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NUTRITIONAL ANEMIAS IN SELECTED COUNTRIES

by

Wun-Yuan Mei

A report submitted in partial fulfillment
of the requirements for the degree

of

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in

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INTRODUCTION

Blood is a constantly changing, highly complex tissue which is concerned with the transport of cell nutrients, the elimination of wastes, and the maintenance of chemical equilibrium. The mature red blood cell takes about seven and half days to develop, then has a life cycle of 120 days. Many factors are involved in this complicated process. Many nutrients are required for the framework of the red blood cells and in the hemoglobin within these cells.

Anemia is a condition in which there is a reduction in the total circulating hemoglobin. Anemias may be described biochemically in terms of lowered hemoglobin levels, number of red blood cells, and hematocrit. They are also differentiated on the basis of appearance of red blood cells; normocytic, macrocytic, or microcytic; nucleated or nonnucleated; normochromic, hyperchromic, or hypochromic. It is also possible to measure the iron reserves and the change in the level of plasma iron and of transferrin. When anemias become more severe, the symptoms are more consistent. They include skin pallor, weakness, easy fatigability, head aches, dizziness, sensitivity to cold, and paresthesia. Cheilosis, glossitis, loss of appetite, and loss of gastrointestinal tone with accompanying symptoms of distress are seen in severe anemias. With increasing severity of anemia, the oxygenation of tissues is reduced--hence the feeling of fatigue. The heart rate increases, palpitation occurs, and there is shortness of breath.

Faulty nutrition occasioned either by failure to provide the essential nutrients, such as protein, iron, folic acid, vitamin E, vitamin B₁₂, pyridoxine, copper, ascorbic acid, etc., or by poor utilization of dietary constituents may lead to anemia, the type being dependent upon the initiating defect. Anemia due to iron deficiency is presently recognized as the most common type of nutritional anemia.

The treatment of anemia is dependent upon a determination of the cause and eliminating it whenever possible. Nutritionally, specific supplements may be required to improve the formation of red cells and hemoglobin. A normal diet to restore good nutrition is usually emphasized to support the specific therapy.

NUTRITIONAL ANEMIAS IN SELECTED COUNTRIES

Middle East

The availability of iron from isotopically labeled wheat, okra chickpea, and broad bean in 37 iron deficient adult men with a history of repeated blood donation in Middle East was determined by Mameesh et al. (1970). The absorption of food iron in each subject was compared with that of ferrous ascorbate. Anemic blood donors exhibited a high rate of absorption of inorganic as well as food iron. Whole wheat iron was significantly less available than the iron in the other vegetable sources examined. They suggested that a high degree of dependence on wheat as a source of dietary iron may contribute to iron deficiency anemia.

In Lebanon, Sabry (1961) reported that cereals and legumes contribute most of the dietary calories with a small portion of the calories being derived from animal protein. In 1964, Cowan et al. made two seven-day dietary surveys, one in spring and one in autumn, which were conducted among a group of rural families in the northern Beka'a area of Lebanon. Bread was eaten at every meal. Fresh meat was normally served only once or twice weekly. Fresh vegetables and fruits were available throughout the year in Lebanon; but in winter, production was limited to the coastal areas and prices in the Beka'a was high. The results of the first survey indicated that the per capita

intake of the population studied met the 1964 National Research Council recommendations for calories, protein, thiamin, riboflavin and niacin, but values for calcium, iron, vitamin A, and ascorbic acid was low. In the autumn survey, the per capita intake of calcium, vitamin A, and niacin was low.

Nigeria

Fleming et al. (1969) studied 151 pregnant and 25 delivered Nigerian women in one year with a hematocrit of 23 percent or less, not associated with hemorrhage hemoglobinopathies, or heavy hook worm infection. The etiology of the anemia was complex, but two factors, hemolysis and megaloblastic erythropoiesis, were of prime importance. Megaloblastic erythropoiesis showed a close correlation with low serum folate activity, and the deficiency was the result of low intake not meeting the requirements of pregnancy and erythroid hyperplasia secondary to hemolysis. Malabsorption of vitamin B₁₂ is uncommon, and this nutritional deficiency has not been recognized to date in Nigeria. The principle cause of folate deficiency is malnutrition. There is a higher incidence of anemia during pregnancy in western Nigeria between May and August each year, and the serum folic acid (SFA) of Nigerian blood donors has been shown to be significantly lower at the same time of the year. Fleming (1970) proposed that the variation of incidence was the result of folate deficiency at a time of food shortage, and was not due to an increase of malaria transmission or hemolysis. He suggested that the fall in the incidence of folate deficiency seen about August follows the harvesting of the new yam crop.

Turkey

Pica, a habit of eating unnatural substances, is an important health problem in Turkey, particularly among villagers who represent the majority of the population. The most common forms of pica are eating of dirt and plaster by children and clay by women (Okeuoglu et al. , 1966).

According to Okeuoglu et al (1966), the relationship of anemia to pica was found in 182 Turkish villagers. Anemia was three times more frequent in pica practicing subjects than in control subjects. The incidence of anemia was greater in children from 4 to 15 years of age than at any other age. The average hemoglobin concentration of 69 control subjects of all ages without a history of pica was 13 gm per 100 ml; 17 percent were anemic. The average hemoglobin concentration of 73 persons of all ages with a history of pica was 9.8 gm per 100 ml and 64 percent of this group was anemic. Anemia associated with pica appeared to be reversible since the average hemoglobin level of 40 subjects who were former dirt or clay eater's was 12.6 gm per 100 ml and only 20 percent had anemia.

The role nutrition plays in the development of pica has not yet been established. Lanskowsky (1959) reported that 83 percent of his patients with pica had worms, ascaris being the most common.

The cure of pica with iron medication is still a controversial problem. In a double-blind study of 16 children with pica, Gutelius (1962) found that iron was not more effective than saline solution in curing or improving the habit of pica. But Lanzkowsky's study (1959) in South Africa showed that pica could be cured permanently by intramuscular injections of iron. Okeuoglu

et al. (1966) indicated that four children with severe anemia associated with pica discontinued their habit in the hospital without iron medication. Changes of environment, diet, attention, interest and supervision might all be factors in the cure of the habit in these patients.

Israel

Rachmilewitz et al. (1966) studied 890 women in Israel in the second and third trimesters of pregnancy and 196 had a hemoglobin value of 10 gm per 100 ml or less. There was some correlation between parity and the incidence and severity of anemia. Anemia was more common in women of North African or Middle Eastern origin and in those with a low family income. Hypochromia was the main feature in most cases of anemia, the mean red cell volume was within the normal range. The anemia women had significantly lower values for iron in serum, folate activity in serum and whole blood estimated with *Lactobacillus casei* and folate activity in whole blood estimated with *Pediococcus cerevisiae*. Possible contributory factors causing these deficiencies are due to gastrointestinal infections and loss of iron by profuse sweating.

Levy et al. (1970) performed complete hematological examinations on 247 health children age one day to six years in Kiryat Shmoneh, Israel. The anemia in children born to anemic mothers was much frequent than among those delivered by nonanemic women. Poor iron and folate stores at birth, malnutrition, and frequent gastrointestinal infections may have contributed to the wide spread deficiency state observed in this group of children. Anemia

seemed to be much less frequent among children whose parents are born in Europe or Israel, but the number of children in this group was too small for a definite conclusion to be drawn.

India

Venkatachalam (1968) reported that the extent of the problem of iron deficiency among the Indian population is much greater than the results of hemoglobin surveys would indicate. The circumstances under which iron deficiency might arise in the Indian population are numerous. Defective absorption is one of the most important. Patwardhan (1961) proposed that the Indian diet based on cereals and pulses has been shown to contain more than 40 percent of the total phosphorus as phytin phosphorus. It may be the factor that interferes with digestion and absorption of food iron.

The effect of calcium on absorption of iron in presence of phytin in the diet was studied by Apt and Venkatachalam (1964). They used three experimental diets, having almost the same amounts of iron 15.54 to 17 mg per day, were given to four healthy men with different levels of calcium about 400, 1000, 1500 mg, respectively. Each subject was under experiment continuously for 30 days, 10 days on each diet, and took throughout a typically Indian cereal rich diet in which the proportion of phytin phosphorus was content at 40 percent of total phosphorus. It was found that with an intake of 1000 mg of calcium, the mean absorption of iron was only about 4 percent, which increased to over 16 percent when the intake of dietary calcium was raised to 1500 mg per day. The

higher levels of dietary calcium probably counter balanced the complexing effect of excess phosphorus and phytate ions on the iron.

METABOLISM OF IRON

Normal values as related to hemoglobin and red blood cells

The amount of iron in the body of the adult male is about 50 mg per kilogram, or a total of 3.5 gm; in the woman it is about 35 mg per kilogram, or a total of 2.3 gm. Approximately 70 to 80 percent of the iron is functioning and the rest is held in storage as ferritin or hemosiderin by the liver, spleen, and bone marrow. Of the functioning iron, 80 percent is in the hemoglobin, and the remainder is in the myoglobin and iron-containing enzymes (Robinson, 1972).

The red blood cells hold a remarkable concentration of iron. They are 33 percent hemoglobin. Consequently their content of iron is high, about 112 mg per 100 gm on a wet weight basis, or 320 mg per 100 gm on a dry weight basis. Normally the concentration remains within narrow limits, so that, although the concentration of iron in the liver and spleen sometimes exceeds that in the red cells, the average concentration of iron in the red cells is much higher than that in any other cells of the body (Hawkins, 1964).

The following table shows that the allocation of iron in the body of a normal man of 70 kg, based on figures by Hawkins (1964).

Table 1 . Distribution of iron in the body of a normal man of 70 kg.

Compound	Iron in total amount of the compound (gm)	Percent of the total iron of the body
Hemoglobin	3.0	75.0
Myoglobin	0.13	3.0
Heme enzymes	0.07	0.2
Ferritin and hemosiderin	0.8	20.0
Siderophilin	0.003	0.1
	4	98

From Hawkins, 1964, p. 315.

Absorption, transportation and excretion

While absorption can occur from the stomach and from any portion of the intestinal tract, it seems to be greatest in the duodenum and progressively less in a descending gradient (Hahn, et al., 1945, and Brown, 1963).

Moore et al. (1944) and Venkatachalam et al. (1956) established the fact that ferrous iron is much more efficiently absorbed than is ferric iron. In all probability the ferric form must always be reduced before absorption can occur under physiologic conditions. Since iron is absorbed in the ionic state, it is reasonable to suppose that gastric acidity can be an important factor in preparing it for absorption. *In vitro* and *in vivo*, gastric juices can

solubilize and reduce iron, but the significance of these findings is questionable. In 1959, Williams reported that iron absorption is normal when gastric dysfunction is present. Hematocrits and plasma iron levels have been found to be the same in women with and without achlorhydria, and plasma iron tolerance tests are not influenced by acid secretions in iron deficiency anemia. Thus, achlorhydria alone would rarely lead to iron deficiency without the intervention of other factors that either increased the requirements or decreased the amount of iron available (Bothwell and Finch, 1962).

Until recently, control of iron absorption had been explained by the "mucosal block" theory proposed by Granick (1951) as shown in Figure 1. According to this theory iron absorption was mediated obligatorily by an iron-accepting protein of the intestinal cell. This protein is apoferritin. The apoferritin-iron complex is ferritin. Iron absorption was thus limited under normal circumstances by the availability of apoferritin in the mucosal cell. It was postulated that, in times of increased iron need, the oxidizing potential at the mucosal border of the luminal cells is lost, allowing ferrous iron to diffuse directly across the cell and into the plasma without mediation of the apoferritin-ferritin system.

According to Conrad and Crosby (1963) and Wheby and Crosby (1963) ingested iron was transported rapidly across mucosal cells to blood stream or was converted to the storage form of ferritin. The iron needs of the body were supplied by the storage iron; the remainder was kept in the cells until they desquamated into the gut. Thus the ferritin formed within the mucosa appears to control the absorption of iron.

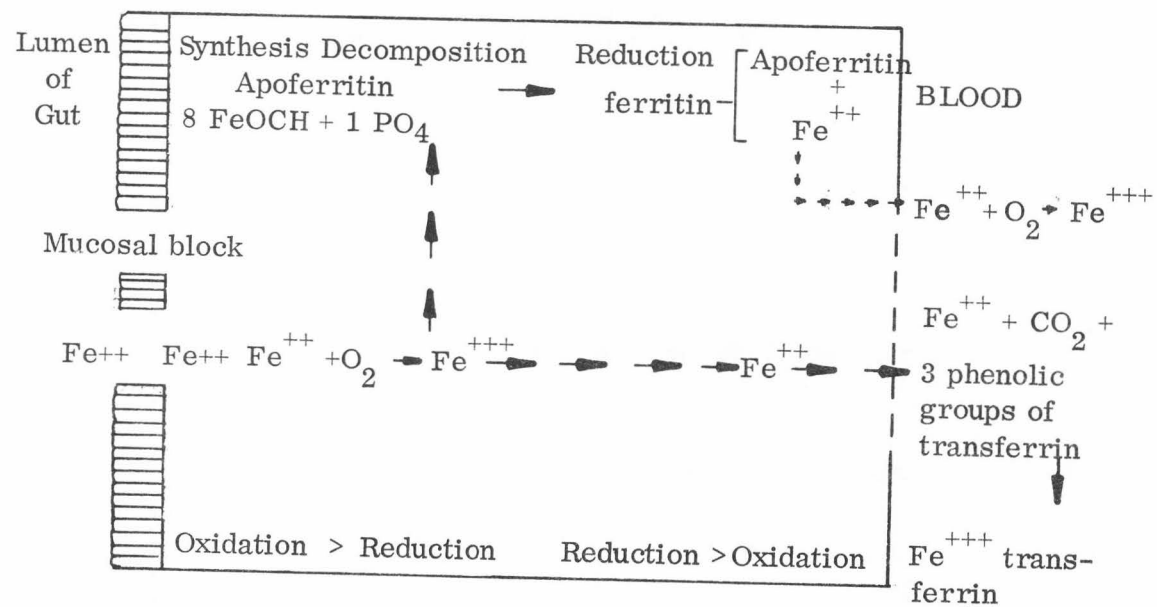


Figure 1. Schematic representation of the "mucosal block" hypothesis (Brown, 1963, p. 206).

