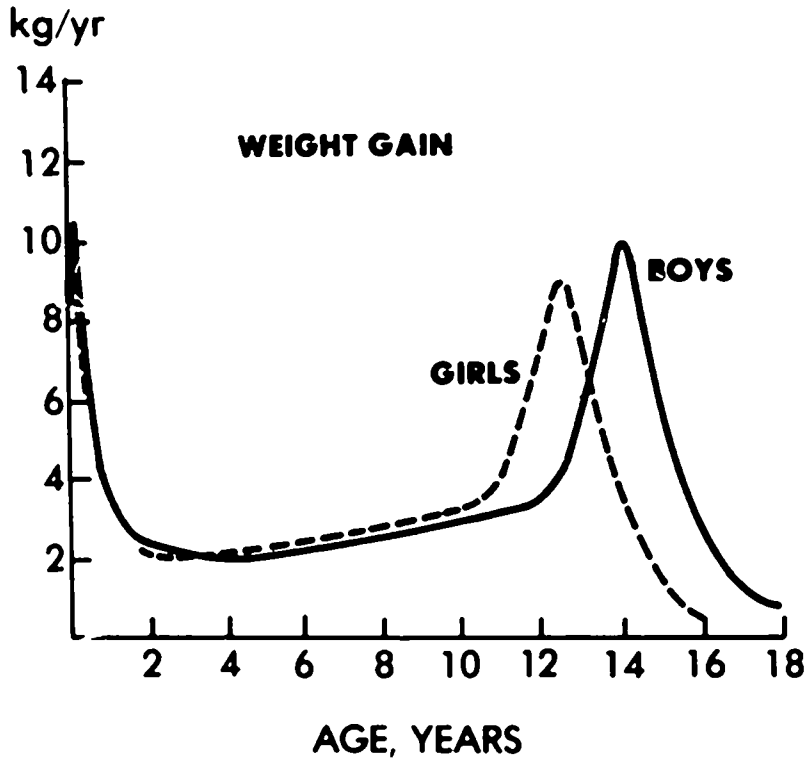

FIGURE 7—AGE, YEARS

TYPICAL INDIVIDUAL WEIGHT-GAIN CURVES FOR INFANCY AND CHILDHOOD. Note the adolescent peak weight gain of 10 kg/year for boys and 9 kg/yr for girls (drawn from Tanner et al[15])

do not peak as sharply as in boys because the maximum yearly weight gain is somewhat less than in boys and because the concentration of hemoglobin in girls increases only slightly during this period. The greatest average weight gain of 9 kg/yr in girls requires about 280 mg of iron for the maintenance of a constant concentration of hemoglobin. The onset of menses usually follows the peak of adolescent growth. The median menstrual loss of about 30 ml per menstrual period of 15-year-old girls involves a net loss of about 175 mg iron/year.[38] However, there are great variations among individuals, and those with the largest losses are at highest risk of iron deficiency.

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IV.
Iron Requirements

Recommendations for dietary iron in infancy and childhood have been partly derived from estimates of the increase in total body iron with age, with the assumption that the average iron absorption is 10 percent.[1] Because of the questionable nature of the latter assumption, it is advisable to validate such estimates by the pragmatic test of determining the amount of dietary iron necessary to prevent iron deficiency anemia under various dietary conditions. The bioavailability of iron will differ according to the nature of the diet, and it is helpful to take this into account in setting dietary standards. In 1972, the World Health Organization's recommendations for iron[2] were varied according to the amount of animal-derived food in the diet (Table II) on the basis of the finding that meat enhances absorption of iron from

TABLE II
RECOMMENDED DAILY INTAKES OF IRON (mg/d)*

		USRDA (1974)	*FAO/WHO (1970)*		
			Percent Calories From Animal Foods		Absorbed Iron
	Age Years	*Iron, mg/day*	<10%	>25%	
			Iron, mg/day		
Infants	0.5-3	15	10	5	1.0
Children +	4-10	10	10	5	1.0
♂	11-18	18	18	9	1.8
♀	11-18	18	24	12	2.4
Men	19	10	9	5	0.9
Women	19-50	18	28	14	2.8
Women	51	10	9	5	0.9

* Recommended daily intakes are estimates of "upper limits" of requirements in normal individuals; they do not take into account any additional requirements imposed by unusual environmental stress, by disease or by parasitic infection

\+ Age ranges for children are those of the USRDA; ranges in the FAO/WHO recommendations differ slightly as follows 4-12, 3-16 yr

the diet as a whole. In the light of more recent studies it may be more appropriate to vary iron recommendations specifically in relation to meat intake, since animal-derived dairy products and eggs do not enhance iron absorption. This consideration is especially pertinent to iron recommendations for infants because their diet rarely contains much meat but often consists primarily of dairy products.

The iron requirements of the infant and child, despite their much smaller body size, are almost as high or higher than those of the adult. This relationship is evident in the WHO recommendatons as well as the U.S. Recommended Dietary Allowances.[3]

The recommendation of the Committee on Nutrition of the American Academy of Pediatrics provides more detailed guidelines for iron nutrition in infants.[4] The suggested intake for term infants is 1 mg iron/kg body weight/day to a maximum of 15 mg, starting no later than four months of age and continuing until three years of age. The recommendation for low birth weight infants is 2 mg/kg/day to a maximum of 15 mg starting no later than two months of age. These recommendations need not be interpreted too rigidly, since dietary composition has such a great influence on iron absorption. Furthermore, the amounts are generous enough to encompass most individual variations, as is generally true of nutritional requirement guidelines.

IV.
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V.
Methods of Preventing Iron Deficiency

A. *Goals and Justification* It is generally agreed that an optimal iron intake is one that allows the unrestricted production of hemoglobin. This can be accomplished with ordinary dietary constituents under most conditions and with only modest iron fortification of foods under other circumstances. The establishment of iron reserves is also a desirable goal, but it should be recognized that the relatively large iron stores of the well-nourished adult male are not a physiologic or desirable reference standard for infants, children, and women.

It may be difficult to justify public health programs focused on the prevention of iron deficiency when they compete with other programs designed to prevent more obviously handicapping or life-threatening disorders. For this reason it may be more practical to incorporate the goal of preventing iron deficiency into a nutritional program of broader scope. For example, the distribution of special weaning foods may be primarily intended to combat protein-calorie malnutrition. However, such products can also serve as convenient vehicles for iron fortification at virtually no additional cost. Alternatively, prevention of iron deficiency may be an unanticipated by-product of programs that are not directly aimed at nutrition as such. For example, improved environmental sanitation may improve iron absorption by decreasing the prevalence of infection and inflammatory disease or it may decrease blood loss through elimination of intestinal parasites.

In justifying the prevention of iron deficiency on its own merits, it is important to emphasize that it is a prevalent disorder, that it can be inexpensively avoided in many settings, and that prevention involves broader benefits in terms of improved general nutrition and health. The evidence for deleterious effects of iron deficiency have been reviewed recently.[1,2] Among the consequences of iron deficiency, decreased exercise tolerance and decreased capacity to do work are handicaps demonstrable even with mild anemia.[3] Retardation of growth with recovery following treatment with iron has also been described.[4] In addition, evidence of systemic consequences that are independent of anemia is being recognized in man and in the experimental animal. These include compromised cellular immunity, an effect on muscle function that is independent of anemia, and possibly, impaired intellectual function.[2]

B. *General Approaches.* Methods for preventing iron deficiency within the scope of unfortified food products include: 1) the encouragement of prolonged breast feeding; 2) avoidance of excessive fresh cow's milk; 3) inclusion of facilitators of iron absorption (meat, fish, or poultry or ascorbic acid-rich foods) when solid foods are fed; and 4) avoidance of excessive weight gain.

Since compliance with these recommendations may be restricted by social and economic factors, and because some infants and children may have increased iron needs, it may be necessary to use iron-fortified foods for the prevention of iron deficiency in some circumstances. How some of these approaches can be combined under various conditions will be outlined in subsequent paragraphs.

C. *Role of Milk Feeding* 1. *Breast milk:* In term infants, prolonged breastfeeding offers substantial protection against the development of iron deficiency anemia. Term infants who were breast fed for at least six months retained substantial iron stores at that age, based on their serum ferritin values. However,

during the remainder of the first year of life, an increasing percentage of infants depleted their iron stores as indicated by serum ferritin values below 10 μg/liter (Fig. 8). In addition their mean hemoglobin concentration fell slightly (0.5 g/dl), but significantly, below that of infants receiving iron fortified formula.* Only eight infants in the above study were given breast milk as their exclusive source of milk for

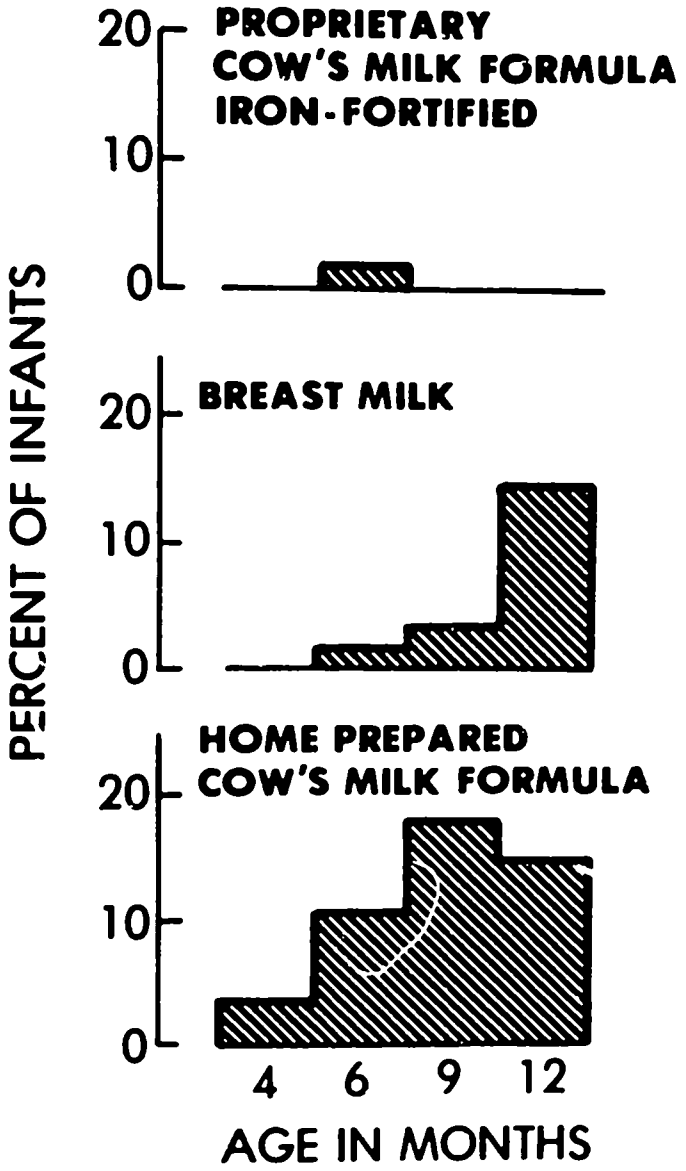

FIGURE 8--AGE IN MONTHS

DEPLETION OF IRON STORES DURING INFANCY. The vertical axis shows the percentage of term infants with serum ferritin values below 10μg/liter. The percentage of infants with low serum ferritin values increases during the latter part of the first year of life in infants fed unfortified formula and to a lesser degree in infants fed human milk (Saarinen and Siimes, 1978).*