In a study of normal and iron deficient pregnant women, Apte and Lyenger (1970) demonstrated that dietary iron absorption is increased in the latter half of pregnancy in normal pregnant women.

Iron absorption from food of animal origin and vegetable origin has been compared with the absorption from a meal in which both vegetable (corn and black bean) and animal food (veal and fish muscle) were combined. Findings indicate that the absorption of iron from meals consisting of veal and corn or black beans was less than that when veal was given alone. The decreased absorption was found less striking when veal was mixed with black bean. Corn did not inhibit the absorption of fish iron. Three times as much corn or black bean iron was absorbed when combined with food from animal origin than when given alone. Amino acids in the same number and proportion as were present in 100 gm of fish muscle enhanced the iron absorption from black beans (a three-fold increase). Their data appeared to indicate that the interaction of vegetables with animal food during digestion may change the pattern of iron absorption of that shown by these foods when given alone. Thus, a certain proportion of animal food should be included in the diet to enhance iron absorption from vegetable food.

After it was absorbed, iron entered the portal circulation bound to transferrin of plasma (Brown, 1963). Similarly, iron from old erythrocytes destroyed predominantly in the spleen was split from the hemoglobin molecule within cells of the reticuloendothelial system and, after a variable stay in these cells as ferritin or hemosiderin, was released to the plasma transferrin and reentered

circulation in the portal bloodstream. Most of the transferrin-bound iron entered the liver and later was circulated to the bone marrow.

Excretion of the metal was exceedingly difficult to measure, but Moore (1970) suggested that excretion was small. The major pathway of iron loss from the body was by hemorrhage, either as a result of disease, accident, blood letting, or menstrual and pregnancy losses in the female population. They suggested that urinary excretion was about 0.1 mg or less per day; in patients with proteinuria, hematuria, hemoglobinuria or iron overload, the values might be several times higher. This occurred by loss of iron carried by excreted transferrin, hemoglobin, or shed renal tubular cells. The daily fecal iron excretion was found to be about 0.3 to 0.5 mg which was from blood destruction into the alimentary canal, unabsorbed ingested or biliary iron and desquamated intestinal mucosal cells.

The loss of iron in sweat and from dermal surfaces has been estimated from one to six mg per day which depended on the weather and the area of skin surface (Moore, 1970).

When the above mentioned several sources of iron excretion were added together, an estimate of 0.5 to 1 mg seemed reasonable.

Function as related to hemoglobin and red blood cells

Iron is the central ion of the hemes (Burnham, 1969). The iron complexes (hemes) serve as prosthetic groups of the cytochromes and hydroperoxidases including the oxygen-carrying heme proteins. Basically, they played

an essential role in the transport of electrons to activate oxygen and the free energy used to meet energy requirements.

The biosynthesis of the heme was completed by the insertion of iron into protoporphyrin, as seen in Figure 2. The exact way that heme and the protein globin were joined to form hemoglobin was not clear (Brown, 1963). However, there seemed to be enhancement of heme synthesis from protoporphyrin in the presence of globin. The suggestion has been made that protoporphyrin and globin combine before the insertion of iron.

Symptoms of deficiency as related to anemia

The anemia of iron deficiency is characterized by small, pale erythrocytes, depleted iron stores, a plasma iron of less than 40 ug per 100 ml, an elevated iron-binding capacity and less than 15 percent saturation of the transferrin. Because of the limited ability of the body to excrete iron except as shed blood, iron depletion occurs slowly in the absence of frank hemorrhage. When iron stores are nearly depleted the plasma iron falls to subnormal levels and erythropoiesis slows. Then the production of red cells by the erythroid marrow is retarded. The anemia which results, is first normocytic, normochromic and then became microcytic, hypochromic. Red cell protoporphyrin increased as an indication of imbalance between iron supply and porphyrin synthesis (Moore, 1970) as seen in Figure 3. Severe anemia developed as the speed of iron depletion increased. When hemorrhage was acute, the production of red cells could be restricted before all iron was mobilized from the

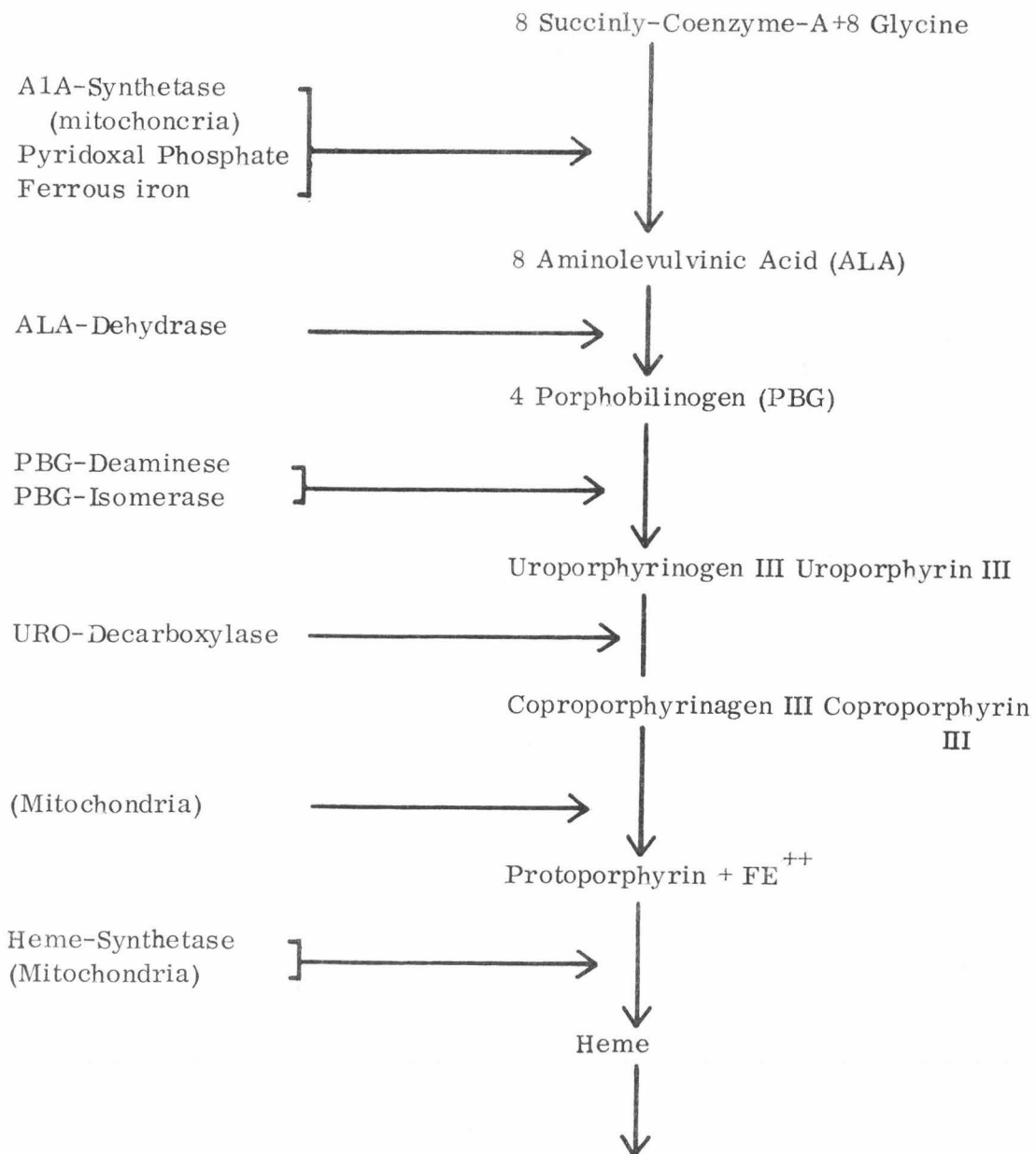


Figure 2. Scheme of heme biosynthesis (from Brown, p. 83, 1963).

reticuloendothelial cells and the anemia would be normocytic or even slightly macrocytic. In recent medical practice, about one-half of the cases of iron deficiency anemia were normocytic (Finch, 1969).

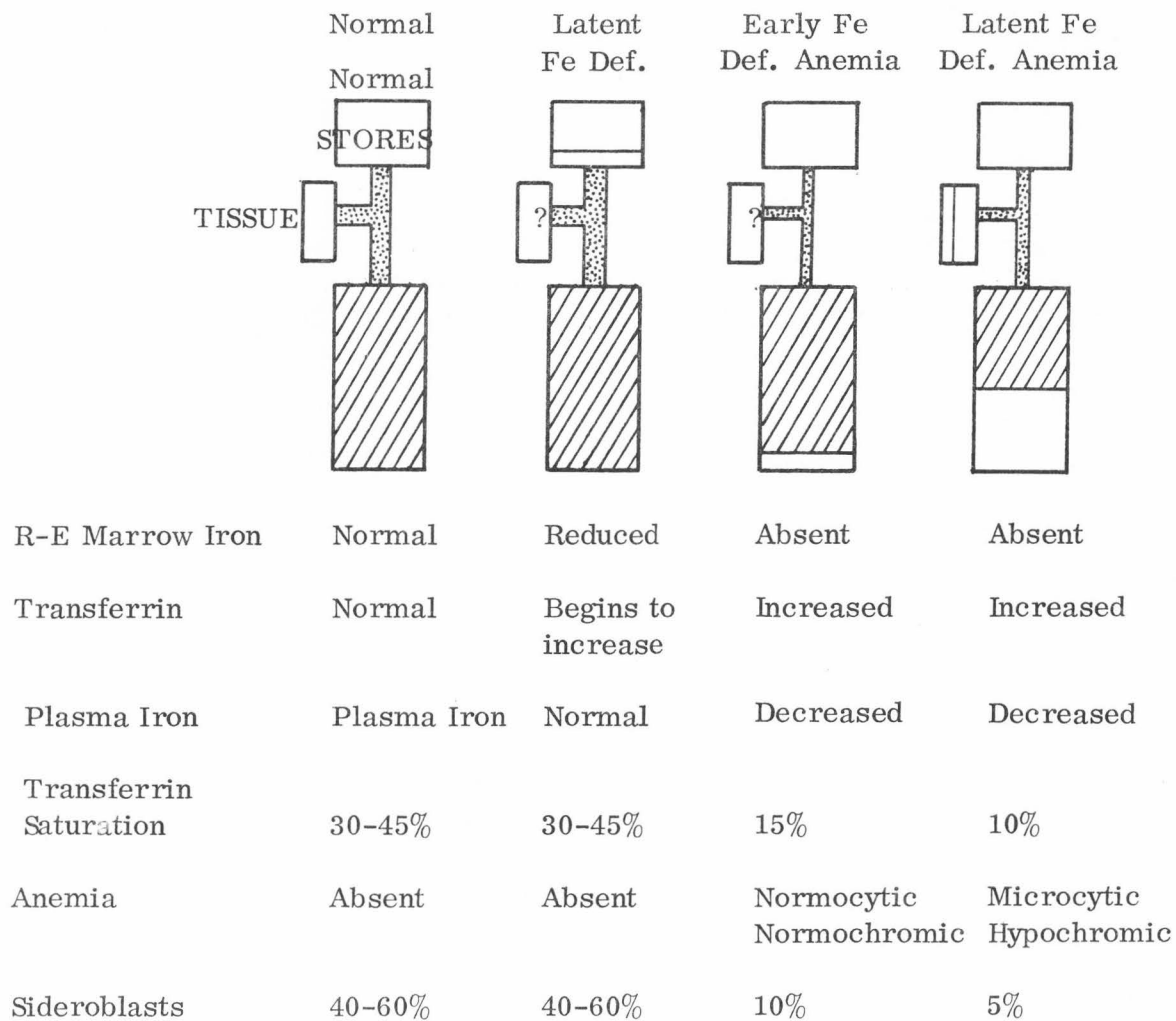


Figure 3. Schematic representation of the development of iron-deficiency anemia. The "?" in the tissue iron blocks indicates the uncertainty about when depletion occurs. (Taken from Moore, 1970, p. 352.)

METABOLISM OF FOLIC ACID

Normal values as related to hemoglobin and red blood cells

Frank et al. (1963) reported that the concentration of folate derivatives in the serum of cattle, sheep, horse, rabbit and chicken is similar to that in man but in the rat, serum concentration of normal animals are significantly higher. Lynch and Moloney (1963) suggested that the range of serum concentration encountered was 55 to 190 ng per ml with a mean value of 129 ng per ml and Bird et al. (1965) proposed that the range was 53 to 80 ng per ml with a mean of 62 ng per ml.

Herbert (1965) has pointed out that significance of the concentration of folate derivatives in whole blood cannot be determined unless the hematocrit is reported. He cites as an example the gradual fall in whole blood folate derivatives in infants during the first eight postnatal weeks which may merely reflect the drop in the hematocrit that is known to occur during this period, rather than folate depletion.

Absorption, transportation and excretion

Burgen and Goldberg (1962) have measured the absorption of tritium-labelled folic acid from segments of small intestine of the rat in situ. They found that the absorptive capacity of the jejunum was only slightly greater than

that of the ileum and that the flux from the mucosal (interior) surface to serosal (exterior) surface of the intestine was about 14 times the serosal to mucosal flux, so that the uptake of folate is an active process.

A preliminary report by Herbert and Shapiro (1962) summarizes results obtained with everted sacs of rat small intestine. They appear to indicate that in the rat the major site of absorption of folic acid the proximal third of the small intestine, and that folate absorption is an active process. Passage of folate from the serosal to the mucosal surface does not appear to involve this process and may be due to diffusion.

In 1953, Spray and Witts demonstrated rapid clearance of 5-formyl tetrahydrofolio acid (THFA) from the plasma of normal human subjects after intravenous injection of 1 mg of this derivative. An average of about 15 percent of the injected dose was excreted in the urine and this excretion had ceased within 24 hours after the injection. Most of the injected material must, therefore, have been rapidly taken up by the tissues. Baker et al. (1965) have reported rapid clearance of 5-formyl THFA from plasma after intravenous or intramuscular injection of 10 mg of this derivative. The urinary excretion of folate derivatives in the 24 hours after such a dose of 5-formyl THFA is 13 percent of an intravenously administered dose and 10 percent of an intramuscularly administered dose.

Herbert and Zalusky (1962) found that with intravenous injection of folate there was no significant increase in the erythrocyte content of folate derivatives assayed by *Streptococcus faecalis* or *Lactobacillus casei*. They also observed that a sample of erythrocytes containing 26 percent of

reticulocytes contained about nine times more folate derivatives active for *Lactobacillus casei* than was present in another sample of erythrocytes (from the same blood) containing six percent of reticulocytes. On the basis of these results they concluded that the mature erythrocytes is relatively impermeable to folic acid, and that the young erythrocyte is more permeable.

Function as related to
hemoglobin and red blood cells

The folic acid coenzymes are extremely important biologic catalysts as indicated by studies with microorganisms, liver slices and laboratory animals. Herbert (1967) indicated a number of the reactions in which the folate coenzymes are involved. These enzymatic reactions involve removal of one-carbon units from one compound and their delivery to another. Any of these reactions may be inhibited not only by nutritional lack of folate but also by blockade of conversion of one folate enzyme to another due to lack of apoenzyme transferase or lack of formiminotransferase due to liver disease.

Folio acid is one of the important hematopoietic agents necessary for proper functioning in animals and man. Its deficiency will result in nutritional anemia for which folic acid is a complete therapy. Folic acid will bring about reticulocytosis and hemoglobin regeneration in pernicious anemia patients, however, but will not correct the neurologic degradation. This disease is due to vitamin B₁₂ deficiency (Chow, 1964).

Symptoms of deficiency
as related to anemia

Herbert (1962) reported the biochemical and hematologic sequence of events in experimentally induced dietary folate deprivation in a healthy 35 year old, 77 kg male. After only three weeks of dietary folate deprivation, serum *Lactobacillus casei* folate activity had fallen from normal (above 7 ng per ml) to the value associated with folate deficiency (below 3 ng per ml). However, tissue deficiency of folate, manifested by a low erythrocyte folate value, did not appear until four months after the onset of dietary folate deprivation.

Herbert (1967) showed the next easily ascertainable sign occurs within the second month which was an increase in the average number of lobes of the nuclei of the neutrophilic polymorphonuclear leukocytes. About 40 to 50 percent of neutrophils normally have three lobes, 20 to 40 percent have two lobes, and 15 to 25 percent have four lobes. Finding a substantial increase in the percentage with four lobes or more than five percent neutrophils with more than four lobes, or even a single neutrophil with more than five lobes suggests the possibility of early folate or vitamin B₁₂ deficiency. After more than three months of folate deprivation, gross tissue depletion of folate becomes evident, as manifested by fall of *Lactobacillus casei*-measurable erythrocyte and liver folate stores and a rise in urinary excretion of formiminoglutamate, urocanate, formate, and amino-imidazolecarboxamide, and overt megaloblastic anemia.

METABOLISM OF VITAMIN B₁₂Normal values as related to
hemoglobin and red blood cells

Plasma vitamin B₁₂ levels in patients with pernicious anemia are about one-tenth of the normal plasma vitamin B₁₂ level. High serum vitamin B₁₂ levels have been found in patients with cirrhosis and myeloid leukemia (Vilter, 1970). Wright et al. (1955) determined the total serum vitamin B₁₂ concentrations in 528 individuals. The individuals studied were "normal" as far as physical health is concerned, and the major variable was age. In such person there is a trend toward lower serum concentration of vitamin B₁₂ in the aged than in younger age groups. Although this difference is statistically significant, the biological importance of this finding is not apparent. There is wide individual differences of values in serum vitamin B₁₂ concentration at all age levels, but in the "normal" group, values below 200 ng per ml and above 1000 ng per ml were uncommon. The average for the entire 528 individuals was 560 ng per ml, the 95 percent confidence bands being 70 and 1060 ng per ml respectively.

Swendseid et al. (1957) reported the range and average values for the vitamin B₁₂ content of liver tissue obtained from 132 subjects. The average value for all age groups was approximately 0.7 μ g per gm. It was concluded that there are no apparent differences in vitamin B₁₂ stores in the various age groups.

Absorption, transportation
and excretion

Intrinsic factor is produced by glands in the fundus and cardia of the stomach. Vitamin B₁₂ is linked with the intrinsic factor and carried through the small intestine to special sites in the ileum. There, the vitamin is attached to the special epithelial cells by the intrinsic factor in the presence of calcium and is transported across the cell into the blood circulation. The intrinsic factor remains in the intestine. In the blood circulation vitamin B₁₂ is combined with serum proteins (Hobinson, 1972).

Bozian et al. (1963) presented a study about vitamin B₁₂ absorption, distribution and excretion of a metabolite. The range of absorption in the subjects without pernicious anemia is 45 to 80 percent with a mean of 70 percent and in those with pernicious anemia 0 to 17 percent with a mean of 3.25 percent. Losses from the body through urine, stool and desquamating skin amounts to 0.1 percent of total body stores daily.

Swendseid et al. (1954) showed the extreme limitation of vitamin B₁₂ absorption from the gastrointestinal tract of individuals with normal gastric function. Within the dose range used, an upper level of absorption appears to have been reached which averages 1.6 ug vitamin B₁₂. This level is not increased by giving the vitamin with a test meal or in conjunction with intrinsic factor sources. In patients with a total gastrectomy, a graded response to intrinsic factor is obtained using 0.5 ug vitamin B₁₂ but at a 5 ug level, increasing the amount of intrinsic factor does not increase vitamin B₁₂ absorption beyond levels obtained in normal subjects.

Doscherholmen et al. (1957) found that radioactive vitamin B₁₂ that has been absorbed after injection or oral administration is mainly stored in the liver. This radioactive store is extremely resistant to flushing out with non-radioactive vitamin B₁₂. They gave an experimental subject 100 doses each of 1 mg during seven months and found that the radioactivity measured over the liver was still 20 percent of the initial value.

Function as related to
hemoglobin and red blood cells

Vilter et al. (1950) suggested that vitamin B₁₂ may catalyze a reaction which results in the formation of thymidine. Koch-Weser et al. (1949) found that vitamin B₁₂ in large doses protects the ribonucleic acid content of the liver cells of rats poisoned with carbon tetrachloride. This observation links vitamin B₁₂ with ribose as well as desoxyribosenucleic acid metabolism. Nieweg et al. (1954) studied the relationship of vitamin B₁₂ to folic acid in the megaloblastic anemias. They cited evidence to support the idea that in the human, vitamin B₁₂ was particularly concerned with pyrimidine formation RNA-protein synthesis.

Symptoms of deficiency
as related to anemia

Hamilton et al. (1952) reported that fresh normal erythrocytes are damaged in a few days in the milieu of the human body deficient in vitamin B₁₂ that they are destroyed in random fashion, presumably by the trauma of circulation. The cell damage incurred in the first few days is not reversible by the subsequent administration of vitamin B₁₂.

In vitamin B₁₂ deficiency, erythrocytes, white blood cells and platelets are formed at slow rates. Erythrocytes grow large and have only half their normal life span. Many other biochemical abnormalities occur such as negative nitrogen balance, decreased choline esterase activity in erythrocytes and plasma, moderate hypoprothrombinemia, hyperferremia, slightly lowered erythrocyte protoporphyrin, and low uric acid and cholesterol levels (Vilter, 1970).

METABOLISM OF PROTEIN

Normal value as related to
hemoglobin and red blood cells

The total amount of body protein is approximately 19 percent of the fresh weight. Approximately 45 percent of this protein is present in the muscle and 18 percent in the skeleton, while skin and adipose tissue account for another 10 and 4 percent respectively (Albanese and Orto, 1970).

The following table shows that the allocation of protein in the body of a woman weighing 53.8 kg.

Table 2. Large protein stores in the body of a woman, 168.5 cm and 53.8 kg

	Grams
Total protein (N x 6.25)	100,006
Striated muscle	4,680
Skeleton	1,864
Skin	924
Adipose tissue	361
Estimate of blood	750
Albumin	250

From Cooper, Forbes and Mitchell, 1953, p. 365.

Absorption, transportation and excretion

Proteins are composed of a variety of different alpha-amino acids. In the protein molecule the amino acids are connected by the peptide-linkages formed between the carboxyl and amino groups of two adjacent amino acids. The breakdown into the component amino acids occur in the stomach and in the upper part of the intestinal tract. There the proteins are split into smaller fragments by the "proteinases" such as pepsin, trypsin, chymotrypsin and the resulting smaller peptides are further digested by different "peptidases" (Albanese et al. , 1970).

Schlüssel et al. (1953) used ^{35}S -labeled proteins to show that 11 percent of the material was taken up by the stomach wall, 60 percent by the small intestine and 28 percent by the colon. They suggested that both the stomach and the colon may be involved in amino acid absorption.

The amino acids liberated from the food proteins by enzymatic processes within the intestinal tract are transferred to the tissues. After absorption, the amino acids are translocated through-out the body via the circulatory system. At this stage amino acids can enter into one of two metabolic pathways. Anabolism is the pathway utilized to synthesize cellular protein. Catabolism is the reverse process involving the breakdown of body protein to amino acids, followed by the breakdown of the amino acids with the excretion of urea, or with the excretion of nitrogen containing compounds, such as creatinine.

Spencer (1969) reviewed the intestinal transport of amino acids with reference to both in vivo and in vitro studies and their limitation. He

mentioned that active transport of amino acids by the small gut can be inhibited by agents interfering with energy production, by other amino acids, by carbohydrates in some cases, and by low sodium concentrations or alteration of adenosine triphosphatase. Transport also responds to other factors, such hormone administration.

Function as related to hemoglobin and red blood cells

All enzymes so far identified are protein. Coenzymes necessary for the action of enzymes have a protein structure usually associated with a specific vitamin. Hemoglobin, the substance in blood responsible for its oxygen and carbon dioxide-carrying properties so vital in respiration, is a protein complex (Guthrie, 1971).

Scrimshaw et al. (1961) studied the anemia of untreated kwashiorkor. They found that the total iron binding capacity is, however, markedly reduced, resulting in a high percentage saturation. The reduction in iron binding capacity is probably due to decreased transferrin, and iron binding protein present in the beta-globulin fraction of plasma, which fraction tends to be relatively and absolutely decreased in protein malnutrition.

Symptoms of deficiency as related to anemia

Anemia is an important manifestation of kwashiorkor and is most frequently normocytic in type, but hypochromic, macrocytic, hypoplastic, and megaloblastic anemia have been reported singly or in combination.

Although the major deficiency in kwashiorkor is of protein, the syndrome is often associated with multiple nutritional deficiencies and intercurrent and parasitic infestations (Nutritional Reviews, 1968).

Adams et al. (1967) recently stressed that although evidence of iron deficiency is uncommon in the anemia of kwashiorkor prior to treatment, the pattern of iron deficiency commonly emerges after treatment with a high protein diet. According to Finch (1969), the major defect in the anemia of protein malnutrition is a failure of red cell production due to decreased stimulation of the erythroid marrow. This decreased stimulus is thought to be a natural consequence of the reduced oxygen requirement of the protein depleted subject. With protein feeding, metabolism and growth are speeded up, and new complications may be caused by the rapid production of plasma protein and expansion of plasma volume. The need for other nutrients needed for red blood cell production, such as iron and folate, is increased, and amounts previously adequate for the stunted malnourished child may now precipitate deficiency symptoms.

METABOLISM OF VITAMIN E

Normal value as related to
hemoglobin and red blood cells

Quaife and Dju (1949) examined the distribution of alphotocopherol and delta and gamma-tocopherol in tissues of apparently normal person (accident cases, one woman and one man). Body fat was found to be the major storage site. Tocopherol levels based on fat content were quite similar in a variety body contained several grams of total tocopherol, most of which was located in the adipose tissue.

Absorption, transportation
and excretion

Experiments with cholsdochocolostomized rats with bile fistula show that bile is required for optimal absorption of vitamin E (Greaves and Schmidt, 1937).