nine or more months: their hemoglobin and other parameters of iron nutrition did not differ significantly from those of the other breast fed infants who were weaned earlier. There is little information regarding the iron status of infants who are exclusively breast fed for longer periods. Four infants who were fed no food other than breast milk were reported to have normal hemoglobin values when examined between 9 and 12 months of age.[7] These data indicate that breast milk alone contains an adequate supply of iron for term infants for at least the first six months, but that additional sources of iron are probably desirable subsequently.

In *preterm infants* breast milk alone does not provide an adequate supply of iron (Fig. 9). In infants fed breast milk, additional iron in the form of drops should be supplied, starting no later than two months of age and maintained at least until one year of age. After four to six months of age, iron-fortified weaning foods may provide an alternative.

Worldwide trends in breastfeeding have been discussed in a recent review.[8] In many developing countries, infants are being weaned at an earlier age to facilitate the mothers return to work or to conform to the mistaken view that early weaning is consistent with ideal practices of modern infant feeding. The reversal of these trends would improve both iron nutrition and general nutrition of the infant. In addition, longer breastfeeding should decrease the prevalence of enteric infections in areas where environmental sanitation makes it difficult to prepare and preserve infant foods in an uncontaminated state.

2 *Cow's Milk Formula* Proprietary infant formulas are modified in composition to resemble breast milk, contain added vitamins and minerals and are available either fortified or unfortified with iron. Formulas are available in powdered or liquid form. Iron (II) sulfate, gluconate, or saccharate are commonly used in a concentration that supplies about 12 mg of elemental iron per liter. When iron-fortified formula accounts for the major source of caloric intake, it has the advantage of delivering a relatively constant and predictable amount of iron.

Iron fortified formula is effective in preventing iron deficiency anemia in the first year of life both in term[8,9,10] and preterm infants.[11,12] Based on recent experience (see Case Study), it appears that the ascorbic acid added to proprietary formulas plays a role in their effectiveness.[11]

Although proprietary infant formulas are excellent vehicles for iron fortification, they should be recommended only with certain qualifications. Where families cannot afford the high cost of proprietary formulas and where lack of refrigeration and running water favor bacterial contamination, improper storage and excessive dilution of formulas can lead to diarrhea and undernutrition. For this reason, aggressive marketing techniques that might decrease breast feeding should be discouraged. In the developed world, a disadvantage of formula is the ease with which excessive calories can be provided to the infant. This can lead to obesity and discourage the gradual diversification of the diet that is desirable after six months to one year of age. Consequently, it is considered best to limit the volume of infant formula to about one quart or liter per day in order to encourage the introduction of iron-rich foods into the diet and to set the pattern for a more varied diet during the second year of life.[14]

There is no demonstrable advantage in providing iron fortification or supplementation during the first four months of life for term infants and during the first two months for preterm infants. During these periods a smaller percentage of iron is likely to be absorbed by the body than at a later age.[15]

A consideration peculiar to the premature infant is an *interaction between vitamin E deficiency and iron administration* that can result in hemolytic anemia during the first two months of life.[16,17] Premature infants, particularly those with a birth weight less than 1500 g, absorb very little of the vitamin E that is present in breast milk or formula or that is administered in the form of alpha tocopherol acetate. Vitamin E is effective as an antioxidant that protects the lipids of the red cell membrane against oxidative destruction. The major clinical manifestation of vitamin E deficiency is a mild hemolytic anemia. Iron functions as a co-factor in lipid peroxidation and aggravates hemolysis in vitamin E deficiency. The risk of hemolysis also depends on the saturation of dietary fatty acids. When the diet is relatively rich in polyunsaturated fatty acids as in many infant formulas, the composition of the lipids in the red cell membrane undergoes a corresponding change, and the cell becomes more subject to oxidative damage and hemolysis. The interaction between these three factors—vitamin E deficiency, iron administration, and a diet rich in polyunsaturated fatty acids—became evident when small premature infants were fed certain iron supplemented proprietary formulas.[18] Due to the recognition of this problem, vitamin E deficiency has become less common due to the manipula-

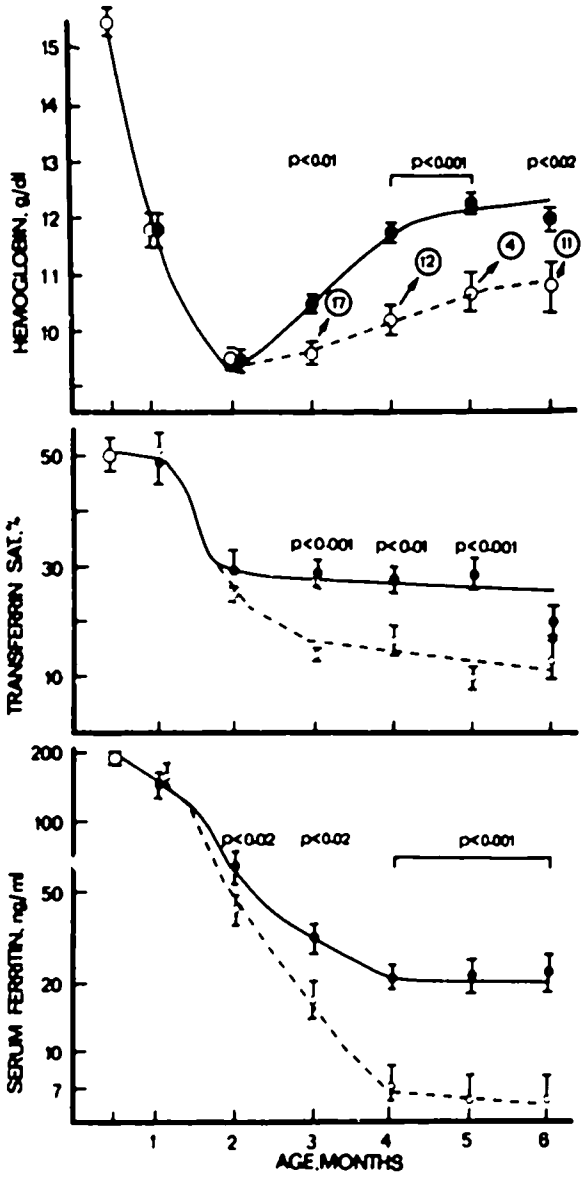

FIGURE 9

CONCENTRATION OF HEMOGLOBIN, TRANSFERRIN SATURATION, AND SERUM FERRITIN IN LOW BIRTH WEIGHT INFANTS. All infants were given banked breast milk during their hospital stay. They were randomly assigned to groups receiving no iron supplementation (--O--) or 2 mg/kg per day starting at two weeks of age (--●--). Means ± SEM are indicated. The number of unsupplemented infants at each age who were started on iron supplementation is shown within circles. The first detectable difference in the unsupplemented group was a lower mean serum ferritin concentration at two months of age. Anemia and decreased transferrin saturation were not significant until three months of age (Lundström et al. 1977).

tion of one or more of the three pertinent variables: 1) iron administration may be delayed;[17] 2) a bioavailable form of vitamin E can be administered;[18] or, 3) breast milk or a formula that is comparably rich in saturated fatty acids can be fed to small, premature infants.[19]

3. *Fresh or Pasteurized Cow's Milk.* The use of unmodified fresh or pasteurized cow's milk during the first six months of life should be avoided for several reasons.[20] The composition of cow's milk differs drastically from human milk, particularly in providing excessive amounts of protein and minerals. Cow's milk appears to decrease absorption of iron from other foods.[21] In addition, the consumption of excessive amounts of fresh or pasteurized cow's milk may be associated with occult intestinal blood loss during infancy[21,22] and contribute significantly to the development of iron deficiency.[23] Blood loss is usually reversed by substituting heat-processed cow's milk formula or soy-based formula (Fig. 9) for fresh cow's milk formula or soy-based formula (Fig. 10) for fresh cow's milk, whether or not iron is administered.[21,22,24] This suggests an intestinal sensitivity to a protein component of milk, such as lactalbumin, which can be modified by heat processing. Some data suggest that subclinical blood loss of this type is common[24,25] and that only the most severe cases come to clinical attention. However, it is difficult to exclude the possibility that significant blood loss