Vitamin E does not seem to be esterified during absorption. The serum contains free tocopherol even after feeding of alphotocopherol acetate or phosphate. Absorbed vitamin E is deposited in various tissues. D-alpha-tocopherol is stored to a somewhat larger extent than the dl-form and to a much larger extent than d-gamma-tocopherol (in rats) (Dam and Sondergaard, 1964).

Mason (1942) examined the deposition of vitamin E in various tissues of the rat at different dietary levels, using the rat antisterility test as criterion. Only a relatively small portion of the ingested amount was stored.

Function as related to hemoglobin and red cells

Vitamin E is probably involved in some synthetic processes. One of these is the incorporation of pyrimidines into nucleic acid, especially in the formation of the red blood cells in the bone marrow (Robinson, 1972).

Nitowsky et al. (1956) studied the relationship between catalase activity and hydrogen peroxide hemolysis. The correlation between the ability of various antioxidants to inhibit both hemolysis and catalysis of unsaturated fatty acid oxidation by lipoxidase or hematin compounds lends support to the hypothesis that vitamin E may play a role in maintaining the integrity of the erythrocyte by inhibition of an oxidase action on the unsaturated fatty acids of the cell membrane.

Tocopherols function as reversible antioxidants, it has been proposed that primary biologic role of vitamin E is as a physiologic antioxidant in vivo, particularly in inhibiting the oxidation of unsaturated fatty acids (Gordon and Nitowsky, 1970).

Symptoms of deficiency as related to anemia

Low plasma tocopherol levels and increased susceptibility of erythrocytes to hemolysis in the presence of hydrogen peroxide were used as criteria of insufficient vitamin E (Herting, 1966).

Majaj et al. (1963) showed the malnourished children with vitamin E deficiency in whom low plasma tocopherol levels have been associated with macrocytic anemia. Treatment with vitamin E has been shown to induce a favorable hematologic response in malnourished infants with macrocytic anemia. These changes include typical therapeutic induced increase in reticulocytes, followed by erythropoiesis and abatement of macrocythemia and conversion of megaloblastic marrow to normoblastic one.

METABOLISM OF PYRIDOXINE

Normal values as related to
hemoglobin and red blood cells

Pyridoxine, pyridoxal, pyridoxamine and pyridoxal phosphate, collectively, are called the vitamin B₆ group. Vitamin B₆ occurs in animal products largely in its pyridoxal and pyridoxamine forms. Pyridoxine is the largest component in products of vegetable origin. Each of the three forms is equally active in animals (Rabinowits and Snell, 1948).

Baker et al. (1966) studied two young, normal adult men who were given 30 μ c of 2-¹⁴C-labeled pyridoxine orally. Over 97 percent of the orally ingested labeled pyridoxine was absorbed, with no conversion to carbon dioxide, as noted by monitoring the radioactivity of the expired air. The best estimate of the total body pool of vitamin B₆ in the young adult is approximately 16 to 25 mg.

Persons on an adequate diet have, on the average, 5 μ g per 100 ml of vitamin B₆ in the blood as determined by *Streptococcus Carlsbergensis* assay. Most of the vitamin B₆ is in the blood cells. Administration of pyridoxine increases the pyridoxal phosphate level of whole blood within 3 days and leukocytes within 10 days. A total of 7 mg of pyridoxine per day appears to be all that the human body can convert into the coenzyme form (Vilter, 1970).

Absorption, transportation
and excretion

Bain (1959) has shown that all these members of the vitamin B₆ group are probably rapidly converted in the intact animal into pyridoxal phosphate and pyridoxamine phosphate within two hours.

Hurwitz (1953) suggested that the phosphorylation of pyridoxal requires the activity of a specific enzyme pyridoxal kinase utilizing a mole of adenosine triphosphate to produce pyridoxal phosphate. To reverse this reaction, phosphatase is needed to remove the phosphate, leaving free pyridoxal. Coursin (1961) said that pyridoxal in turn may reenter the same cycle or may proceed by transamination to pyridoxamine and thence through pyridoxamine phosphate by oxidation or transamination to pyridoxal phosphate. On the other hand, pyridoxal may be changed by the aldehyde oxidase of the liver to 4-pyridoxic acid. This compound is an inactive degradation product and is excreted by the kidney.

The schematic diagram to provide a working hypothesis for understanding the relationships of the various members of the vitamin B₆ group are shown in Figure 4.

Function as related to
hemoglobin and red blood cells

Synderman and co-workers, in 1953, first demonstrated an essential role for vitamin B₆ in human erythropoiesis, when microcytic, hypochromic anemia appeared in eight month old boy after 130 days on a vitamin B₆ deficient diet.

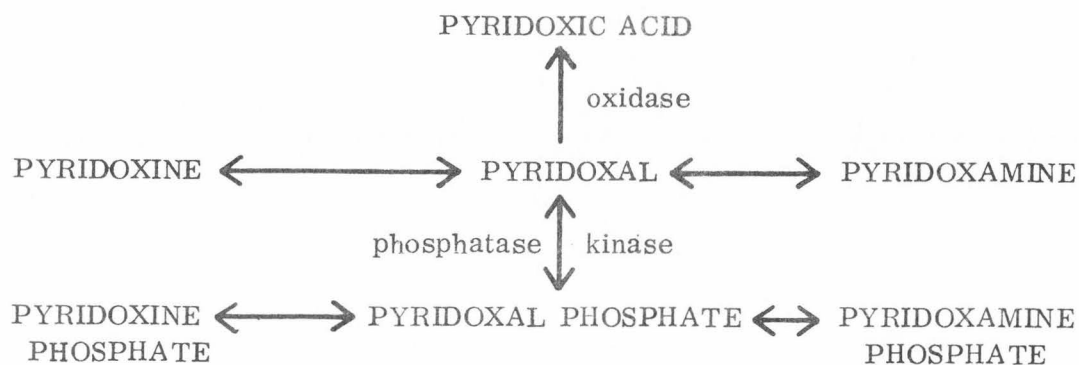


Figure 4. The metabolism of the members of vitamin B₆ group (From Snell, 1958, p. 111)

Cartwright and Wintrobe (1957) measured the erythrocyte protoporphyrin contents of 31 normal pigs and 14 pyridoxine-deficient pigs. The mean plus or minus standard deviation for the normal group was 118 ± 43.4 per 100 ml of red cells and for the pyridoxine-deficient group 47 ± 13.6 per 100 ml of red cells. They suggested that the fundamental disturbance in pyridoxine deficiency anemia in swine is failure to synthesize protoporphyrin.

Symptoms of deficiency as related to anemia

Hines and Harris (1964) studied three elderly patients (sixth decade or beyond) with pyridoxine-responsive anemia. In each megaloblastic erythropoiesis was found. The pyridoxine-responsive anemia has usually been considered characterized by normoblastic erythropoiesis, hypochromic, microcytic adult erythrocytes and evidence of iron overload.

Horrigan and Harris (1964) reported the ferrokinetic studies in patients with pyridoxine-responsive anemia confirm the failure of bone marrow to utilize iron effectively in the delivery of hemoglobin to the peripheral blood. The appearance of intravenously injected tracer amounts of ^{59}Fe in the erythrocytes of the peripheral blood is almost always decreased, with 70 to 80 percent incorporation noted during transfusions and vitamin B_6 therapy. Marked iron overload, including erythroblastic iron content and plasma transferrin and transferrin-bound iron that increase the nonhemoglobin iron pool, undoubtedly influences interpretation of data on the percentage of ^{59}Fe label utilized for the hemoglobin synthesis. Plasma turnover rates of iron in patients reflect a very rapid clearance of iron from the peripheral blood.

METABOLISM OF ASCORBIC ACID

Normal value as related to the hemoglobin and red blood cell

The normal concentration of ascorbic acid in various tissues and body fluids of all the higher animals tends to follow a similar pattern but there are many moderate differences. The tissue distribution and patterns of change during a deficient dietary intake are similar, so far as studied, in man and all the animals depend upon the dietary intake. The normal muscle tissue is relatively low in ascorbic acid content, compared with glandular tissues. During depletion blood and urinary concentration fall more rapidly and in greater degree, approaching zero, compared with blood cells and fixed tissues. The normal pituitary, adrenal cortex, corpus luteum, embryonic tissue, leucocytes, and glandular cells of the intestinal tract are particularly high and vary with the degree of activity. Pancreas, kidney, liver, thymus, salivary, spleen, and brain tissue as intermediate (Nutrition Reviews, 1968).

Absorption, transportation and excretion

Ascorbic acid is absorbed in humans in the upper part of the intestine, possibly by simple diffusion, and is circulated in the blood. From the blood stream it is picked up by the tissues, passing readily into the adrenal tissue with 66 mg per 100 gm of tissue, kidney with 12 mg per 100 gm of tissue,

liver with 32.8 mg per 100 gm of tissue, and spleen with 41.9 mg per 100 gm of tissue, most of which appears to be equilibrium with the serum. The eye, muscle, testes, and brain also accumulate some, but the concentration is markedly different from that in the blood serum (Guthrie, 1971).

In 1958, Hellman and Burns studied the turnover of L-ascorbic acid- 1-C^{14} in man and have compared their results with representative animal studies. In man, all the ascorbic acid given appeared in the urine in the form of ascorbic acid, diketogulonic acid, or oxalic acid, and none was found in the expired CO_2 . In the guinea pig, respiratory CO_2 is a major route for the excretion of derivatives of ascorbic acid.

Abt et al. (1963) first experimentally reported a new excretory pathway of ascorbic acid catabolism in man, the respiratory tract. The human subjects, two men and two women, were normal adult volunteers on estimated daily dietary intakes of 25, 30, 40 and 60 mg of ascorbic acid. The hourly excretion of C^{14} for human subjects was followed for a seven-day period. Initially exhaled C^{14}O_2 was high in all subjects but leveled off thereafter. The higher the blood level and intake of ascorbic acid, the greater the amount excreted in the urine. This is also true for the rate of hourly excretion. The cumulative amount of C^{14} excreted from administered radioactive ascorbic acid was compared for a seven-day period for guinea pig, monkey and man. The total amount excreted was great for the smallest subject; otherwise the curves for the three species were similar.

Function as related to hemoglobin
and red blood cells

Terroine (1962) demonstrated that ascorbic acid can assist in the utilization of iron and often to afford a significant sparing or protective effect on several vitamins in the B-complex including thiamin, riboflavin, folio acid and pantothenic acid, and on vitamins A and E. The affects appear to depend on its reducing or antioxidant action.

Moderate injections of diphtheria toxin cause losses of ascorbic acid from guinea pig glandular tissues in the range of 33 percent; and a prior intake of the vitamin in the range of 5 to 10 mg per day affords greater protection than lesser intakes against the resultant injury, particularly as seen in the odontoblasts and subcutaneous tissue (Nutrition Reviews, 1968).

Symptoms of deficiency
as related to anemia

Roy and Guha (1958) proposed that among all animal studies thus far, only man and other primates, guinea pigs, the red vented bulbul bird and the fruit eating bat are dependent on food sources for ascorbic acid. They are genetically deficient in the enzyme L-gulonolactone oxidase. Other animals synthesize ascorbic acid from glucose, via glucuronic acid and gulonic lactone and keep it under physiologic control if at all essential nutrients are supplied.

Anemia does not occur in pure ascorbic acid deficiency of mild degree, such as has been observed in human subjects who have undergone five months or more of a diet deficient in ascorbic acid but adequate in all other essential

nutrients. Even blood donations of 6000 cc or more during the period is insufficient stress to induce anemia (Vilter, 1970).

Zulzer et al. (1949) studied 36 cases of infantile scurvy with the purpose of investigating the relation between ascorbic acid deficiency and anemia. The anemia usually noted in case of scurvy does not present a uniform picture. Macrocytic, normocytic, normochromic, and microcytic hypochromic pictures were observed with corresponding bone marrow patterns. Correlation of the clinical and hematologic features indicated that the anemia found in scurvy represents a variety of mechanisms; namely, (1) response to hemorrhage, (2) infection, and (3) coexisting specific deficiencies, such as liver principle deficiency in megaloblastic anemia and iron deficiency. It could be shown that in the presence of continued ascorbic acid deficiency good hemopoietic responses were obtained with folio acid when the marrow pattern was megaloblastic, but no secondary reticulocyte response following the subsequent administration of ascorbic acid was noted.

METABOLISM OF COPPER

Normal values as related to hemoglobin and red blood cells

The adult human body contains about 110 to 150 mg of copper. In terms of concentration it is highest in liver, kidney, heart, and brain, and comparatively low in lungs, spleen, and muscle. During growth, the highest concentration of copper appeared in the rapidly developing structures (Hawkins, 1964).

Over 90 percent of the copper in mammalian plasma is associated with the alpha-globulin, ceruloplasmin, while the bulk of erythrocyte copper is presumed to be associated with erythrocyte copper. Human whole blood contains about 100 ug per 100 ml of copper distributed about equally between erythrocytes and plasma. A small amount of the plasma copper is bound to amino acids, such as glutamin, histidine, and threonine, apparently in equilibrium with that bound to albumin (Li and Vallee, 1970).

Absorption, transportation and excretion

Dowdy (1969) suggested that, in man, copper absorption occurs in the stomach or the upper small intestine, or both. A schematic representation of the major metabolic pathways of copper in the body is presented in Figure 6.

From the intestine, copper moves into the blood serum (Reaction 2). Serum copper can be generally divided into two forms. The copper which reacts directly with the copper colorimetric reagent, sodium diethyldithiocarbamate, is loosely bound to serum albumin. This is the small fraction of serum copper. The larger fraction is tightly bound to the serum copper enzyme, ceruloplasmin. The copper in the enzyme does not react directly with carbamate (Cartwright and Wintrobe, 1964).

Studies with radioactively labeled copper administered by mouth have revealed that absorbed copper appears rapidly in the plasma, bound loosely to albumin, reaching a peak concentration at approximately two hours. There is then some fall in serum copper levels followed by a secondary peak at approximately 24 hours, with the copper then tightly bound to ceruloplasmin. When equilibrium is reached, about 93 percent of serum copper is in ceruloplasmin and 7 percent in the albumin and amino acid-bound fraction. Albumin bound copper is the transport fraction and copper bound to ceruloplasmin is not exchangeable with ionic copper in vivo and is released only when the protein molecule is catabolized (Cartwright and Wintrobe, 1964).

The albumin-bound serum copper fraction is transported to the various body tissue. The reactions which were involved are numbers 3, 5, 11, and 14 in Figure 6. Generally, tissue copper was in equilibrium with direct reacting serum copper (Reactions 4, 12, and 15). Some of the tissues need to be considered separately.

Copper was found in the red blood cell. The red blood cell copper concentration was not influenced by either total serum copper or ceruloplasmin

levels. Two forms of copper have been found in the red blood cell: (a) erythrocyuprein, a copper protein that accounts for about 60 percent of the red blood cell copper and (b) a labile fraction known as nonerythrocyuprein copper that accounts for the 40 percent. Erythrocyuprein is probably synthesized in normoblasts in the bone marrow (Reaction 6). Presumably the nonerythrocyuprein fraction of erythrocyte copper is likewise supplied at the normoblast stage, however, copper moves freely from the direct-reacting fraction of serum into mature, nonreticulated erythrocytes, and vice versa (Reactions 9 and 10). The erythrocyuprein and nonerythrocyuprein fraction in red blood cell were in equilibrium (Reactions 7 and 8).

The liver is the key organ in the body economy of copper (Reaction 14). The major pathway for the excretion of copper is through the biliary system (Reaction 20 and 22). The small amounts are excreted in the urine (Reaction 13).

Any extra copper will move to liver compartments A and B (Reaction 16 and 17), where biliary excretion and ceruloplasmin synthesis occurs, respectively. Ceruloplasmin is emptied into the blood serum (Reaction 18). Although it has not been demonstrated, ceruloplasmin probably returns to the liver for catabolism (Reaction 19).

Function as related to hemoglobin and red blood cells

Experiments on a number of animal species have shown that copper functions in the utilization of iron in an early stage of hematopoiesis. Copper

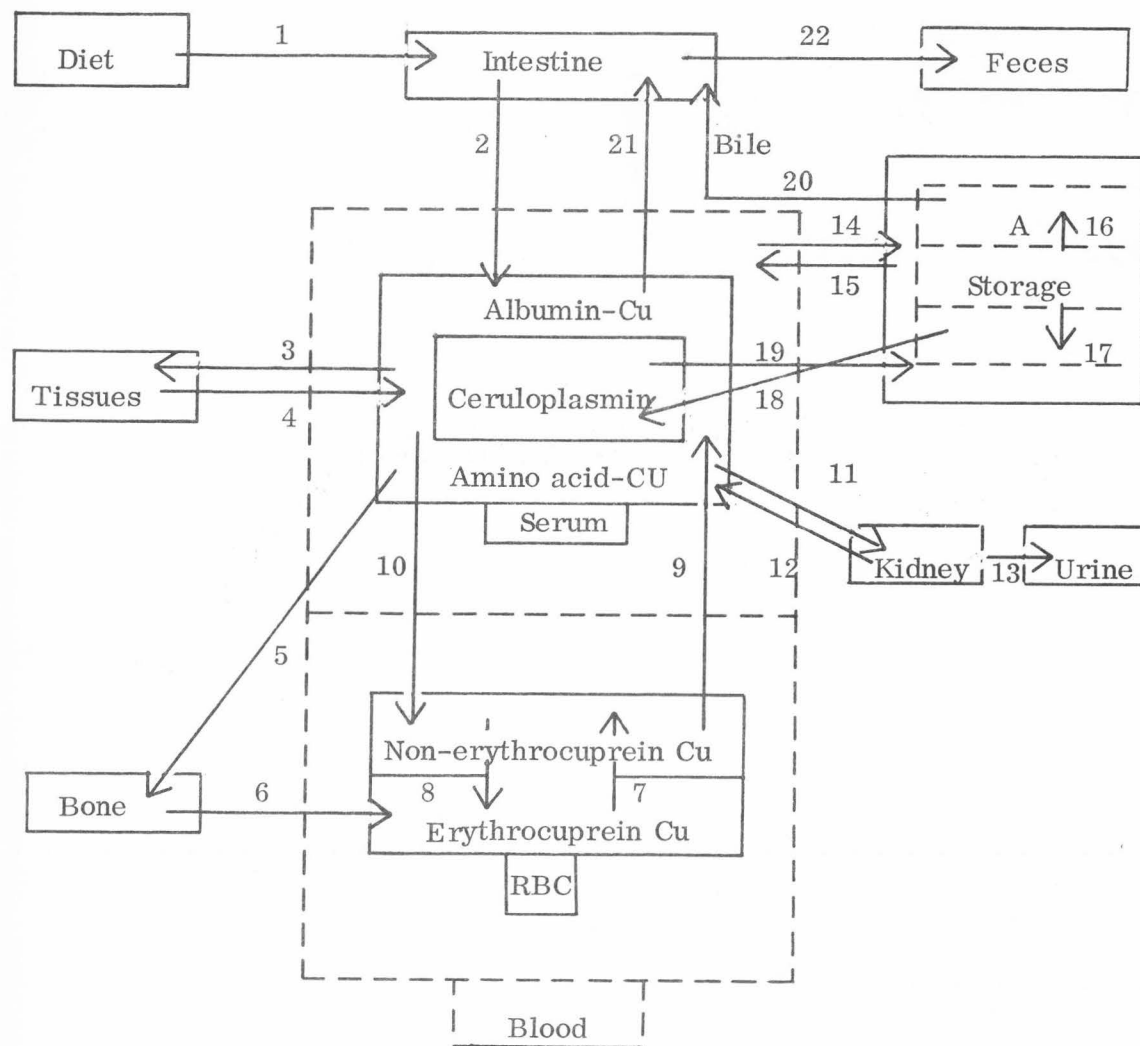


Figure 5. Schematic representation of the major metabolic pathways of copper in the human body (Dowdy, 1969, p. 88).

appears to be involved with the development of young erythrocytes, in contrast to iron, which governs the development of the size of the cell and its hemoglobin content (Hawkins, 1964).

Matrone (1960) suggested that copper affects iron absorption in an indirect manner. In a copper deficiency, hemoglobin synthesis was decreased and iron accumulated in body tissues. The absorptive sites were then signaled to block further iron absorption. When copper was given hemoglobin synthesis would be initiated, body iron stores would be reduced, and the absorptive sites would begin to function. Thus, the increase of iron absorption by copper was controlled by the body need (Crosby, 1968).

Symptoms of deficiency as related to anemia

Cordano, Baertl and Graham (1964) have described the occurrence of biochemical and clinical signs of copper deficiency in infants recovering from severe malnutrition. The infants ranged in age from 8 to 15 months upon admission to the hospital. The patients were given a milk formula designed to provide an adequate intake of protein and calories. Iron supplements were also given, yet all showed a progressive fall in the blood hematocrit and a progressive neutropenia, with little evidence of reticulocytosis.

In 1956, Sturgeon and Brubaker studied the five infants with hypochromic anemia and laboratory findings of marked hypocupremia, hypoferremia and hypoproteinemia. Although this syndrome was rare, its occurrence in young infants has produced a revival of interest in the role of copper in infant nutrition.

Preliminary observations in this laboratory have revealed five cases of iron deficiency anemia associated with marked reduction in serum copper concentration. In association with the hypocupremia and iron deficiency anemia were findings of decreased serum iron concentration, decreased percent saturation of the iron-binding protein, and increased free erythrocyte protoporphrin concentration. The total serum iron-binding capacity was not increased.

Lahey et al. (1952) and Cartwright et al. (1956) reported that either iron or copper deficiency brought about a normoblastic hyperplasia of the bone indistinguishable from each other. They suggested that a common factor, lack of available iron, was involved in either iron or copper anemia.

CONCEPTS OF INTERRELATIONSHIPS

Ranke et al. (1961) demonstrated that vitamin B₁₂ absorption is impaired by vitamin B₆ deficiency. Fifty millimicrograms of radioactive vitamin B₁₂ labeled with Co⁶⁰ (B₁₂) were given by mouth to vitamin B₆ deficient and B₆ injected animals. They found that deficient and treated animals absorbed 32 and 64 percent of the administered dose, respectively. Data are also presented to show that feeding rats a diet deficient in vitamin B₆ will result in the lowering of vitamin B₁₂ reserve as manifested by the low vitamin B₁₂ serum level and hepatic storage.

Jeejeebhoy et al. (1965) show that in the patients with vitamin B₁₂ deficiency, the unconjugated folate levels were high and varied between 5.7 and 44.5 ng per ml with a mean value of 26.1 ng per ml. The conjugated folates were, however, low and varied between 11.5 and 50 ng per ml with a mean value of 29.8 ng per ml. Unlike control subjects and folate deficient patients, the average ratio between conjugated and unconjugated folate levels amounted to about 1.3. These changes could be reversed by giving vitamin B₁₂. The results probably indicate a way by which vitamin B₁₂ and folic acid are interrelated at the cellular level.

Moore et al. (1944) found that iron is absorbed more easily in its ferrous form and indeed it has been clearly shown that the trivalent ion penetrates the intestinal wall more slowly than the divalent ion.

Ascorbic acid increases the absorption of iron from the intestinal tract. Initially, the increased absorption of iron in the presence of ascorbic acid was attributed to a reduction of ferric to ferrous ions (Nutrition Reviews, 1966). The usual cause of macrocytosis and megaloblastosis in kwashiorkor is probably folate deficiency. Megaloblastic changes occur with greater frequency when conditions are present which cause increased depletion of tissue folate store, such as infection and rapid regeneration of red cells following hemolysis in hemolytic anemias and in malaria. During treatment of kwashiorkor with a high protein diet, the marrow may change from normoblastic to partially megaloblastic when folate stores are depleted by the increased demands of rapid erythropoieses (Nutrition Reviews, 1968).

Vitale et al. (1966) proposed that iron deficiency may also result in a functional defect in folate utilization. The activity of the enzyme formimino-transferase was significantly reduced in iron deficient animals and was associated with a marked increase in urinary excretion of formiminoglutamic acid.

Copper deficient swine failed to absorb dietary iron at the normal rate (Lee et al., 1968). Increased amounts of stainable iron were observed in fixed sections of duodenum from such animals. When iron-59 was given orally, the mucosa of copper deficient pigs extracted iron from the duodenal lumen at the normal rate, but the subsequent transfer to plasma was impaired. Intra-muscular iron supplements given to copper deficient pigs, increased iron in the reticuloendothelial systems, the hepatic parenchymal cells and in normalblasts. When red cells that were damaged by prolonged storage were administered,

the reticuloendothelial system failed to extract and transfer the erythrocyte iron to the plasma at the normal rate. Administration of copper to copper deficient animals with normal iron stores increased the plasma iron immediately.

Sandstead et al. (1965) studied 39 Egyptian infants with protein-calorie malnutrition (marasmus). Plasma copper levels were extremely variable. Initially 25 percent were below 50 ug percent, 65 percent were below 100 ug percent, and 9 percent were 200 ug percent. Hypocupremia were commonly found in the acute phase of the disease. Elevated plasma copper levels were seen occasionally, more frequently during the recovery phase. The occurrence of hypocupremia was perhaps related to the hypoproteinemia.

Majaj et al. (1963) reported the occurrence of vitamin E responsive megaloblastic anemia in Jordanian infants with kwashiorkor. Sandstead et al. (1965) found that the serum vitamin E levels were generally low in kwashiorkor. Megaloblastic changes in the marrow were associated with low serum vitamin E levels in six patients, and probable megaloblasts and/or giant stab cells were observed in five. Three patients with normoblastic marrows also had low serum vitamin E levels.

During the study of the role of nutritional factors in the anemia of chronic liver disease an apparent relationship between vitamin E and B₁₂ was found by Oski et al. (1966). Subjects with low levels of serum tocopherol had elevated levels of vitamin B₁₂. In 26 adults with chronic liver disease of various etiologies an inverse relationship between levels of serum vitamin B₁₂ and levels of serum tocopherol was observed. It appeared that vitamin E may

play a role in the transport of metabolism of vitamin B₁₂ and that in its absence Vitamin B₁₂ accumulates extracellularly with the development of a possible intracellular deficiency.

Herbert and Zalusky (1962) reported that vitamin B₁₂ is required for normal folic acid metabolism. Vitamin B₁₂ is necessary for the formation of methionine from homocystin, a reaction in which N⁵-methyl tetrahydrofolic acid serves as a methyl donor, N⁵-methyl tetrahydrofolic acid may accumulate in the serum when vitamin B₁₂ is deficient. The "piling up" of N⁵-methyl tetrahydrofolic acid reduces the amount of folic acid available to travel via other metabolic pathways. This may explain much of the apparent folic acid deficiency in many patients with vitamin B₁₂ deficiency (Figure 7).