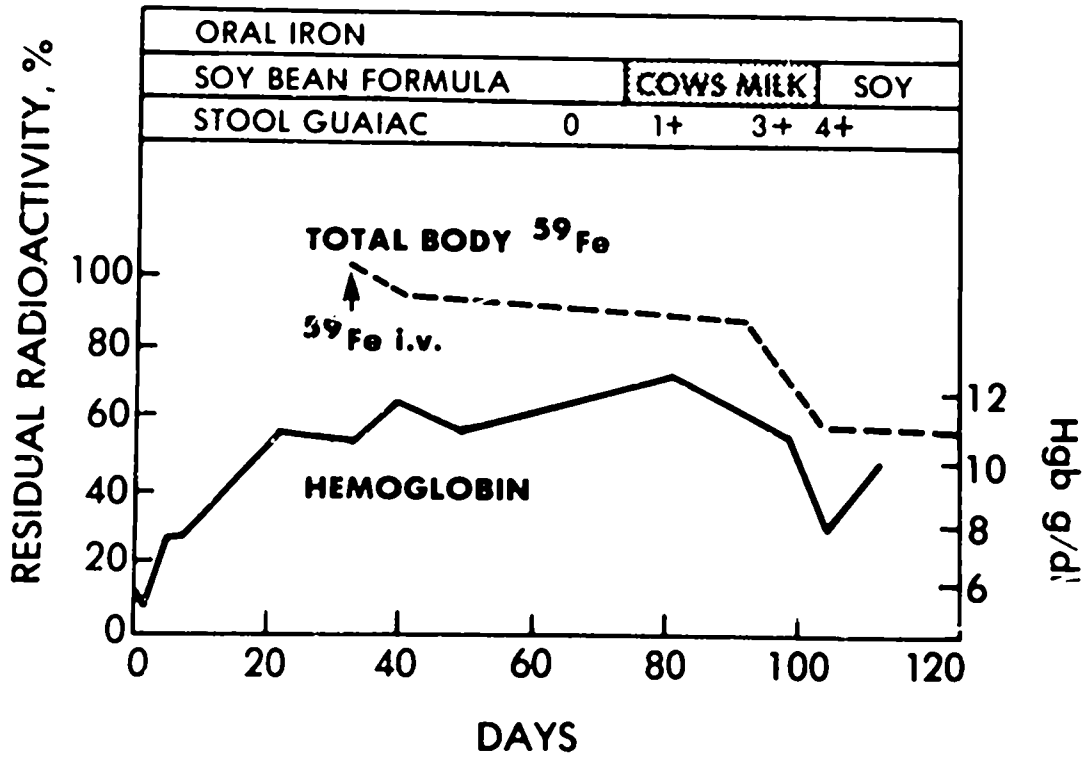

FIGURE 10—DAYS

ENTERIC BLOOD LOSS RELATED TO COW'S MILK INGESTION. An unusually severe example is illustrated by a 3 month old infant with iron deficiency anemia at an age when it is unlikely to be due to dietary iron deficiency. For this reason, he was investigated for intestinal blood loss in relation to diet. After intravenous administration of ^{59}Fe II, the loss of total body radioactivity was normal when the infant was fed a soy formula but increased to the equivalent of 22 ml of blood loss per day after challenge with pasteurized cow's milk. Hemoglobin concentration fell and stool guaiac became positive concurrently. (from data of Mentzer, 1972, taken from ref. 24.)

represents a relatively rare idiosyncratic reaction.

After six months of age, unmodified fresh or pasteurized cow's milk is an excellent food, provided that excessive volumes are avoided. The consumption of 0.75 liter/day, equivalent to that supplied by breast milk, in the average infant, is a reasonable volume. As in the case of cow's milk formulas, larger amounts discourage the development of a diversified diet. Even in adults, there is evidence for an association between iron deficiency and a high intake of dairy products.[7]

4. *Evaporated Milk and Powdered Whole Milk.* These are relatively inexpensive and easily stored products which can be used to prepare an infant formula. However, they contain no added iron. Powdered skim milk is less expensive than powdered whole milk, but has the disadvantage of a low caloric density and a marginal supply of essential fatty acids. With either product, it is desirable to add iron to the diet from another source after four months for term infants and after two months for preterm infants.

D. *Transitional Foods.*[8] Foods that are specially for the infant and that bridge the period between exclusive breast or bottle feeding and sharing of the family meals can be termed transitional foods, weaning foods, or beikost. These foods are most readily introduced into the infant's diet after about four months of age when his neurological development begins to become adapted to chewing as an alternative to sucking, and when spoon feeding becomes more readily accepted. By four to six months, most infants have developed sufficient head control to indicate either that they are hungry or that they no longer wish to accept food. Prior to this time, there is an element of forced feeding when solid foods are given. Earlier initiation of transitional foods is also discouraged because of the likelihood that this predisposes to the development of obesity and allergy. Where environmental sanitation is poor, it may be best to delay the use of transitional foods until after a year of age.

The traditions surrounding the selection of transitional foods differ in various parts of the world. Although initiation of transitional foods is often considered detrimental to the maintenance of breast feeding, this need not be the case. In some countries, breast feeding may be maintained two or more years with concurrent feedings of solid food. The first transitional food may be a cereal product prepared as a gruel or it may be pureed fruit or vegetable. In certain countries, specially prepared infant cereals are heavily fortified with sufficient iron to meet almost half of the estimated dietary iron needs in one serving. Indeed, the feasibility of preventing iron deficiency by relying on iron-fortified infant cereal as the major source of iron during the first year of life has been demonstrated (Fig. 11). In some countries fortification is at a much lower level, similar to that used to fortify other grain products; often it is the policy to add iron and other nutrients only to the level originally present in whole grain. In other areas, no cereal products are iron-fortified.

If iron-fortified dry infant cereal is used as one of the first transitional foods, this will take advantage of the improved iron absorption that characterizes the period after four to six months of age when iron stores become diminished.[9] The principal advantages of dry infant cereal as a vehicle for iron during the pre-school period are its low cost and ease of storage. These advantages also apply to the transitional foods available from international and certain bilateral agencies, e.g. United States Agency for International Development products such as Wheat Soy Blend, Corn Soy Milk, and Instant Corn Soy Milk, which contain added ferrous fumerate to a level of 18 to 21 mg/100 g dry product.[10] The fortification of cereal products with both iron and ascorbate enhances the absorption of the iron. Settings in which cereal products are most suitable as the major vehicle for fortification iron are those in which serving size and feeding practices are relatively uniform or where the food accounts for a large percentage of the caloric intake. Large variations in consumption of cereal products make it difficult to supply a relatively predictable amount of iron in the diet.

Excessive weight gain during the first year of life may occur in formula-fed or cow's milk-fed infants, presumably because mothers are apt to encourage the infants to finish their feedings. Another factor that may lead to excessive milk ingestion is the lack of opportunity to drink water to quench thirst. Heavier infants at a year of age seem to be at greater risk of iron deficiency, possibly because of a concommitant increase in red cell mass.[11] For these reasons, it is currently recommended in Sweden and Finland that infants after six months of age be given one meal a day in which milk is not included. This meal will include one or more solid food and water as a beverage in order to allow the infant to satisfy his thirst without being required to ingest excessive calories at the same time, as is the case with milk. A meal of this type appears to be effective in avoiding unnecessary weight gain.

E. *School Lunch Programs.* School lunch programs are established in many parts of the world and provide an opportunity for supplying additional iron to the diet. A realistic goal is to supply about one-half of the estimated daily iron requirement in that meal. This may be accomplished by combining smaller amounts of food that are enhancers of iron absorption, with iron-rich foods such as whole grain cereal products, legumes, and foods made with iron-fortified flour. The increased expense of these

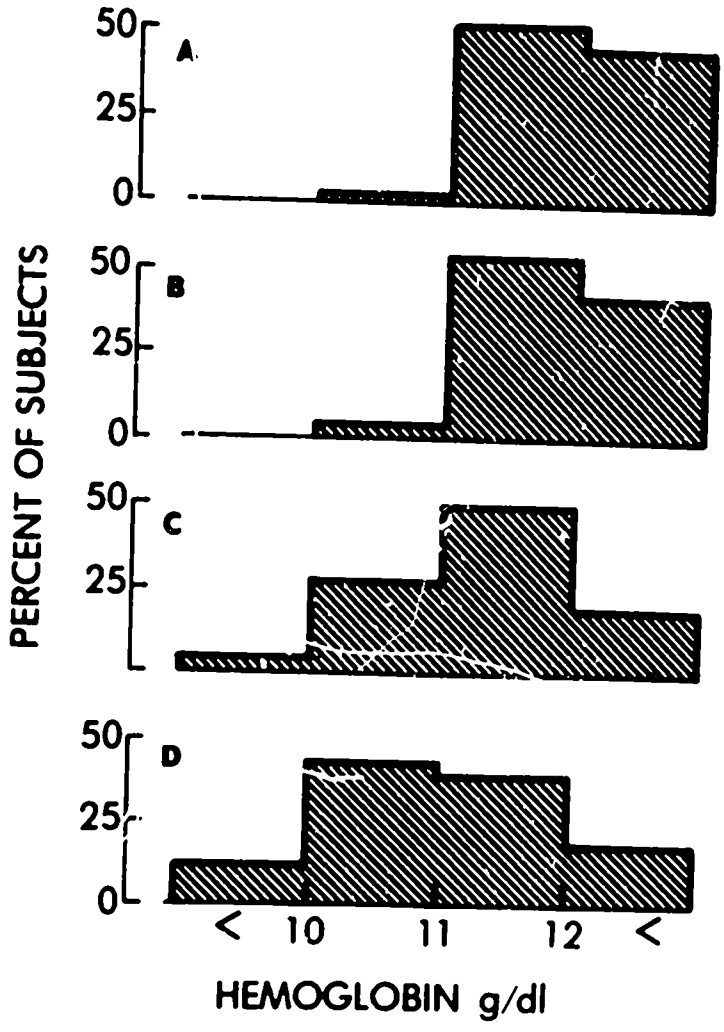

FIGURE 11—HEMOGLOBIN g/dl

IRON-FORTIFIED INFANT CEREAL IN THE PREVENTION OF IRON DEFICIENCY ANEMIA. The distribution of hemoglobin concentration is shown at 1 year of age in infants who received different levels of iron intake starting at 3-5 months of age. Group A was given a more than adequate iron intake including a supplement of 15 mg of elemental iron/day as Fe(II) sulfate and two feedings per day of Fe(II) sulfate-fortified cereal providing an additional 20 mg/day. Groups B and C received the same infant cereal, fortified with Fe(II) saccharate at a level to provide iron intake of 10 to 16 mg/day in group B and 5 to 10 mg/day in group C. Group D received an uncontrolled iron intake that included no highly fortified cereals. The results showed that a total iron intake of 10 to 16 mg/day in group B supplied primarily in the form of iron-fortified cereal was adequate to prevent iron deficiency anemia (drawn from Mok, 1963).

enhancers, such as meat and ascorbic acid-rich foods (fruit or fruit juice) can be justified in terms of their overall nutritional benefits.

F. *Non-Targeted Food Fortification* Children and women are the intended beneficiaries of iron-fortified foods that are available to the entire population. The considerations involved in implementing successful fortification programs have been reviewed recently.[1] Iron-fortified flour, cereal products, sugar, and salt have been used or studied. The most most suitable vehicle for iron is a food that is consumed by a large segment of the population in amounts that will consistently deliver the desired amount of iron, particularly to the target population.

A reasonable prerequisite for fortification iron is that its anticipated absorption under normal conditions of storage and use be at least 50% of the absorption of an equivalent amount of ferrous sulfate without storage. The lack of standards for the use of iron in food fortification fosters the continued use of forms of iron fortification that are unlikely to be effective. The use of compounds that are poorly absorbed or that are oxidized to poorly absorbed forms under the usual conditions of storage is likely to lead to disappointing results in food fortification programs and should be discouraged.

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VI.
Laboratory Diagnosis of Iron Deficiency[1-5]

A. *Special Considerations in Infants and Children.* The laboratory diagnosis of iron deficiency in infants and children requires special attention to the use of age-specific reference standards. There are marked developmental changes in normal values for hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and transferrin saturation. These must be taken into consideration when interpreting results and are discussed below in relation to the individual laboratory tests.

The nature of the population from which laboratory reference standards are derived will profoundly influence estimates of the prevalence of iron deficiency and the accuracy of diagnosis in individual subjects, especially in infants and children. Until recently, "normal" values were compiled from a superficially healthy population, but one that was not systematically screened to exclude individuals with mild deficiencies of iron or folate or with conditions such as thalassemia minor. In the case of hemoglobin, for example, this type of sampling can lead to a non-Gaussian distribution skewed toward low values[6-8] since individuals with marginal deficiencies are not excluded. This helps to explain why, until recently, a hemoglobin concentration of 10 grams deciliter (g/dl or g/100 ml) was the accepted lower limit of normal in children. If individuals making up the lower portion of a frequency distribution curve respond to iron treatment by an increase in concentration of hemoglobin as shown in some studies,[9-11] then the original values are not a legitimate basis for establishing a normal range.

A sounder method for establishing normative data is the selection of a reference population that excludes individuals with other laboratory evidence of iron deficiency and/or other common marginal conditions. Without this type of exclusion the "lower limit of normal" hemoglobin concentration among a group of clinically healthy infants in one study[7] might have been estimated to be about 8 g/dl. However, after excluding all subjects with low or borderline values for transferrin saturation, the estimated lower limit of normal approximated 11 g/dl. The reference standards described in this section were in almost all cases derived by using a similar approach.

Particular attention to blood sampling is also required in infants and children, especially since improperly collected capillary samples can be a major source of error. Fingerstick blood can readily be obtained from infants by health workers after a little training. The key points in obtaining a good sample are that the extremity be warm in order to facilitate a free flow of blood, and that any squeezing of the finger be avoided in order to minimize contamination of blood with tissue fluid. Samples of venous blood can be obtained in larger volume and with no more pain than capillary samples. However, a second person is needed to immobilize the extremity of infants and young children for purposes of venipuncture. Health workers with limited experience will also have more difficulty in entering a vein, particularly in children between six months and two years of age when the vein is likely to be obscured by subcutaneous fat.

B. *Selection of Laboratory Tests.* The diagnosis of iron deficiency must of course begin with an accepted definition. One practical definition is to consider iron deficiency present when the iron supply is inadequate for the normal synthesis of essential iron compounds.[1] Since hemoglobin is the most readily measured essential iron compound, an increase in

hemoglobin concentration in response to iron administration can be regarded as presumptive evidence of prior iron deficiency (provided that recovery from anemia of infection can be excluded).

Although it seems conceptually acceptable to base the definition of iron deficiency on the concentration of hemoglobin, there are practical difficulties in determining whether any given individual does or does not have a hemoglobin below *his* normal level. This is so because normal and abnormal values in a population overlap to a considerable degree. Using the customary values to separate normality from iron deficiency, many potentially iron-responsive individuals may go undetected while many of those identified as anemic may not prove to be iron-deficient.[9] Thus, large errors are introduced when hemoglobin is used as the only laboratory test. This overlapping of hemoglobin values is an obstacle to detecting those individuals whose hemoglobin is no more than 2 g/dl below their potential level; there is less chance that values will overlap into the normal range when anemia is more severe.

Fortunately, other tests can be combined with the analysis of hemoglobin to improve discrimination between normal and iron-deficient individuals.[1,4] These tests include the mean corpuscular volume, the transferrin saturation, the red cell protoporphyrin, and the serum ferritin. Each of these measurements behaves somewhat differently and is subject to different errors. Consequently, the most suitable combination of tests will vary in different situations, depending for example, on the prevalence of iron deficiency, whether co-existing infection or lead poisoning is common, or whether thalassemia minor (a genetic trait) is prevalent in the group being evaluated.

One factor affecting the selection of tests is the volume of the blood sample and the manner in which it is obtained. With the exception of the transferrin saturation, each of the tests can be performed on 0.2 ml of blood or less, an amount that is easily obtained by fingerstick. The determination of serum iron and total iron binding capacity, from which transferrin saturation is calculated, requires a greater amount of blood than is readily obtained by methods other than venipuncture.

The selection of tests will also depend on the equipment available, whether analyses are done locally or in a central laboratory, and the level of training of the laboratory technician. Another important consideration in selecting laboratory tests is the time required to obtain the results of the analysis. It is desirable to use procedures that allow the detection of an abnormality and initiation of treatment during a single visit.

1. *Hemoglobin.* The concentration of hemoglobin is most reliably measured after accurate dilution of the blood sample in a solution that converts hemoglobin to cyanmethemoglobin, which is then quantified spectrophotometrically.[10] A simpler but less reproducible method involves a visual color comparison.

The normal postnatal changes in hemoglobin concentration were discussed above. Figure 12 and Table III show reference values for hemoglobin concentration after six months of age.* Laboratory testing for iron deficiency is rarely done prior to this age. The reference values are based on venous blood from a white, non-indigent population living at sea level, excluding individuals who have other laboratory evidence of iron deficiency or thalassemia minor. There is a gradual rise in values throughout childhood, and at puberty there is a further increase in concentration of hemoglobin in adolescent boys. An approximate correction for altitude based on data from adults[10] can be obtained by increasing the values by 4% per 1,000 meters elevation.

An unresolved question relates to the application of the reference standards shown on Figure 12 and Table III to blacks. Evidence to date indicates that blacks normally have a hemoglobin concentration that is about 0.5 g/dl below that of whites at all ages, except perhaps in the perinatal period.[11,12] This difference does not appear to be due to iron deficiency, thalassemia minor, or differences in socio-economic status.[12] If further studies confirm that this difference exists and is independent of health factors, it may be necessary to adjust the reference standards shown on Figure 12 and Table III downward by about 0.5 g/dl in blacks. Failure to do so might result in falsely identifying about 10% of the normal black population as anemic.

Further study is required to determine the extent to which the hemoglobin concentration might differ in capillary and venous samples. In the first week of life, capillary values are substantially higher than venous concentrations,[13] presumably due to pericapillary flow of plasma and a consequent increase in the concentration of red cells. There is some evidence that venous hemoglobin values are higher than capillary concentrations in infants at about a year of age,[14] but this finding requires further

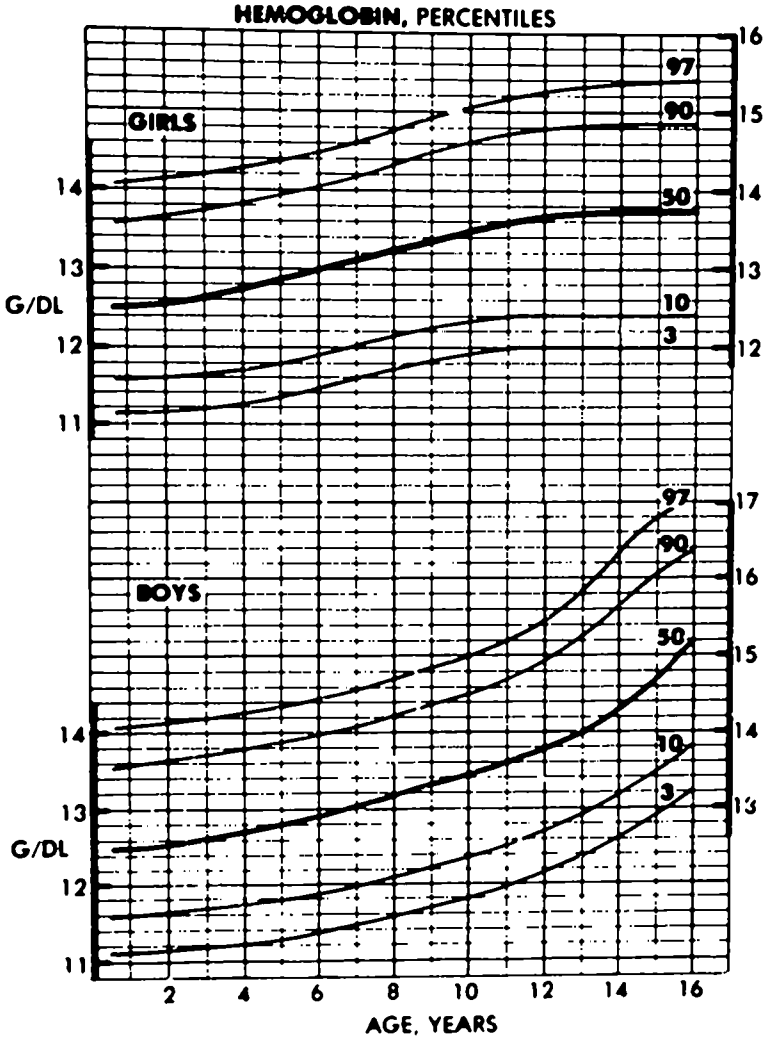

FIGURE 12—AGE, YEARS

HEMOGLOBIN CONCENTRATION IN INFANTS AND CHILDREN. The percentile curves were derived from non-indigent white children living at sea level or after exclusion of subjects with laboratory evidence of iron deficiency, thalassemia minor, and/or hemoglobinopathy (Dallman and Siimes, 1979).*

substantiation with special attention to assure that capillary samples are obtained under ideal circumstances.