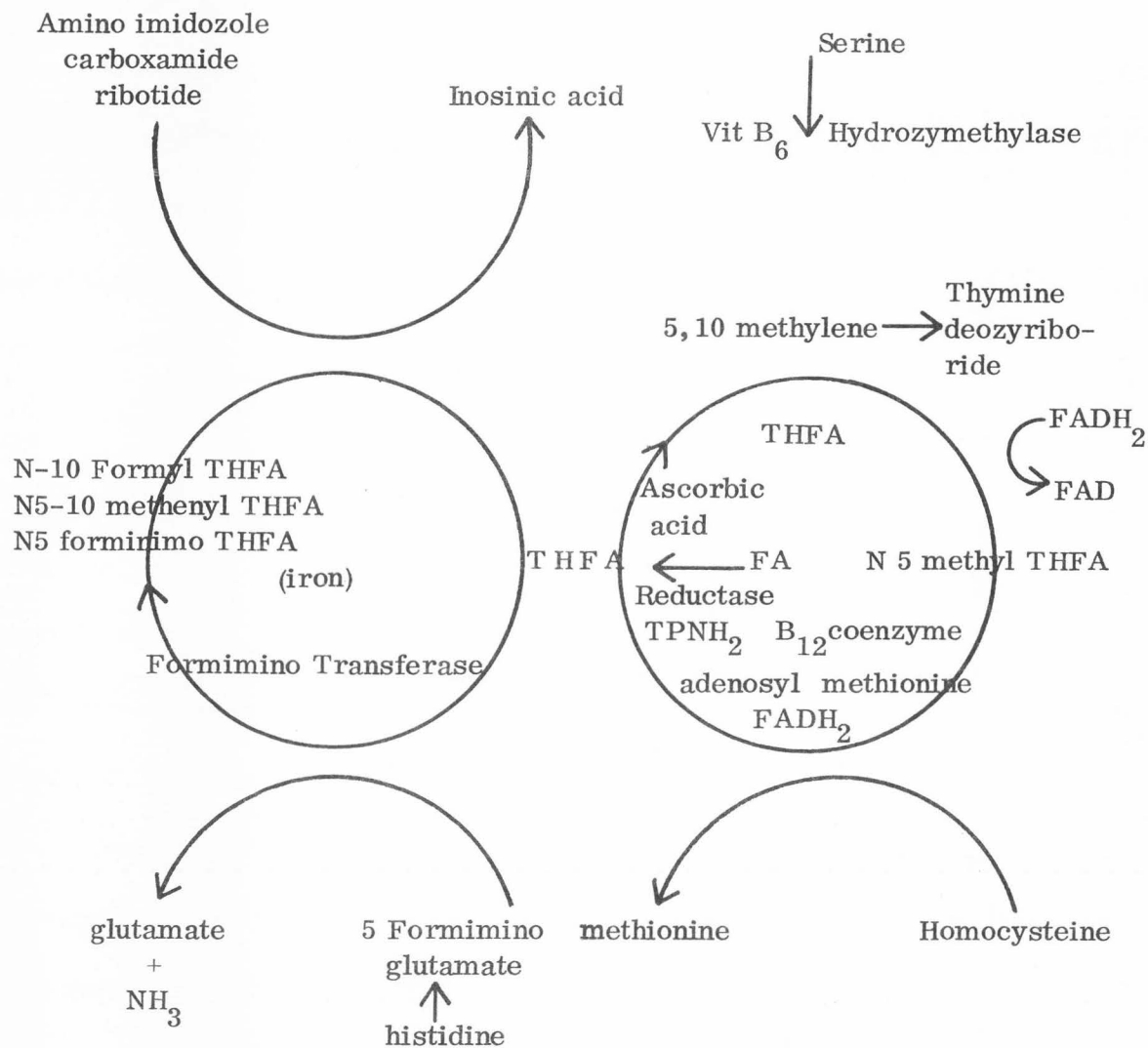


Figure 6. A scheme illustrating the interrelations of the folic acid coenzyme and the impact on these reactions of vitamin B complex, ascorbic acid and possibly iron (from Vilter, 1970, p. 279).

DIETARY REQUIREMENTS AND FOOD SOURCES OF IRON

Requirement

Since iron already present in the body is utilized over and over again, the amount of iron used daily for hemoglobin formation (26 to 27 mg) is far in excess of the actual daily requirement for iron in the diet (Drabkin, 1951). Drabkin proposed that the requirement represents only that iron which was lost to the body through excretory channels.

The total daily iron loss (gastrointestinal and urinary tracts and skin) for the adult man was taken by whom as 0.91 mg, based on an average body weight of 65 kg. For women at the menopause, the rate of iron loss was also taken by whom to be 14 ug per kg body weight per day as in man, based on the average body weight of 55 kg, while for women of child bearing age, menstrual losses of iron must be added to the basal iron loss (FAO/WHO, 1970).

Hallberg et al. (1966) reported that in 90 percent of normal women the menstrual iron loss amounts to less than 1.4 mg per day and in 95 percent to less than 2 mg per day. Therefore, adding the latter figure to the basal loss of 0.8 mg per day, the total iron losses to 2.8 mg per day in women.

In pregnant and lactating women, iron is required not only to replace basal physiological losses but also to allow for the expansion of the red cell mass, to provide for the needs of fetus and placenta and to breast feed the baby.

Therefore, the iron requirement during the first half is about 3 mg per day; during lactation is about 2.4 gm per day (Table 4).

The FAO/WHO Expert Group (1970) also reviewed information about absorption from mixed diet. Radioactive iron absorption studies indicated that the amount of iron absorbed was affected by the presence of different foods. So experts agreed to express recommended intakes for iron on the basis of three types of diet (Table 3).

Table 3. Recommended daily intakes of iron

		Absorbed iron required	Recommended intake according to type of diet		
			Animal foods below 10% of calories (mg)	Animal foods 10-25% of calories (mg)	Animal foods over 25% of calories (mg)
Infants	0-4 months	0.5	a	a	a
	5-12 months	1.0	10	7	5
Children	1-12 years	1.0	10	7	5
Boys	13-16 years	1.8	18	12	9
Girls	13-16 years	2.4	24	18	12
Menstruating women ^b		2.8	28	19	14
Men		0.9	9	6	5
Pregnancy			see Table 4		
Lactation					

^aBreast-feeding is assumed to be adequate.

^bFor non-menstruating women the recommended intakes are the same as for man.
Taken from FAO-WHO, 1970, p. 54.

Table 4. Distribution of iron requirements during pregnancy and lactation

Period	Requirements (mg)	
	Total	Daily average
First half of pregnancy	110	0.8
Second half of pregnancy (925-500 mg)	425	3.0
Lactation ^a		
Repayment of blood loss 250 mg		
Milk iron loss 45 mg	440	2.4
Basal losses 145 mg		

^aAssuming a six months lactation period. (From FAO/WHO, 1970, p. 52)

Food sources--enrichment levels

Rich sources: organ meats (liver, kidney, and heart), egg yolk, dried legumes, cane molasses, cocoa, shellfish and parsley.

Poor sources: milk, milk products, fats and oils, white flour and bread (unenriched), polished rice, potatoes, sago, white sugar, and fruit juices.

Intermediate sources: muscle meats, fish, poultry, nuts, green vegetables, wholemeal or enriched flour, wholemeal bread and roots.

DIETARY REQUIREMENT AND FOOD SOURCES OF FOLIC ACID

Requirement

Herbert (1968) suggested that the minimal daily adult requirement for folic acid is about 50 ug. This is the quantity of folic acid that, when administered orally or parenterally daily as pteroylglutamic acid to patients with folate deficiency uncomplicated by other systemic diseases, will produce a relatively rapid return toward hematologic normality, accompanied by reticulocytosis, and rise of hematocrit toward normal.

A woman with folate deficiency megaloblastic anemia was sustained on a diet almost devoid of folate for two years, supplemented by 75 ug of pteroylglutamic acid daily. On this diet, macrocytosis disappeared after 282 days. This dose was probably greater than required to sustain normality, since there was evidence that her tissue folate stores were gradually replenished on this dose (Sullivan and Herbert, 1964).

Supporting 50 ug of folic acid as in the range of the minimal daily adult requirement are several other studies: (1) three healthy adult female medical research technicians were given a diet containing approximately 5 ug of folate daily, supplemented with a single daily oral tablet of pteroylglutamic acid. In the space of slightly more than a month, a clear fall of the normal *Lactobacillus casei* serum folate level of 10.3 ng per ml to a subnormal level of 4.2 ng per

ml occurred in the technician whose daily tablet contained 25 ug of pteroylglutamic acid; however, the normal serum folate level of 6.5 ng per ml of the subject receiving a daily tablet containing 50 ug of pteroylglutamic acid daily was essentially the same (6.2 ng per ml) at the end of the study, and the normal serum folate level of 11 ng per ml in the subject receiving a daily tablet containing 100 ug of pteroylglutamic acid was also essentially the same (9.4 ng per ml) at the end of the study (Herbert, 1962).

Folic acid is widely distributed in foods in both free and conjugate form. FAO/WHO, in 1970, suggested that only the "free" folate is available for absorption in healthy individuals and that the absorbability of the monoglutamate portion of the "free" folate is absorbed by normal individuals. Therefore, the FAO/WHO recommended a higher daily dietary intake of 200 ug of folate per day.

The recommended daily intakes of folic acid are given in Table 5.

Table 5. Recommended daily intakes of "free" folate

Age or status	Recommended daily intake in ug per day
0-6 months	40
7-12 months	60
1-12 years	100
13 years and over	200
Pregnancy	400
Lactation	300

From FAO/WHO, 1970, p. 46

Food sources--enrichment levels

Rich sources: liver, kidney, yeast, deep-green leafy vegetables.

Intermediate sources: lean beef, veal, eggs and whole-grain cereals.

Poor sources: root vegetables, dairy foods, pork and light-green vegetables.

DIETARY REQUIREMENTS AND FOOD SOURCES
OF VITAMIN B₁₂

Requirement

Heyssel et al. (1966) suggested an obligatory daily loss rate of vitamin B₁₂ is 0.1 percent of the total body pool (2 to 5 mg) regardless of how large or small the pool may be. Normal adults eating a diet containing 5 to 15 ug of vitamin B₁₂ divided into three meals can and probably do absorb at least 5 ug of the vitamin daily. A minimal daily dietary intake of vitamin B₁₂ of 0.6 to 1.2 ug is adequate to maintain health and normal hemopoiesis in normal subjects operating with low body store. Any additional needs not known. It is valid to recognize the value of increased of 3 to 5 ug of vitamin B₁₂ as the dietary requirement for a population appears reasonable.

Herbert (1968) reported that the minimal daily adult male requirement for vitamin B₁₂ was about 0.1 ug. Although reticulocytosis occurred and hematocrit rose, serum vitamin levels of vitamin B₁₂ deficient subjects were not always raised by intramuscular injection of 0.1 ug of the vitamin daily for periods up to a month. Preliminary studies suggested that a gradual rise did occur when the parenteral dose was 0.2 ug daily. Daily parenteral doses of 1 ug of vitamin B₁₂ raised the serum vitamin B₁₂ level of such subjects into the clearly normal range with two weeks. Thus, the minimal daily adult

requirement for vitamin B₁₂ is definitely below 1 ug, is not below 0.1 ug, and will probably prove to be approximately 0.1 ug (and not above 0.2 ug).

The recommended daily intakes of vitamin B₁₂ are given in Table 6.

Table 6. Recommended daily intakes of vitamin B¹²

Age or status	Recommended daily intake in ug
0-12 months	0.3
1-3 years	0.9
4-9 years	1.5
10 years and over	2.0
Pregnancy	3.0
Lactation	2.5

From FAO-WHO, 1970, p. 40.

Food sources--enrichment levels

Rich sources: meat, poultry, fish, cheese, milk, and eggs.

Poor sources: cereal grains, vegetables, fruits.

DIETARY REQUIREMENTS AND FOOD SOURCES OF PROTEIN

Requirement

The amounts of amino acids present in the diet are not necessarily all available to the body. Availability can be reduced by incomplete digestion and absorption; by the presence of inhibitors of digestive enzymes found in some foods; and by damage to proteins and amino acids from heat treatment and other processing. The amino acids of most animal proteins are very efficiently absorbed, but this is not necessarily so for many proteins of vegetable origin. On the vegetable diets, the fecal nitrogen may amount to 20 percent or more of the intake. The reason for this is not always known; the low apparent absorption is probably only partly explained by a high fiber content of the diet (FAO/WHO, 1965).

Table 7 showed the requirement for reference protein derived from the calculation of nitrogen requirement ($N \times 6.25$). In the table an indication is given of levels 20 percent below and 20 percent above the average.

Table 7. The protein requirements of children and adults in terms of reference protein

Age (Years)	gm per kg body weight per day		
	Average	-20%	+20%
Children:			
1-3	0.88	0.70	1.06
4-6	0.81	0.65	0.97
7-9	0.77	0.62	0.92
10-12	0.72	0.58	0.86
Adolescents (boys and girls):			
13-15	0.70	0.56	0.84
16-19	0.64	0.51	0.77
Adults	0.59	0.47	0.71

Additional allowance for pregnancy: 6 gm per person per day in the second and third trimesters.

Additional allowance for lactation: 15 gm per person per day.

Taken from FAO/WHO, 1965, p. 22.