Iron deficiency is by far the most common cause of anemia. However, in planning public health programs for the prevention of iron deficiency, it is important to base estimates of prevalence on the combination of other laboratory tests with the assessment of anemia and, if possible, to confirm the estimate by therapeutic trial with iron. Anemia cannot be equated with iron deficiency because inflammatory disease and thalassemia minor also commonly cause anemia, and also because of unresolved issues in the application of reference standards, as for example in blacks. In addition, because of the large overlap of values between normal and iron-deficient populations, a hemoglobin value within the "normal" range

TABLE III

Hemoglobin and Mean Corpuscular Volume (MCV) at Various Ages. Means and lower limits of the 95% range are tabulated (based on Dallman and Siimes, 1979).*

Age (yr.)	Hemoglobin (g/dl) Median	Hemoglobin (g/dl) Lower limit	MCV (fl) Median	MCV (fl) Lower limit
0.5-2	12.5	11.0	77	70
2- 4	12.5	11.0	79	73
4- 8	13.0	11.5	81	75
8-11	13.5	12.0	83	76
11-14				
Female	13.5	12.0	85	78
Male	14.0	12.5	84	77
14-18				
Female	14.0	12.0	87	79
Male	15.0	13.0	86	78
18-49				
Female	14.0	12.0	90	80
Male	16.0	14.0	90	80

does not assure the absence of iron deficiency.[2]

2. *Hematocrit* The hematocrit is measured after centrifugation of a minute amount of blood that has been collected in a heparinized capillary tube.[12] The hematocrit is then calculated by comparing the height of the column of packed red cells with the height of the entire column of red cells and plasma. An advantage of this method is its technical simplicity, particularly when applied to capillary blood samples. A disadvantage lies in the uncertainty about whether the hematocrit is as sensitive an indicator of iron deficiency as the hemoglobin concentration.[13] When mean values in large groups of subjects are compared, the hematocrit is generally equivalent to the hemoglobin multiplied by three. For example, this relationship exists between a hemoglobin of 11 g/dl and a hematocrit of 33%, the lower limits of normal for infants shown on Table III.

The determination of the hematocrit has been assumed to be a convenient and reliable alternative to the analysis of hemoglobin concentration; however, recent evidence suggests that this assumption requires reexamination.[13] When hemoglobin and hematocrit were determined in children between one and two years of age, almost three times as many subjects had low hemoglobin values as would have been predicted on the basis of the number of low hematocrit values. However, because of the convenience and widespread use of the hematocrit method, it will be important to obtain confirmation of these findings before concluding that the hematocrit is less useful than the hemoglobin concentration in screening for anemia.

3. *Mean Corpuscular Volume (MCV).* Electronic counters measure red cell volume directly and have made the MCV an accurate and practical laboratory test. Formerly, the determination of the MCV was a time-consuming and poorly reproducible procedure since it was derived from the ratio of the hematocrit to the red cell count obtained by microscope. The direct determination of red cell volume is highly reproducible and actually less subject to sampling error than the hemoglobin determination; dilution by tissue fluid or fluctuations in plasma volume do not affect red cell size. Although the initial purchase of an electronic counter involves a large expenditure, it allows many samples to be processed by one technician. The ability to determine hemoglobin concentration and red cell volume with the same instrument is

in additional advantage.

Reference values for MCV are shown on Figure 13 and Table III.* Red cells are normally larger at birth than in the adult but red cell size decreases during the first six months of life. Red cell size is smallest during the remainder of infancy and gradually increases during childhood. Mean values in girls are slightly higher than in boys after seven years of age but no sex differences are reported in adults.

A low MCV with anemia is most commonly associated with iron deficiency but is also characteristic of thalassemia minor. This is not an important consideration among populations where thalassemia minor is rare. However, where thalassemia minor is common, e.g. in blacks, Mediterranean populations, and in Southeast Asia, it must be taken into consideration. Other conditions rarely result in the combination of a low hemoglobin and a low MCV.

Other red cell indices can also be obtained by electronic counter, but they are not directly measured like the MCV. The mean corpuscular hemoglobin or MCH, which undergoes similar changes in iron deficiency as the MCV, is derived by dividing hemoglobin concentration by red cell count. The mean corpuscular hemoglobin concentration (MCHC) is the least directly measured and least

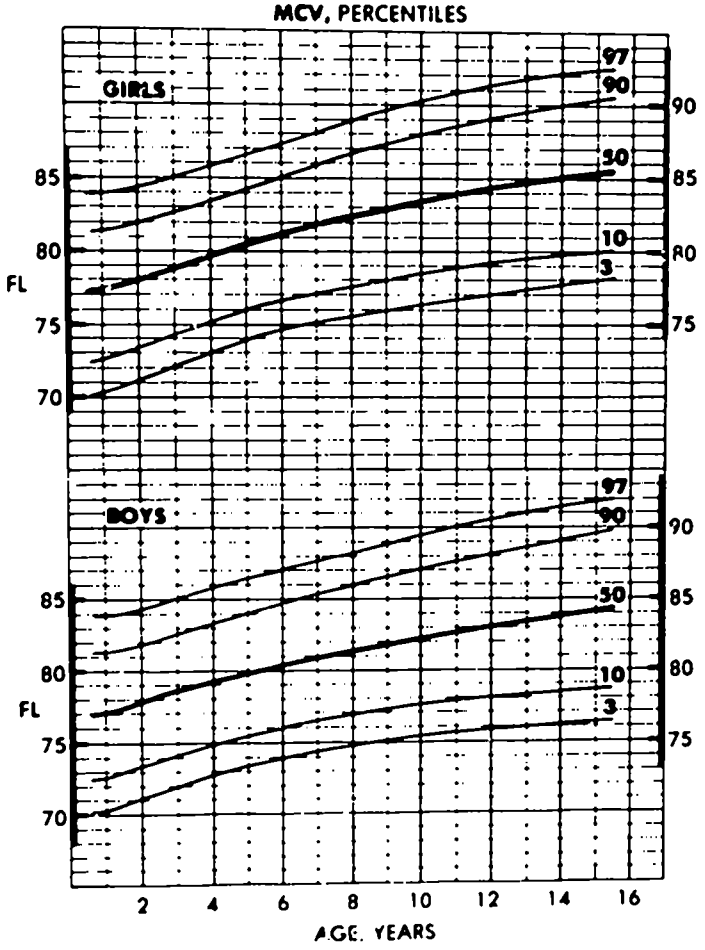

FIGURE 13—AGE, YEARS

MEAN CORPUSCULAR VOLUME (MCV) IN INFANTS AND CHILDREN. The percentile curves were derived from a portion of the same population as in Fig. 12 (Dallman and Siimes, 1979).*

useful of the indices obtained by electronic counter. because the MCHC can be calculated by dividing hemoglobin by hematocrit, it is the only red cell index that is readily obtained without electronic counters. However, it is the least sensitive of the indices in the diagnosis of iron deficiency.[22]

As iron deficiency develops, the decrease in MCV appears to occur at about the same time as anemia starts to develop and may actually represent a greater relative deviation from normal.[23] If the results of both hemoglobin and MCV determinations are available, they can be used to decrease errors due to overlap of normal and abnormal values when only one test is used. For example, the likelihood of an abnormality is great if both values lie below the 10th percentile because this would rarely occur by chance in a normal individual.

Free Erythrocyte Protoporphyrin (FEP). When insufficient iron is available to combine with protoporphyrin to form heme, there is an accumulation of protoporphyrin in the red blood cell. The FEP can be measured rapidly by a simple fluorescence assay.[24] This can be done either by employing a fluorometer or an instrument especially designed for directly determining red cell protoporphyrin by the fluorescence of a thin film of blood.[25] The latter requires little technician time or training. The FEP is useful in distinguishing iron deficiency from thalassemia minor: it is elevated in iron deficiency but normal in thalassemia minor.[26] However, elevated protoporphyrin values are obtained not only in iron deficiency, but also in inflammatory disease and with exposure to lead. There also appear to be normal developmental changes in FEP values. High FEP values are present at two months of age without being indicative of iron deficiency,[27] iron stores are abundant at this age. During the remainder of infancy, FEP values are reported to be somewhat higher than in the adult, but during this period of development, it has not been conclusively determined whether or not iron deficiency was responsible for the difference.[28]

All of the tests discussed so far (hemoglobin, hematocrit, MCV, MCH, and FEP) can be performed rapidly. Under ideal circumstances, if the laboratory analysis can be done shortly after blood sampling, it is possible to make a diagnosis of iron deficiency and initiate treatment in a single visit. These laboratory tests also have the advantage of being interpretable even after recent ingestion of medicinal iron and of reflecting the degree of repopulation of the blood by normal red blood cells during the course of treatment.[29]

5. *Transferrin Saturation.* The transferrin saturation is calculated by dividing the concentration of serum iron by the total iron-binding capacity and multiplying by 100 to express the result as percent. Almost all of the iron in the serum is bound to the iron-binding protein, transferrin. Serum iron[24] and iron-binding capacity[25] are most commonly measured by spectrophotometric techniques. The assay when done manually is difficult, time-consuming, and subject to error due to contaminating iron in the environment. Automated techniques provide more reproducible results and permit the analysis of a large number of samples.[30] However, the initial investment in equipment is high.

During the neonatal period, the transferrin saturation is higher than at any other age.[31] However, after about four months of age, it decreases to values that are lower than in the adult; this is true even after large therapeutic doses of iron have been administered.[32] In adults, a transferrin saturation of 16% is commonly considered the lower limit of normal. A corresponding value in children between 6 months of age and adolescence is 7 or 10%.[27,28] Seven to 16% in children can be considered an intermediate zone with overlapping of deficient and normal individuals.[28] The measurement of transferrin saturation is widely used as a confirmatory test in the diagnosis of iron deficiency. For survey purposes, it is essential that the assay be used in combination with other tests and that normally lower values in infants and children be taken into account in the interpretation.

There are large biological variations in serum iron that result in many false positive and negative diagnoses when transferrin saturation is used as the only test of iron deficiency. After about three years of age, there is a diurnal variation in serum iron,[29] usually with high values in the morning and low values at night. In a group of healthy adults, the magnitude of this fluctuation was reported to fall from an average high of 47% in the morning to 13% at night.[29]

The transferrin saturation may decrease in inflammatory disease, as well as in iron deficiency. In some cases, the total iron binding capacity (TIBC) may be helpful in distinguishing the two conditions.[31] A high TIBC suggests iron deficiency, whereas a low value is characteristic of inflammatory disease.