Food sources--enrichment levels

Rich sources: milk, fish, meat, poultry, cheese, and nuts.

Intermediate

sources: eggs, legumes, flours and cereals.

Poor sources: fruits and vegetables.

DIETARY REQUIREMENTS AND FOOD
SOURCES OF VITAMIN E

Requirement

Body tocopherol requirements appear to depend on a variety of factors. As the intake of fat and polyunsaturated fatty acids (PUFA) increased, the requirement for vitamin E increased. The adult needs about 10 IU when PUFA intake is high (over 35 gm per day). The Food and Nutrition Board of the National Research Council (1968) recommended daily intake of 5 IU during the first year of life, 25 IU for women, 30 IU during pregnancy and lactation, and 30 IU for men. These allowances are based upon metabolic body size.

A convenient rating for the contribution to vitamin E nutriture by diets and individual food is "E: PUFA" the ratio of the amount of vitamin E (milligram of d-alpha-tocopherol) to the amount of polyunsaturated fat (grams of polyunsaturated fatty acids). The E: PUFA ratio of the average 1960 diet in the United States (average per capita amount of foods available for consumption) is 0.6. Published data on man and on animals indicate that this ratio is not much higher than that of diets inducing definite symptoms of vitamin E deficiency. Thus, diets with E: PUFA ratio above 0.6 are predicted generally to protect against vitamin E deficiency, but diets with ratio lower than 0.6 are expected to have a depleting diet (Harris and Embree, 1963).

Herting and Drury (1963) measured the vitamin E content of vegetable oils and fats. Average values for refined in hydrogenated oil of cotton seed, maize, safflower and soya were 0.31, 0.19, 0.31, 0.16 mg d-alpha-tocopherol per gram of oil, giving 0.65, 0.36, 0.45, 0.28 mg d-alpha-tocopherol per gram of polyunsaturated fatty acid. Increased consumption of such oils might cause depletion or deficiency of vitamin E in man.

Booth and Bradford (1963) estimated tocopherol content in raw edible parts of plants. Values differed with season, rose with maturity, and were often high in parts not normally eaten, such as skin or faded leaf. They were generally high in dark green tissue, lower in light green, and low in some roots and fruits.

Food sources--enrichment levels

Rich sources: vegetable oils (corn, soya bean, peanut, coconut, cottonseed), dark-green leafy vegetable, nuts and legumes.

Poor sources: animal origin.

DIETARY REQUIREMENTS AND FOOD
SOURCES OF PYRIDOXINE

Requirement

Healthy young adult male subjects (ages 18 to 22 years) were placed on a liquid formula diet made up of purified foodstuffs sufficient in all known nutrients except vitamin B₆. Throughout the experiment, the subjects were divided into two groups: the first group received a low protein diet providing 30 gm of protein per day; the second group was given a high protein diet supplying 100 gm of protein per day. The results indicated that the pyridoxine requirement was increased with the higher protein intake. Under the conditions of this study, it appeared that the minimal daily vitamin B₆ requirement for subjects on a high protein intake would be 1.5 mg per day, while the optimal daily vitamin B₆ requirements for these same subjects would be 1.75 to 2 mg per day. On the other hand, the subjects on the low protein diet would have a requirement of slightly greater than 1 mg per day and an optimal requirement of 1.25 to 1.5 mg per day (Baker et al., 1964).

The need for vitamin B₆ is proportional to the amount of protein metabolized. The recommended allowance for the reference man and woman is 20 mg daily. This provides a reasonable margin of safety and permits a protein intake of 100 gm or more. Those who ingest a high protein diet (125-150 gm) may need

more, whereas those who have a low protein diet (40-50 gm) may require only 1.2 to 1.5 mg (Robinson, 1972).

The allowance for infants is 0.2 to 0.4 mg; for children from 1 to 10 years, it increases gradually from 0.5 to 1.2 mg; and for adolescents are 1.4 to 2 mg. During pregnancy and lactation 2.5 mg is recommended (Food and Nutrition Board, 1968).

Food sources--enrichment levels

Rich sources: meat, poultry, fish, potatoes, sweet potatoes, vegetable, whole-grain cereals, and egg yolk.

Poor sources: sugars and other sweeteners, fats and oils, coffee and cocoa.

DIETARY REQUIREMENTS AND FOOD SOURCES OF ASCORBIC ACID

Requirement

After a period of six weeks for which all received a daily supplement of 70 mg ascorbic acid, 19 men and 1 woman lived on an diet deficient in vitamin C, seven receiving a daily supplement of 10 mg, three of 70 mg, and the remainder did not receive a supplement. No signs of scurvy appeared in those receiving 10 or 70 mg daily, though, in the former, blood values were very low. It is concluded that a daily dose of 10 mg ascorbic acid is slightly greater than the minimum protective dose, and that, as long as there is no evidence that an intake of more than 30 mg daily has veneficial effects, there is no basis for recommending an amount larger than this to meet the daily requirement (Medical Research Council, 1948).

The rate of metabolism of ascorbic acid in normal male adults has been shown to be in the range of 0.4 mg per day per kg of fat free body weight. This is the equivalent of 20 to 40 mg for the majority of presumably healthy men, amounts that are in excess of quantity that prevents scurvy (Griffith, 1967).

Martin et al. (1957) studied the intakes and serum levels of vitamin C in 2129 pregnant women. In general, serum levels decreased during pregnancy except in the group at a high level of intake. Values were further decreased postpartum, and were lower for lactating than for nonlactating women. Evidence

was presented that on the average intakes of 80 to 100 mg daily supported high levels of ascorbic acid in the serum during pregnancy. The serum levels of nonlactating mothers averaged 0.7 mg per 100 ml during the puerperium on intakes of 100 mg or over per day; the serum concentration of lactating mothers did not average greater than 0.3 mg even on intakes exceeding 120 mg daily.

The recommended daily intakes of ascorbic acid are given in Table 8.

Table 8. Recommended daily intakes of ascorbic acid

Age	Recommended daily intake in mg
Birth-12 years ^a	20
13 years and over	30
Pregnant women (second and third trimesters)	50
Lactating women	50

^aFor infants aged 0-6 months it is accepted that breast-feeding by a well-nourished mother is the best way to satisfy requirements for ascorbic acid.

Taken from FAO/WHO, 1970, p. 28.

Food sources--enrichment levels

Rich sources: citrus fruits (oranges, grapefruits and lemons), fresh strawberries, cantaloupe, pineapple, melon, raspberries, guavas, broccoli, brussels sprouts, spinach, kale, green peppers, cabbage and turnips.

Intermediate sources: peaches, pears, apples, bananas, blueberries.

Poor sources: organ meats (liver, kidney, heart, and brain), cod roe, milk, and eggs.

DIETARY REQUIREMENTS AND FOOD

SOURCES OF COPPER

Requirement

Dietary copper strongly influences the levels of copper in the blood and liver of most species fed either toxic or copper deficient diets (Milne and Weswig, 1968).

A deliberate attempt has been made to induce copper deficiency in young infants by Wilson and Lahey (1960). They found that varying the dietary intake of copper was without effect during the first two months of life. The babies fed the low copper diet appeared normal in every respect. By six month of age, however, this group had a somewhat lower hemoglobin level, but hypocupremia was not present, nor were other manifestations of copper deficiency. Wilson and Lahey's study did not establish the minimum requirement of copper for young infants; it did document the fact that infants might thrive, for short periods of time at least, on intakes well below those currently recommended. How long they could thrive on such a diet was a most important question. The majority of infants reported in the clinical literature as suffering from hypocupremia had subsisted largely on milk for periods of six to twelve months or longer.

On a normal diet copper is not accumulated preferentially by any tissue. Gubler (1956) suggested the normal intake of man was 2.5 to 5 mg per day.

This amount was adequate for the maintenance of positive copper balance. Children required about 0.05 to 0.1 mg copper per kg of body weight daily. Underwood (1962) suggested 2 to 3 mg per day for an adult diet. It appears that approximately 3 mg of copper per day is needed by either children or adults for a high quality diet. This amount should be easy to obtain from common foods on any well-balanced diet.

Food sources--enrichment levels

Rich sources: organ meats (liver, kidney, heart, and brain), crustaceans and shellfish (especially oysters), nuts, dried legumes, dried vine and stone fruits, and cocoa. The copper content of these foods ranges from 20 to 30 ppm to as high as 400 ppm.

Intermediate sources: wholemeal flour and bread, the green leafy vegetables, eggs, muscle meats, fish, and poultry.

Poor sources: milk, butter, cheese, white sugar, honey, margarine, nonleafy vegetables, most fresh fruits and the refined cereal products. The copper content of these foods ranges from 0.5 to 2 ppm.

OCCURRENCE OF ANEMIAS DURING THE LIFE CYCLE

Little information currently exists about the prevalence of iron deficiency without anemia. All incidence figures relate to the prevalence of anemia and assume, probably correctly, that iron deficiency is the principle cause. The anemia is most frequent in those areas of the world where dietary intake, particularly of animal protein, is low, where infection with intestinal parasites is common and where medical care is inadequate. Frequency is greatest among the poor classes and at those times in life when iron requirements are highest, during growth and during the reproductive years in women (Moore, 1970).

It is true that iron deficiency is not the only cause of anemia. In survey population, the nature of any anemia encountered is seldom identified. Therefore, in a given population group, 10 percent of the subjects may be anemic. This does not necessarily mean that all 10 percent have iron deficiency anemia. In a study of 1052 pregnant women, Benjamin et al. (1966), found that 49.1 percent of the patients were mildly anemic (so classified when hemoglobin concentrations were between 10 and 12 gm per 100 ml; hematorcrit between 30 and 35 percent, or erythrocytes between 3,500,000 and 3,750,000 per cubic milliliter), and 22.8 percent of the cases were moderately or severely anemic (hemoglobin below 10 gm per 100 ml; hematocrit below 30 percent, or erythrocytes below 3,500,000). There were 239 patients in the latter group. Of these 130 were investigated by serum determination of iron, folic acid, and vitamin

B₁₂. Four of these were in the first, 51 in the second, and 75 in the third trimester of pregnancy. Based on the serum concentrations of these nutrients, 80 percent were classified as iron deficient, 30.8 percent as vitamin B₁₂ deficient and 64.6 percent as folic acid deficient.

Evaluation of bone marrow stores of iron is considered by White (1967) the best indication of iron status. Use of this method in a limited number of studies has indicated a relatively high incidence of iron depletion in the absence of anemia in girls and women.

Mosen et al. (1967) found that the iron intake of nonanemic university women 19 to 37 years of age averaged 9.2 mg, or only about one-half the 1968 RDA for iron (18 mg). Many showed evidence of unsatisfactory iron status, including absent or low bone marrow stores, low plasma iron, low percent saturation of transferrin and high iron absorption.

White (1969) reported a similar low intake of iron in high school and college women. She attributed the low intake of this age group to concern over calories as well as factors in food processing and preparation, such as decreasing use of iron containing cooking vessels. These observations are consistent with the inability of many pregnant women to meet their iron needs with medicinal iron.

Herbert (1968) suggested an incidence of folate deficiency that appears to encompass many of economically disadvantaged people of the world, especially pregnant women of low socioeconomic status, and also to indicate that deficiency of vitamin B₁₂ is common among vegetarians, patients with sprue, and the elderly. The more finely divided the food, and the more it is heated

(especially in water) prior to being eaten the larger the percentage of its initial folate content that is destroyed. As a general rule this means that vitamin B₁₂ is found only in foods of animal, fish, and fowl origin. This means that vegetarian will have a incidence of vitamin B₁₂ deficiency. A high incidence of folate deficiency is seen in frequent pregnancy, resulting in recurrent drainage of residual folate stores (normal stores are 5 to 10 mg), which are taken by the fetus at the expense of the mother.

The prevalence of iron deficiency anemia among children aged six months to three years seen at a ambulatory clinic in Iowa was found to be 4.1 percent. Among a variety of epidemiologic and dietary variables, only age and birth weight had statistically significant relationship to the development of anemia. The mean age of all the anemic children was 16.6 months in contrast to a mean age of 21.3 months among all those with higher levels of hemoglobin. This peak prevalence of anemia was 7.1 percent in the 12 through 17 months age group and 6.3 percent for the entire second year. This contrasts with the finding of less than one percent anemic children in the two to three-year-age group. Mean birth weight of anemic children of this age was 6.6 lb; among the "normals," mean birth weight was 7.3 lb (Kripke and Sanders, 1970).

Futrell et al. (1970) reported a nutritional status study of Negro pre-school children in Oktibbeha County, Mississippi. The iron intake of the children was so low that all average daily intakes were below 8 mg except when the mother had some college education. The "low" hemoglobin levels also reported were probably related to this insufficient iron intake. Ascorbic acid showed the same relationship. Approximately 43 percent of the children

whose mothers had either no schooling or less than five grades, had less than 15 mg of ascorbic acid; the percentage decreased to 15 percent when the mothers were high school graduates. In this survey, both dietary intake and laboratory values paralleled the mother's educational attainments. The higher her education, the better were the dietary intake and nutritional status of her child.

Haughton (1963) determined hemoglobin concentrations in a random sample of 286 young children, 15 percent of whom were Negro from low socioeconomic areas of New York City. The data showed that 54, or 18.9 percent, of the preschool children studied in this underprivileged population had hemoglobin levels below 10 gm per 100 ml. Of these 54 children, 44, or 81.5 percent, were under two years of age and only four, or 7.4 percent were over three years of age. It appears that the great majority of the cases of anemia in preschool children in the health district could be expected to occur in children under two years of age.

In a series of 460 preschool Negro children from low income families, 133 or 29 percent, were found to have hemoglobin levels below 10 gm per 100 ml and almost one half were below 10.5 gm per 100 ml. The prevalence of anemia (hemoglobin levels below 10 gm per 100 ml) was high by six months of age, reached a peak of 65 percent in those children who were 12 to 17 months of age, and then fell off rapidly in the older age groups (Gutlius, 1969).

Utilization of dietary iron has been calculated for 1048 infants whose care was supervised under field conditions in a low socioeconomic population where iron deficiency has been shown to be widespread. Subjects were placed

either in a control group receiving an evaporated milk formula with separate vitamin supplementation but with no additional iron was prescribed or in a study group under similar management but furnished for a period of six to nine months with iron containing formula providing 12 mg of elemental iron per quart. Study group infants remained in good iron balance, but iron deficiency anemia was flourishing in the control group. In this latter group, 76 percent of the infants became anemic—most before one year of age. In contrast, only nine percent of the study group infants developed hemoglobin concentration less than 10 gm per 100 ml, more than half after 12 months. Subsequent inquiry revealed the diet to be iron deficient after discontinuance of iron containing formula (Andelman and Sered, 1966).

TREATMENT OF NUTRITIONAL ANEMIAS

Medicinal

In the overwhelming majority of persons suffering from iron deficiency anemia, treatment with orally administered simple iron salts leads to a completely satisfactory rise in hemoglobin levels and symptomatic improvement. It is an exceedingly rare patient who is unable to tolerate treatment with such oral iron preparations. McCurdy (1965) compared the effectiveness of oral and parenteral iron therapies in patients severely deficient in iron. Each patient received either oral ferrous sulfate in a dose providing 180 mg of iron daily, or one or the other of two iron complexes suitable for intramuscular administration. The total dose for parenterally administered iron was 2500 mg if the hematocrit was between 20 to 25 percent, or 3000 mg if the hematocrit was less than 20 percent. Although the mean daily increase in hematocrit value was slightly greater for those patients treated with one of the parenteral iron preparations than it was in either of the other two groups, the differences were not statistically significant. Because of the possibility of serious adverse reactions to intramuscular or intravenous injection of iron compounds, the author noted that there would seem to be no justification for their use, except in those situations where oral iron is poorly tolerated by the gastrointestinal tract or where the patient is incapable of taking oral medication.

Pritchard (1966) reported that severe iron deficiency anemia can be corrected very nearly as fast by several inexpensive preparations of oral iron as by iron dextran injection or saccharated iron oxide. The average rate of increase in hemoglobin concentration in 28 women who ingested ferrous sulfate, ferrous gluconate, or ferrous fumarate in amounts which provided 180 to 220 mg of iron per day was 0.25 gm per 100 ml compared to 0.28 gm per 100 ml per day in 14 who received iron dextran injection intramuscularly and 0.33 gm per 100 ml per day in 14 who were treated with saccharated iron oxide given by intravenous infusion. Ferrous sulfate, ferrous fumarate, or ferrous gluconate in a daily dose containing about 200 mg of iron are very satisfactory for treatment in most cases of severe iron deficiency anemia. He also suggested that addition to a simple iron salt of folic acid, vitamin B₁₂, liver-stomach concentrate, and ascorbic acid certainly increased the cost over that of a similar amount of ferrous sulfate or ferrous fumarate but did not increase the rate of hemoglobin production.

Dubach et al. (1948) proposed that it was not unlikely that as much as 100 mg of a 200 mg oral dose of iron might be absorbed per day. Normal hemoglobin production utilizes approximately 25 mg of iron daily. During the response of the bone marrow to treatment of severe iron deficiency anemia, red blood cell production may be increased by a factor of two or four times. Thus, even at the maximum response, absorption of 100 mg of iron per day from the gastrointestinal tract should provide adequate iron for hemoglobin formation. Parenterally administered iron would therefore not to be expected to provoke a more rapid response than adequate iron dosages administered orally.

Ascorbic acid has been shown to potentiate the absorption of ferrous sulfate in aqueous solution, in standard tablet form, and in a plastic matrix. The potentiation increases with increasing doses of ascorbic acid up to 500 mg and holds with doses of iron up to 120 mg. The use of iron preparation containing ascorbic acid may permit the use of less frequent doses in the therapy of iron deficiency anemia and may refill iron stores better than oral iron salts without ascorbic acid (McCurdy and Dern, 1968).

Lyenger et al. (1970) reported the results of prophylactic trial for the prevention of pregnancy anemia. All the subjects belonged to low income groups. They were divided into four groups: group I received placebo tablets containing lactose; group II received 30 mg of elemental iron daily as ferrous fumarate in a single tablet; group III received 30 mg of elemental iron and 500 ug of folic acid daily in a single tablet; and group IV received, in addition to iron and folic acid, 2 ug of vitamin B₁₂ daily in a single tablet. Of the subjects in the unsupplemented group 60 percent showed a progressive fall in hemoglobin levels with advancing pregnancy, whereas in the other 40 percent the levels remained stable. In the three supplemented groups, only 6, 10, and 14 percent of subjects showed such a fall, whereas the remaining subjects showed either stable levels or an actual increase. There appeared to be little difference between the three supplemented groups in this respect. It is suggested that daily supplements of 30 mg iron given during the last 100 days of pregnancy is adequate to maintain satisfactory hematological status during pregnancy.

The results of the therapeutic trials in fifty-six patients with severe nutritional megaloblastic anemia due to folate deficiency with varying

parenteral doses of pteroylglutamic acid and leucovorin are described. The hematologic response was good in all patients given 1000, 500 and 100 ug pteroylglutamic acid per day parenterally for 14 to 21 days, as well as in patients given 200 ug leucovorin parenterally or 5000 ug pteroylglutamic acid orally per day for the same length of time. No appreciable therapeutic response was elicited in patients given 10 ug pteroylglutamic acid per day parenterally (Izak et al., 1963).

Oski and Barnes (1968) observed a group of eleven infants who had a mean birth weight of 1,480 gm and averaged 57 days of age at the time of tocopherol therapy. Infants in this group received from 200 to 800 mg of d-alpha-tocopherol by mouth over a period which ranged from 7 to 13 days with a mean response time of 10 days. All infants in this group were anemic and all had elevations in their reticulocyte counts. Tocopherol appears to be poorly absorbed from a low-fat diet in the premature infant. Treatment of premature infants from birth with supplemental vitamin E reduces the severity of anemia and prevents the marked reticulocytosis commonly observed in these infants of low birth weight.

Dietary

The gap between intake and needs in certain groups has led to recommendation that fortification of foods with iron be increased and expanded. The absorption of wheat iron in normal and in iron deficient human subjects has been compared to that of hemoglobin, ferritin, and iron salts. It is shown that iron salts, and that wheat iron is less available than either hemoglobin or ferritin.

There is also considerably less enhancement in absorption of wheat iron in the iron deficient subject (Hussain et al., 1965). In 1955, Steinkamp, Dubach and Moore studied the absorption of iron from bread enriched in the baking process by four different forms of radioactive iron (ferrous sulfate, reduced iron, ferric orthophosphate, and sodium ferric pyrophosphate). Absorption was measured from the amount of radioactivity that was not recovered from the feces. In healthy subjects, they found 1 to 12 percent absorption from two to four mg of iron baked into bread and considerably higher absorption (26 and 38 percent) in two who were thought to have latent iron deficiency. Three subjects with overt iron deficiency showed absorptions of 45 to 64 percent. The authors concluded that iron added to bread was a significant source of iron in human nutrition. They also found absorption to be increased two to three times when one gram of ascorbic acid was given with the labeled bread. Callender and Warner (1968) measured absorption of radioactive iron incorporated into bread in the baking process. The bread was given in a standard meal on two to four successive days and absorption was compared with that obtained from a standard dose of 5 mg of ^{59}Fe ferrous iron. The mean absorption from the standard dose was 42 percent, which is similar to that obtained in a larger series of 63 iron deficient subjects in whom the mean absorption was 40.1 percent; the absorption from the bread was only 6.7 percent. This is considerably less than the amount absorbed by the iron deficient subjects in the study of Steinkamp et al. (1955), although the total dose of iron was smaller and might, therefore, have been expected to be more efficiently absorbed.

The study of the availability from chapatti of wheat iron, and of an iron salt added to the flour, given to 21 Indian ladies as part of their usual diet, was reported. The wheat appears to have been better absorbed than the iron salt but the availability of both was very low. The mean absorptions of wheat iron were 4 and 2.2 percent from white and whole-meal chapatti, respectively, and 2.1 and 1.8 percent from the iron salt baked into similar chapatti. According to the data, it seems that small supplements of iron in flour will provide an insignificant benefit to countries where chapatti, and probably other cereal foods, are eaten. Unfortunately, if this is true, it is not possible at present to compensate for low availability by adding much larger amounts of iron. Iron salts induce rancidity in flour, which is promoted by warmth and humidity. Furthermore, iron salts have a harmful effect on the baking qualities of flour. Therefore, it is an urgent need to discover or develop an iron preparation that can be added to flour in relatively high concentrations without seriously affecting its keeping or baking qualities (Elwood et al., 1970).

There is no doubt that the nutritional quality of cereal protein is rather low. That it can be improved by amino acids supplementation, and that lysine is usually the most limiting amino acid. Increasing availability of crystalline amino acids at lower cost has suggested that amino acid fortification or supplementation of cereals might be a useful procedure for combating protein malnutrition. Many authors have the impression that addition of small amounts of lysine, or lysine and threonine, to cereals will produce products with high quality proteins comparable to meat and milk. But this is not generally true (Hegsted, 1968). Howe et al., (1967) stated that the poorest inhabitants of the

cereal-growing areas subsist almost entirely on cereal grains. If they consume a sufficient quantity of any grain to satisfy their caloric requirements, the quantity of protein ingested will be adequate and its quality can also be made adequately lysine supplementation. It is known that other deficiencies besides those of energy and protein exist in the developing countries. If each cereal could be so supplemented that when used as the sole source of food it satisfied all nutritional requirements and further, if a sufficient quantity of such supplemented grain were available to satisfy energy requirements it is highly unlikely that any nutritional deficiencies would develop.

Howe et al., (1967) given the following PER values (protein efficiency ratio): white flour, 0.7; white flour + 0.25 percent lysine, 1.6; and casein, 2.5. Casein has usually been found to have a biological value of NPU (net protein utilization) of 70 percent. White flour plus lysine is approximately 64 percent as effective as casein or would have a biological value of 45 percent.

Human and cows milk contain little iron (about 1.5 mg per quart and 0.5 mg per quart, respectively). About 15 quarts of cows milk would have to be consumed each day to provide enough iron to meet the requirements of normal infants during the first year of life. When milk accounts for a large proportion of the infant's calories, the diet will perforce be grossly deficient in iron. The most important source of iron in the diet of American infants is cereal that has been artificially fortified with reduced iron or iron-pyrophosphate during processing. Such cereals contain 8.6 to 22 mg of iron in each dry ounce. Limited amounts of eggs and meats are ordinarily consumed during the first year of life and so usually contribute a relatively small proportion of the total iron

requirements. Iron requirements of normal infants can be met in a number of ways. Daily consumption of one and fourth oz dry weight of iron enriched baby cereal beginning by six weeks of age and progressively increasing to one and half oz dry weight by six months of age assumes an adequate iron intake for all infants. The consumption of iron-fortified baby cereals decrease markedly during the second six months of life, so that by 12 months of age most infants no longer receive these cereals. This nutrition committee suggests the most effective way to prevent iron deficiency on a large scale is to provide an iron-fortified dietary staple which can be begun by six weeks of age for infant consumption. Milk or carbohydrate staples (cereal, bread, grits, rice) have been shown to be suitable for fortification. The low birth weight infants and infants with reduced iron endowment, need relatively larger amounts of iron. It is doubtful that diet alone, even when iron supplemented cereals are used, can provide sufficient iron for these greater requirements. Iron fortified milk or medicinal iron should be prescribed in order to assure an iron intake of at least 2 mg per kg per day to a maximum of 15 mg per day for infants with reduced iron endowment (Committee on Nutrition, 1969).

SUMMARY AND CONCLUSION

Little information currently is available concerning about the prevalence of iron deficiency without anemia. All incidence figures relate to the prevalence of anemia and assume, probably correctly, that iron deficiency is the principle cause. Survey studies indicate that in certain parts of India and Africa nearly half the population may be affected. Frequency is greatest among the poorer classes and at those times in life when iron requirements are highest, during growth and during the reproductive years in women.

Nutritional anemia results from one or a combination of the following: inadequate diet, defective absorption, blood loss, or repeated pregnancies. Defective absorption can be caused by diets that are grossly deficient in iron or high in cereal content and low in animal protein. Clay-eating is practiced particularly by children and adult women in Turkey: among the poorer classes, its prevalence is probably much greater than is generally realized.

Nutritional anemia is usually characterized as hypochromic and microcytic cells. This broad classification suggests a lack of the pigment hemoglobin and the presence of small cells. Megaloblastic anemia is the primitive red blood cell of large size with large nucleus; present in blood when there is deficiency of vitamin B₁₂ and/or folic acid.

The solution of these deficiencies are to help the developing countries in techniques to produce more food, make better use of their present food,

provide acceptable high protein foods at a reasonable cost, and educate the people with ways to use the principles and modern knowledge of nutritional requirements to obtain better health.

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