6. *Serum Ferritin.* The measurement of serum ferritin allows the estimation of iron stores by non-invasive means. Ferritin is normally present in the serum, but in such small quantities that it remained undetected

ıtil recently. Serum ferritin is measured by radioimunoassay.[34,35] This is most easily performed as a ıe-day assay, employing a kit. However, it still reıires the availability of a gamma counter and a ghly trained technician. For these reasons, the test relatively expensive. The advantage of the serum rritin assay is that it allows evaluation of iron atus within the normal range, as well as in condiıns of iron deficiency and excess,[36-38] information at is invaluable in nutrition surveys and that canıt be provided by other means.

ıanges in the concentration of serum ferritin cording to age parallel known changes in iron ıres.[37] The curves shown on Figure 14 are indicative of average iron reserves at various stages of development: abundant reserves in most newborns, adult men, and elderly of both sexes, and lower reserves in children and women during the childbearing years.[8,37,39,40] At all ages, a serum ferritin value below 10 or 12 μg/liter indicates depletion of iron reserves. A low concentration of serum ferritin is characteristic only of iron deficiency. However, when inflammatory disease and iron deficiency coexist, serum ferritin values may be within the normal range.[33,38]

One area of uncertainty is the reliability of the serum ferritin in diagnosing iron deficiency in infancy. Although a low serum ferritin is present in iron defi-

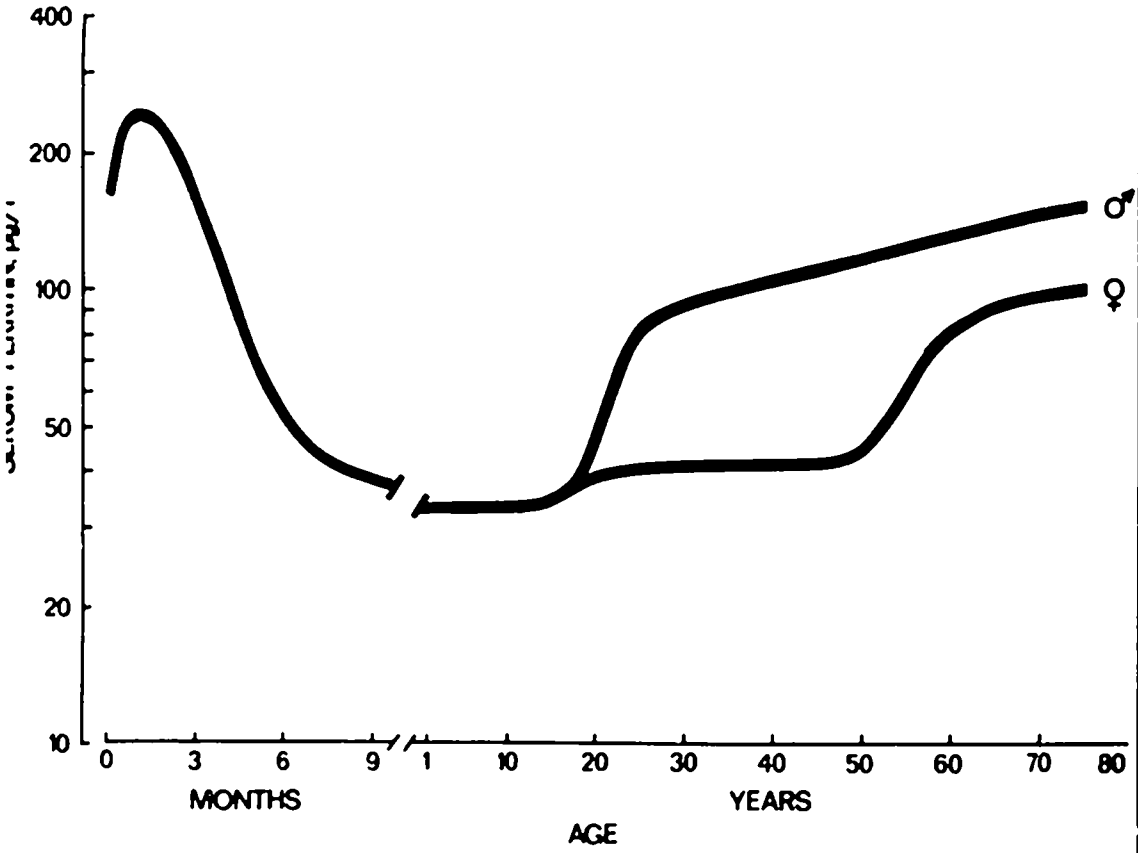

FIGURE 14

DEVELOPMENTAL CHANGES IN CONCENTRATION OF SERUM FERRITIN. Mean values (geometric means) in healthy populations are shown. At all ages, a serum ferritin concentration below 10 or 12 μg per liter is considered indicative of depleted iron stores. (Saarinen and Siimes, 1978,[8] Siimes *et al.* 1974[37] Finch et al, 1977,[40] Cook, *et al* 1976[39])

ciency,[10,11] a normal serum ferritin does not exclude this possibility. In a group of 225 healthy infants who had serial laboratory studies during the first year of life, six of them had developed evidence of iron deficiency by hemoglobin (< 11 g/dl), MCV (<70 fl), and transferrin saturation values (< 10%).[11] All of these infants had serum ferritins that were in the normal range, even though subsequent iron treatment resulted in an increased hemoglobin concentration. This phenomenon has not been apparent in older children or adults. Another caution in the use of serum ferritin applies to the falsely high values that may be obtained during the first few weeks of iron treatment.

7. *Therapeutic Trial* Perhaps the most reliable criterion of iron deficiency anemia is the hemoglobin response to an adequate therapeutic trial of iron. A therapeutic trial allows the recognition of individuals whose hemoglobin values, although within the reference range, are low for those individuals. The therapeutic trial is a classic method for determining the prevalence of iron deficiency in populations, and can be particularly informative when both placebo and iron treatment groups are employed.[5] However, ethical considerations are becoming more of a constraint on using placebo groups. Informed consent is considered appropriate for such studies in many countries, but this procedure may negate the value of the placebo group by calling attention to a readily correctible form of malnutrition. In addition it may be difficult to justify either withholding treatment from subjects who are almost certain to be deficient or conversely, treating those who are almost certain not to require it.

C. *Population Surveys* Surveys to determine prevalence of iron deficiency require careful attention to the selection of an appropriate study population, the development of a well-defined experimental design, and appropriate statistical analysis.[12] In the planning of laboratory analyses centralized laboratory facilities offer the advantages of uniform standards and performance of an assay by the same technician. In these situations, hemoglobin and MCV constitute an excellent combination because extremely large numbers of samples can be handled with an instrument that is used for both of these assays. The serum ferritin, as a third test, offers special advantages in surveys, not only for confirming the presence of iron deficiency anemia, but for estimating the iron reserves of the remaining population. Alternatives to serum ferritin as a third test are the transferrin saturation and the FEP. The best combinations of tests remain to be determined and verified by therapeutic trial. It is likely that different solutions will be found to suit different local requirements.

D. *The Choice of Ages for Detection of Iron Deficiency.* Most cases of iron deficiency are mild and do not result in symptoms that are recognized as requiring medical attention. Iron deficiency is therefore most commonly detected through the routine laboratory assessment of populations that are at particular risk. The usual goal is to identify subjects with anemia who are likely to have a hemoglobin response with administration of iron and/or an improved diet. Among infants and children, laboratory detection of anemia is commonly recommended at about one year of age in term infants and six to nine months of age in preterm infants, ages at which the prevalence of iron deficiency becomes high in many populations.[13] Other suitable ages for the laboratory detection of anemia are between two and three years, at about five years in the pre-school child, and in adolescence. The most appropriate times will depend on the prevalence of iron deficiency at various stages of development, which will differ according to the population. The decision of when to recommend laboratory detection of anemia is ideally based on previous experience with children in the same clinical setting. The value of the procedure in terms of cost versus benefit can only be evaluated by determining the number of subjects that must be seen in order to detect each individual with iron-responsive anemia.

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VII. TREATMENT

Oral administration of ferrous sulfate is the treatment of choice for almost all cases of iron deficiency. This iron compound remains the standard against which the efficacy of a multitude of other compounds is measured, and it is as well tolerated as comparable doses of other therapeutically effective preparations. Some slow-release preparations may be absorbed as well as ordinary ferrous sulfate, but their higher costs would be justified only in settings where gastrointestinal side effects are common. Iron salts and ascorbic acid have been combined in some proprietary preparations with the purpose of enhancing iron absorption. However, ascorbic acid is relatively ineffective in stimulating the absorption of medicinal iron, in contrast to the marked effect that it has in enhancing the absorption of iron in foods.

Dose. The dose of oral iron is based on the amount that will be adequate for the most rapid possible increase in concentration of hemoglobin. Studies in adults who were phlebotomized to a stable hemoglobin of 10 g/dl showed that an optimal hemoglobin response could be obtained with 100 mg of elemental iron in the form of ferrous sulfate divided into two doses, one at breakfast and one at dinner.[1] Administration of iron between meals will increase the percentage absorbed. A comparable dose (about 3 mg/kg per day) is also recommended as adequate for infants and children.[2] A dose of 6 mg/kg/day has been widely used in infants and children, but is probably unnecessarily high. Although the response in hemoglobin should be virtually complete after two months, therapy should ideally be maintained for a longer period to allow accumulation of storage iron. This can be accomplished by continuing treatment for a total of about five months. If intestinal intolerance to iron salt occurs, a reduction in dose or administration with meals should result in improved tolerance.

Side effects do not appear to be common in infants[3] and children, but this has not been as extensively studied as in the adult. It seems relatively rare that medication in children is stopped because of constipation, diarrhea, nausea, and epigastric pain or heartburn, which are common side effects in the adult.[4] This impression could be attributable to the fact that infants and young children are less able to recognize and explain symptoms than adults.

An alternative approach to the treatment of mild iron deficiency anemia, especially in infants, is the use of iron-fortified food. Although this is an accepted method of preventing iron deficiency, there is also ample evidence of the therapeutic effectiveness of some fortified foods. For example, anemia in preterm infants can be rapidly reversed with the use of iron-fortified formula despite the unusually large baseline iron requirements imposed by a rapid rate of growth (Fig. 15).[5] In contrast to medicinal iron, this form of treatment has the advantage of posing no toxic hazard due to overdose or accidental ingestion by siblings. Furthermore, no significant gastrointestinal side effects appear to result from fortification iron added to infant formula.[6]

Therapeutic iron is best absorbed during the first month of treatment and less well subsequently. For example, adults given 100 mg iron as ferrous sulfate twice a day with meals absorbed an average of 14% during the first week of therapy compared to 7% after three weeks and 2% after four months of treatment.[1] Thus, the first month would appear to be the most important in determining the success of treatment; it is also likely to be the best period in terms of compliance.

Medications for young children are generally given in liquid form. Iron in liquid form is apt to cause some staining of the teeth. This is not permanent and can be removed by brushing. Staining can be minimized by administering the medication on the back of the tongue.

A major concern in children relates to the possibility of *accidental overdose*. Serious iron toxicity is a common consequence of accidental drug ingestion, especially between the ages of one and five years if medication is not kept out of reach. All iron preparations should be labelled "Caution! Keep out of reach of children." A high prevalence of this form of toxicity is evident from a report describing 66 children with acute iron poisoning who were admitted to one hospital over a three year period.[10] The risk of acute iron poisoning can be decreased by avoiding preparations that look or taste like candy, by not dispensing excessively large amounts at one time, by using containers that young children cannot readily open, and by warning parents to keep the medication in a safe place.

Risk of Infection. Recent reports have suggested the possibility that oral[14] or intramuscular[11,13] iron therapy can reactivate pre-existing infections such as malaria, brucellosis, and tuberculosis in populations where these infections are common. It has been postulated that iron deficiency suppresses certain infections by depriving invading microorganisms of the iron needed for growth. The implications of this

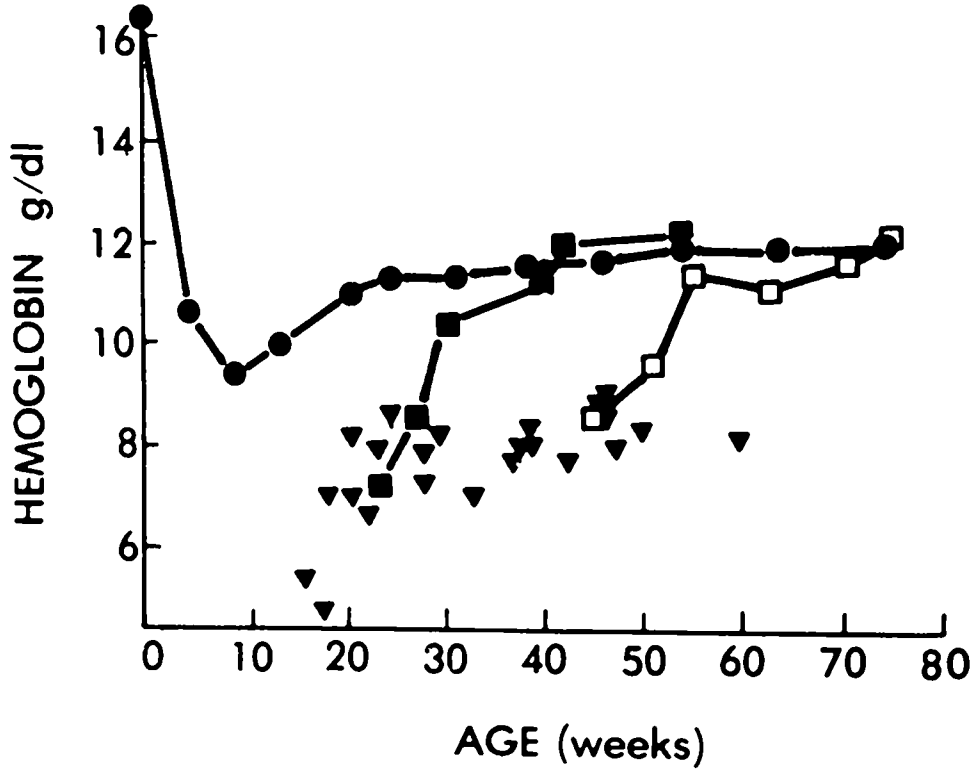

FIGURE 15

TREATMENT OF IRON DEFICIENCY ANEMIA WITH IRON-FORTIFIED FORMULA. Mean hemoglobin concentrations for premature infants changed from formula without added iron to formula with added iron after development of anemia. ■ before 35.0 weeks of age and □ after 35.0 weeks of age) compared to values in premature infants fed from birth with added iron from the first week of life (●). Individual hemoglobin values at the onset of anemia are indicated (▼). (Garty and Cross, 1964.)

hypothesis for infants and children are the subject of two recent reviews.[11,12] At present, the benefits of preventing or treating iron deficiency by fortification of foods or by oral administration of iron seem to outweigh the risks under most conditions. However, the possible risk of reactivating latent infections in susceptible populations should be further evaluated.

Response The laboratory abnormalities of hemoglobin, FEP,[13] and red cell volume are most rapidly corrected within the first month of treatment, and the response is generally complete after two to three months. One or more of these tests can be repeated after either interval to determine whether or not there has been a response to iron therapy. In cases of severe anemia, the reticulocyte response can best be detected after one to three weeks. This response may be delayed where there is a concurrent infection or inflammatory disease. Iron absorption is decreased in febrile children who have minimal or no stainable marrow iron (Fig 16).[14,15] Poor iron absorption is also common in infants with severe protein-calorie malnutrition[16] prior to nutritional rehabilitation. It is also reported in otherwise well nourished iron deficient children[17] but this appears to be rare.

The reticulocyte count is a relatively laborious and time-consuming laboratory procedure, and is therefore not an appropriate test in many clinical settings. In addition, when anemia is mild, as it is in the vast majority of cases, the reticulocyte response is

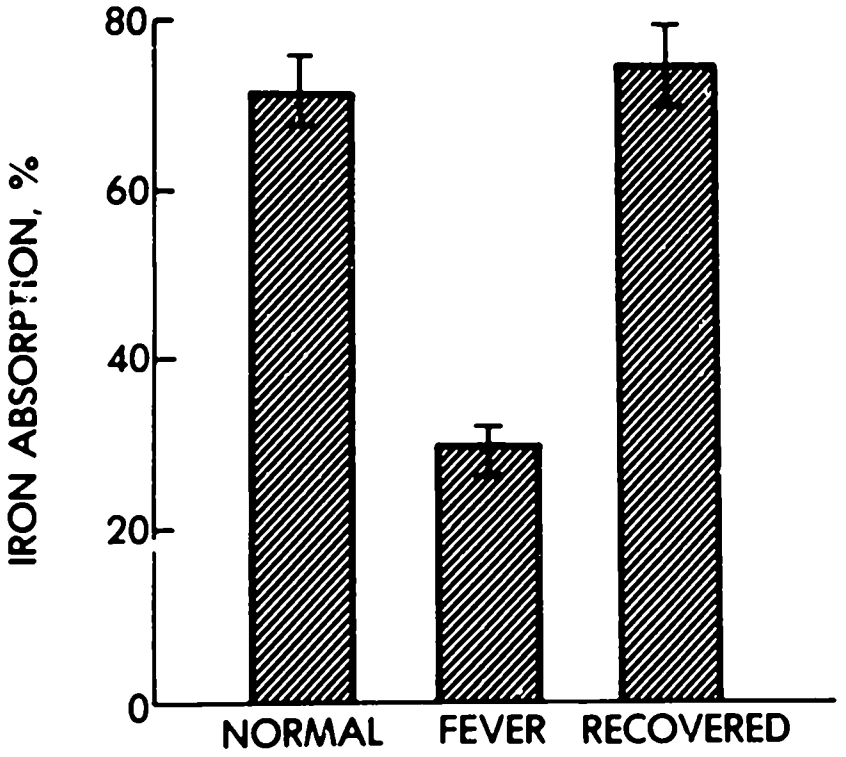

FIGURE 16

IRON ABSORPTION IN THE FEBRILE CHILD. Absorption of a standard dose of 0.5o mg ^{59}Fe(II) in children with little or no stainable marrow iron. Means ± SEM are indicated. Absorption was decreased in individuals with acute febrile infections but returned to control values after resolution of the fever (drawn from Bender-Götze et al. 1976 and additional unpublished data)

usually of insufficient magnitude to be reliably detected.

The use of intramuscular iron (generally in the form of iron dextran) is rarely warranted, except perhaps when administration of oral iron medication cannot be relied upon or when the possibility of follow-up is uncertain. Intramuscular injections may be painful and skin discoloration is common if special care is not taken to avoid back-flow into subcutaneous tissues. The therapeutic response is not significantly more rapid than with oral medication. There is also concern that parenteral iron may predispose to infection.[11,12] The basis for this concern has been derived primarily from *in vitro* studies but some clinical studies are also suggestive. Severe anaphylactic reactions rarely occur with intramuscular or intravenous injections. Therefore, parenteral medication should only be used when there is a good reason to substitute it for oral medication, as for example after there has been an apparent lack of response to oral therapy in a patient who has strong laboratory evidence of iron deficiency. If malabsorption of iron is suspected, a comparison between the concentration of fasting serum irons before and 2 hours after a dose of 1 mg elemental iron as ferrous sulfate is helpful.[14] However, it should be kept in mind that malabsorption of iron is relatively rare, but failure to administer medication is common.

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VIII.
THE PLANNING OF AN INTERVENTION PROGRAM: A CASE STUDY*

Prevalence of iron deficiency in Chile is very high, particularly in infancy. Several surveys conducted mainly among an urban lower class and lower-middle class socio-economic group have shown the presence of anemia in 20 to 40% of infants between the ages of 6 and 24 months. Many factors contribute to this high prevalence of iron deficiency: breast feeding is of short duration, there are essentially no iron-fortified products available for infants, introduction of solid foods in the diet is late, and medicinal iron is seldom prescribed for *prevention* of iron deficiency.

During the first year of life, Chilean infants are fed mainly powdered milk, distributed by the National Health Service (NHS) through a national program of food supplementation. This is a large program that serves about 80% of the total infant population. Each infant (or his nursing mother) now receives 3 kg month of full-fat (26%), unfortified, powdered milk between birth and 6 months and then 2 kg month until 2 years of age.

Efforts to utilize the national program of milk distribution as a means of preventing iron deficiency in infants began several years ago. The simplest way of doing this appeared to be the addition of a soluble iron salt to the product. Consequently, the low-fat (12%) milk powder being distributed at the time was experimentally fortified on a pilot basis with 15 mg of elemental iron as ferrous sulfate per 100 grams of milk powder. Isotopic studies conducted in healthy but primarily iron deficient infants revealed that 3 to 4% (geometric mean) of the fortification iron was absorbed.

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A field trial with this product began in 1972 in three NHS clinics. Healthy term infants who had been weaned before 3 months of age received either the fortified or the unfortified, control milk until 15 months of age. A nurse visited each infant's home every two weeks in order to monitor and encourage the consumption of the product and to perform a dietary survey. Stool samples of the infants were periodically analyzed for iron as a means of determining actual ingestion of the fortified milk. A total of 603 infants entered the field trial, 382 could be studied at 9 months of age and 314 at 15 months. Even though the study involved infants of low socio-economic status, incidence of protein-calorie malnutrition was low and was therefore not likely to have been a complicating factor in the study.

At 9 and 15 months of age, there were significant differences in hemoglobin concentration between the two groups. Anemia (hemoglobin below 11 g/dl) was present in 27.7% of the control infants at 9 months and in 34.6% at 15 months of age. Corresponding figures in the fortified group were 20.7% and 12.7% at 9 and 15 months respectively. Differences between the groups in respect to transferrin saturation and free erythrocyte protoporphyrin were small.

Infants who consistently had more than 15 mg iron/100 g stool were selected as representing a subgroup of subjects in whom the consumption of fortified milk was most likely to have been as directed. Milk distributed to families through the national program is sometimes sold or shared with other members of the family. Mean values for hemoglobin concentration and percent saturation of transferrin were higher in infants who were shown to have consumed the fortified milk than in the fortified group as a whole, but anemia still existed in 14.8% of

the group receiving iron-fortified milk at 9 months and at 15 months of age. The 0.3 to 0.4 mg of fortification iron that these infants would be predicted to absorb per day proved to be inadequate for the prevention of anemia in all cases. The transferrin saturation and free erythrocyte protoporphyrin values supported the conclusion that persistence of iron deficiency was common despite consumption of the iron-fortified milk.

In response to these disappointing results, we considered the use of ascorbic acid as a means of improving the bioavailability of fortification iron in milk. Preliminary double isotope studies showed an increase in iron absorption by a factor of 1.8 and 2.8 when ascorbic acid was added in a ratio of 100 mg and 200 mg, respectively, per 15 mg of iron. As a result, a new fortified formula was developed containing 15 mg of iron as ferrous sulfate and 100 mg ascorbic acid per 100 g of milk powder. The product was also slightly acidified in order to modify the taste sufficiently to discourage its consumption by other members of the family and still have it acceptable to infants. Iron absorption from the new formula varied between a geometric mean of 10.7 and 12.0%, higher by a factor of 2.1 to 2.7 than absorption from the fortified milk without ascorbic acid (Table IV).

A pilot study with this formula was started in 1976 i three communities at the periphery of Santiago. Tl experimental design was similar to the first trial. Tw hundred and eighty infants entered the study in tl fortified group and 278 in a control group receivir the regular unfortified milk. Acceptance of tl acidified milk was very good with about 90% of ir fants still taking it at 15 months of age. Preliminar analysis of laboratory data showed significant di ferences between the two groups in hemoglobir serum iron, TIBC, transferrin saturation, FEP an serum ferritin at 9 and 15 months of age. Anemia w present in 7.5% of infants receiving the fortified mil at 9 months of age, but in only 1.6% at 15 month Corresponding figures in the control group wer 34.7% and 27.8%. At 15 months, only 6.4% of ir fants in the fortified group had a transferrin satur tion below 9% versus 35.2% in the control grou Free erythrocyte protoporphyrin values over 1C μg/dl were present in 22.4% and 4.6% of the cas respectively (Figure 17).

It is clear that improved bioavailability of the iron i the formula with ascorbic acid was confirmed in ou field trial. Iron deficiency was shown to be the majc cause of anemia in the study population, and th anemia could be essentially erradicated by the use c

TABLE IV

ABSORPTION OF FORTIFICATION IRON FROM AN ACIDIFIED MILK FORMULA WITH ASCORBIC ACID

Batch Date	Study Date	n	Hemoglobin (g/dl)	Transferrin Saturation (%)	Iron Absorption (%)* Low-Fat Milk (A)	Acid Milk + vit.C (B)	B/A
7/75	7/75	9	11.8* (10.8-12.8)	9.1 (5.2-13.2)	5.5 (0.8-9.0)	12.0 (4.7-27.0)	2.2 (1.2-6.0)
9/75	7/76	17	11.2 (8.4-13.3)	9.2 (2.1-14.7)	4.0 (0.2-27.6)	10.7 (3.1-51.5)	2.7 (1.0-10.1)
7/76	7/76	13	11.4 (9.5-12.8)	12.1 (2.4-28.6)	4.9 (1.8-25.4)	11.3 (1.9-31.1)	2.1 (0.4-7.7)

*Geometric mean

*Mean values and range in parenthesis

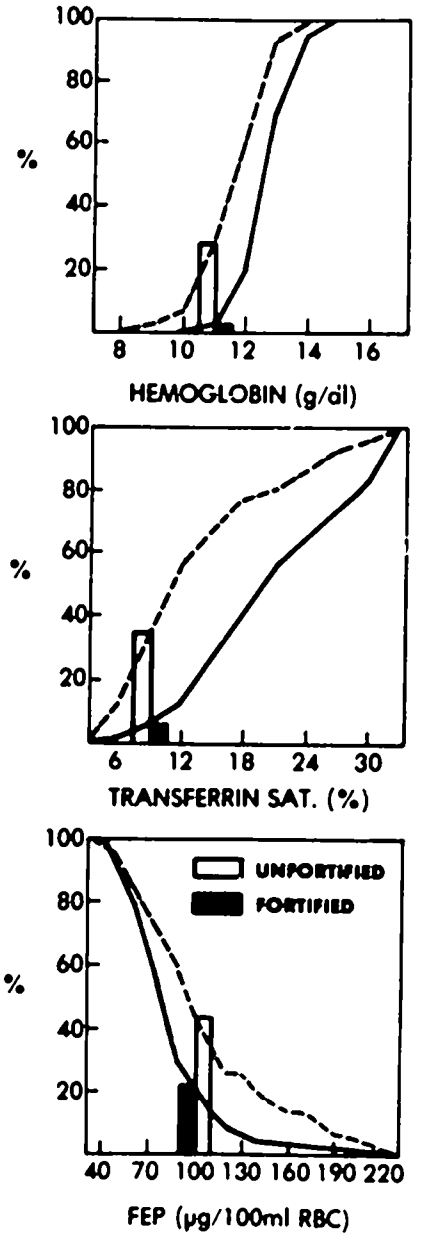

FIGURE 17

Cumulative plots of individual values of hemoglobin, transferrin saturation, and free erythrocyte protoporphyrin (FEP) at 15 months of age in infants receiving unfortified milk (–) or acidified milk fortified with iron and ascorbic acid (—). The height of the columns indicates the percentage of infants with hemoglobin <11 g/dl (top), transferrin saturation <9% (middle), and FEP >100 μg/100 ml RBC (bottom) in each of the two groups.

milk fortified with iron and ascorbic acid. However, a question still to be answered concerns the long-term acceptance of the fortified, acidified milk under the normal operational conditions of the national supplementation program when the close supervision of the pilot studies is no longer a factor. This is now being studied in a section of the city of Santiago with about 400,000 inhabitants.

Various methods of manufacturing and packaging the formula are also under investigation in order to make it possible for the suppliers of the national milk program to adopt uniform, new specifications. Eventually, the nationwide distribution of iron and ascorbic acid fortified milk will depend on a governmental decision regarding the additional expense compared to the presently used milk.

IX.
RESEARCH NEEDS

Although much has been learned about the iron nutrition of infants and children within the last few decades, there are many problems that still deserve study.

The interaction between iron nutrition and infection is a topic of importance, especially in developing countries. The iron-binding proteins, transferrin and lactoferrin, have been shown to have bacteriostatic properties *in vitro* that are lost when these proteins are iron-saturated. If these proteins are major factors in resistance to bacterial infection, they may play a key protective role during the period of physiologically low concentrations of immune globulins in infancy. This possibility is the basis for concern that therapeutic iron, particularly in parenteral form, could predispose to infection. This problem has been the subject of several recent reviews and clearly deserves further investigation.

Another area of continuing interest is the basis for intestinal bleeding induced by cow's milk and the determination of its prevalence, especially in infants. The effects of cow's milk may have broader implications than simply in the realm of iron nutrition. Since milk is a major food in the diet of infants and children in many parts of the world, it would be helpful to know more about its effects on the intestinal mucosa: bleeding may be only one of several manifestations in susceptible infants and children.

The basis for the high bioavailability of iron in breast milk remains unclear, although several possibilities related to its protein, lactoferrin, and mineral content have been suggested. There is also some evidence that the absorptive function of the intestinal mucosa is conditioned by prior diet. These are promising areas of inquiry that may provide information applicable to improving the bioavailability of iron in other foods.

In recent years much information has been obtained on iron absorption from various types of meals in adults. More corresponding information for infants would be valuable. For example, is the iron absorption from breast milk or fortified formula compromised by feeding infant cereal at the same meal; or alternatively, does breast milk or formula facilitate the absorption of the supplemental iron that is contained in infant cereal?

The methods that are used to diagnose iron deficiency require further refinement. Alternative combinations of tests need to be compared in various clinical settings in order to determine how best to assess the prevalence of iron deficiency in populations and to diagnose it in individuals most economically. This would be facilitated by the development of simple and less expensive instruments that can be used by health workers after very little training.

The prevalence of iron deficiency in relation to the adolescent growth spurt has received little attention. In boys, the peak in the growth spurt and a rapid rise in hemoglobin concentration coincide. In addition, with onset of active physical labor or sports, blood volume may increase substantially, with a concomitant increase in total red cell mass. The influence of these factors might be obscured unless it were specifically looked for in nutritional surveys.

Another area requiring further investigation is that of determining the systemic consequences of iron deficiency. In recent years there has been a growing body of evidence to indicate that iron deficiency can com-

promise celiular immunity, muscle function, intestinal absorption, and thermogenesis, all independently of its effects on anemia. Further studies in this area are required to determine the subtle effects of iron deficiency in terms of systemic manifestations. This type of information is important in assigning an appropriate priority to programs aimed at elimination of mild iron deficiency anemia.